The Ability of Olive Rhizogenesis in Correlation with Vegetative Growth

Agriculture

Keywords: Olive, propagation, nebulization, IBA, culture, mist-propagation.

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Abstract

The present study was carried out in a three–year period, to investigate olive varieties propagation (Kaninjot, Mixan, Bardhi Tiranes, Kryps Berati, Himara, and Frantoio), by treatment with indol-3 butyric acid (IBA), (IBA2gl¯¹, IBA5gl¯¹ and Control), in the typical vegetation phases: February, May, September and December, with mist-propagation method. After 70 days, the percentage of rooting of studied cultivars varies from 2 to 95%. The two IBA dosages have not affected rooting in the same way within each period. IBA at IBA2gl¯¹ and IBA5gl¯¹, gave high rooting percentages for Kaninjot, UBT and Mixan cultivars, respectively from 67 to 78%; 63 to 70%; and 56 to 77%. Leafage presence has been an important factor. Defoliation has varied between 8.3 and 44.1%. At high cambial activity, the IBA concentration of IBA2gl¯¹, resulted more effective; whereas under conditions of low cambial activity, high IBA concentrations yielded better results. At active vegetation stage the IBA in high concentrations shows inhibitory and toxic effect. The control gave low rooting percentage and with significantly highlighted changes compared to IBA treatments. Cultivars have their highest endogenous rhizogenic capacity in May and September, period which corresponds to the active cambium activity.

Introduction

The "mist propagation" technique, has a high propagation coefficient, and produces a powerful and resistant seedling to diseases, but its efficiency is influenced by different factors such as endogenous hormonal stimulants, *Fiorino 1980.* exogenous hormonal stimulants (*Leva et all.1999)* hormonal acid concentrations (*Bartolini,et al.* **1988),** the nature of the green cutting and its place in the spring the time applied for propagation, etc, *Caballero 1983.*

A lot of researches for the propagation of the olive varieties have proved different rooting capacities from one variety to the other, *Bartolini et al.1983*. Meanwhile a lot of hormonal acids have been experimented with several hydroalcoholic concentrations and the obtained results have varied according to the genotype used. Due to these reasons, the research is mainly oriented on the study of the effect of the indol-3 butyric acid in extreme concentrations and in the typical vegetation phases: February, May, September and December for 6 main olive cultivars in Albania. in the typical vegetation phases: February, May, September and December

Materials and methods

Experimental scheme: Green cuttings of the six olive genotypes are tested for their rooting capacity with the "mist-propagation" method, in correlation with two concentrations of indol-3 butyrik acid (AIB) and (Control), during four stages of their meristem tic activity. There have been 18 basic treatments: *(1) BT/Cont (2) Him/Cont, (3) Fran/Cont (4) Kan/Cont (5) KB/Cont (6) Mix/Cont (7) BT/IBA2gl¯¹, (8) Him/ IBA2gl¯¹ (9) Fran/ IBA2gl¯¹, (10) Kan/ IBA2gl¯¹ (11) KB/ IBA2gl¯¹ (13) Mix/ IBA2gl¯¹ (14) BT/ IBA5gl¯¹ (15) Him/ IBA5gl¯¹ (16) Fran/IBA5gl¯¹, (17) Kan/IBA5gl¯¹, (18) Mix/ IBA5gl¯¹*

Vegetative material**:** Green cuttings have been taken from 30-year old trees. Rooting period has been every month on the fifth day. (February, May, September, December). The macro explants are 8-10 cm long, having two pairs of leaves at the apical, with all the leaves at the base being cut off. Each treatment has 100 cuttings (4 times x 25 cuttings). The 5 sec treatment with hydro alcoholic solution of indol-butyric acid (ecyberon) in two concentrations of *IBA* $2gl^{-1}$, and *IBA* $5gl^{-1}$, . A Control treatment has been considered and applied as comparative. In every hydro alcoholic concentration, the percentage of alcohol is 24% and H2O 76%.

Temperature and Humidity regimes**:** The cuttings have been planted at nebulization bank. Within the biological green house, with perlite subtract. Temperature has been kept at 20ºC and at 24ºC (±1ºC) in the subtracts. Nebulization has been achieved for 5 sec at every 15 Wh/m², depending on the sun radiation. Illumination at the bank is 4500-5000 lux. Solar integrator module has been set to be automatic, *Rodríguez et al. 2008.*

Indices and statistical analysis*:* The end of the rooting process estimated the following results: (i) Rooting percentage, (ii) number of roots, and (iii) percentage of defoliation. Statistical analysis through Jmp software, for the variance analysis, ($p=0.05$), coefficient of variation, bivariate analysis and the coefficient of regression through the genotypes in correlation with the concentrations of IBA, time, number of roots.

Results and Discussion

The process of rhizogenesis: Rhizogenous processes during 7 weeks in a nebulisation bank, display a lot of modifications on the top and bottom of the green cutting. These were the result of the separation from the parent tree, the application of auxins, base warming and vaporization, to which the cuttings were in the regime. On the top, a considerable part of the cuttings has lost one or several leaves. In the segments of the cuttings base, during the two first weeks the wound in the base was healed, whereas two weeks later there was intensive propagation of cells and the formation of an obvious cellular mass, colored white -into -cream called a “Callus”.

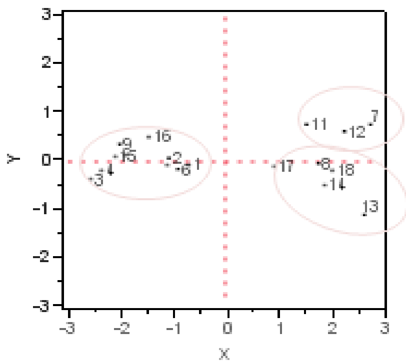
The differentiation of meristem radicles starts immediately after the creation of the callus, depending on the cultivar, the concentration, and the period of time applied for rooting. The cuttings which dropped the leaves, have been associated with callus degeneration and the canker of the substrate tissue. The presence of leaves on top of the green cutting was a really important factor for the success of rooting, especially for the creation of the roots emission, *Caballero 1983.*

***Table 1,** The effect of both IBA concentrations on rooting percentage. **Variance Analysis** Test Tukey-kramer & All Pairs for 18 treatments in (February, may, september, december) on the rooting percentage and number of roots of cv.* Kaninjot standart; Mixan; Himara; Kryps Berati; Bardhi Tiranes; Frantoio. *(Means Comparisons for all pairs using Tukey-Kramer HSD lsd 2.23, alpha 0.05).*

	February		May		September		December	
Treatment	Rooting %	Roots nr	Rooting %	Roots nr	Rooting %	Roots nr	Rooting %	Roots nr
BT/Cont	30.9 qr	2.6 ef	25.9 st	3.5 d	20.9 uv	3.3 c	10.06 xy	3.0 de
Him/Cont	0.0	0.7 h	2.0	1.1 g	0.0	1.0 f	0.0	0.4 h
Fran/Cont	38.8 pq	2.8 def	27.9 rs	3.1 de	20.0 uv	3.0 c	14.0 wx	2.4 efg
Kan/Cont	35.4 pq	2.6 ef	30.9 qr	3.3 d	29.0 rs	3.1 c	17.1 vw	1.9 g
KB/Cont	5.04 z	0.8 h	8.9 xyz	1.2 g	0.0	1.7 def	0.0	0.6 h
Mix/Cont	25.9 st	2.3 fg	29.0 rs	3.9 d	22.9 tu	3.2 c	10.9 xy	2.2 fg
BT/IBA2gl⁻¹	58.8 j	3.6 bcd	72.9 h	5.7 bc	77.1 gh	5.0 b	35.9 p	3.8 abc
Him/ IBA2gl⁻¹	5.0 z	1.6 gh	26.3 st	1.9 fg	0.0	1.4 ef	0.5	0.9 h
Fran/ IBA2gl⁻¹	74.0 h	2.6 ef	80.0 efg	6.1 bc	83.8 de	5.4 ab	47.9 lm	3.0 de
Kan/ IBA2gl⁻¹	83.8 de	3.3 cde	89.0 bc	5.3 c	92.6 ab	4.6 b	50.1 kl	2.8 def
KB/ IBA2gl⁻¹	17.0 vw	1.0 h	22.9 tu	2.3 ef	0.0	2.0 de	0.3	0.9 h
Mix/ IBA2gl⁻¹	78.8 fg	3.0 def	57.9 j	6.5 ab	82.0 def	5.2 ab	42.2 no	4.2 a
BT/ IBA5gl⁻¹	65.9 i	4.6 a	53.3 i	7.2 a	45.9 lm	5.5 ab	40.9 mno	3.9 abc
Him/ IBA5gl⁻¹	9.0 xyz	2.7 def	19.0 uv	3.2 d	0.3	1.6 def	0.0	0.5 h
Fran/IBA5gl⁻¹	82.0 def	4.2 abc	65.0 i	5.5 c	64.0 i	4.6 b	58.1 j	3.3 cd
Kan/IBA5gl⁻¹	95.9 a	2.7 def	52.9 kl	6.1 bc	80.9 fg	4.8 b	77.9 efgh	3.4 bcd
KB/IBA5gl⁻¹	10.2 xy	2.8 def	37.0 op	3.0 de	5.0 z	2.4 cd	9.06 yz	0.8 h
Mix/ IBA5gl⁻¹	85.9 cd	4.4 ab	50.9 klm	6.4 ab	82.9 def	6.0 a	49.0 klm	4.0 ab

Levels not connected by same letter are significantly different. **Rooting %. lsd.1.23 hsd**. **Number of Roots –lsd. 0.97 hsd,** (*P=0.05*). Acronym of the names of cultivars: Kan-s=Kaninjot standart; Mix=Mixan; Him=Himara; KB=Kryps Berati; BT=Bardhi Tiranes; Fran=Frantoio.

The feedback of the olive varieties in function of the IBA dose and period of time. Some cultivars have registered extreme values of the rooting percentages in different concentrations of the hormone. It was proved that not all three treatments affected in the same way the rooting result of the olive cuttings at any tested term. Both concentrations of the Indole -3- Butyric Acid (IBA), have influenced differently. Rooting percentage is considerably obvious, not only for the dose IBA2gl¯¹, but also for IBA5gl¯¹, whereas for other varieties there has been either one dose or the other with regard to only one typical rooting term. The Kan-S cv has had an extremely high rooting percentage in both concentrations: with IBA IBA5gl¯¹ (95.9% in February and 80.9% in September). But also with IBA IBA2gl¯¹ it rooted (83.8% in February, 89.0% in May, and 92.6% in September). A high rooting percentage has also resulted for Fran, Mix, as well as BT cv, Table-1. Whereas the cultivars; KB and Him cv, have displayed low rooting percentage. The above mentioned cultivars although displaying sufficient callus size, after 30-35 days, their callus increases in volume, and becomes like a spongy mass, that it can't reach rooting. Non - differentiation of rooting is a genetic characteristic and occurs because of lack of enzyme activators that synthesize the auxinic complexes, assimilated by the phloem in these cultivars, Caballero 1983.



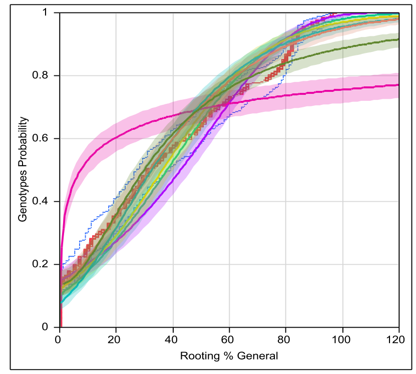
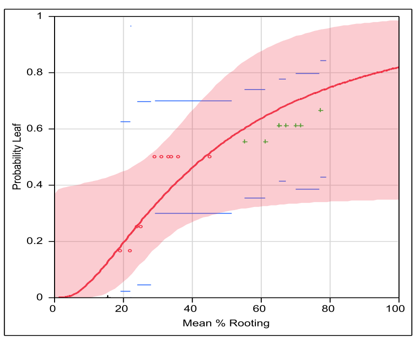


Figure-1., Multivariate Principal Components/Factor Analysis. Analiza e kordinatave kryesore e bazuar ne 18 trajtimet kryesore (genotip+Trajtim). PC1 dhe PC2 zoterojne 99% te variacionit total. Trajtimet jane shperndare ne kater hapesirat perfaqesuese positive dhe negative te PC1 dhe PC2.

Figure-2, Regression orthogonal for distribution of rooting (%) for different propability, about variety, period and the concentration of IBA, analyzed the averages of six genotypes of olive.



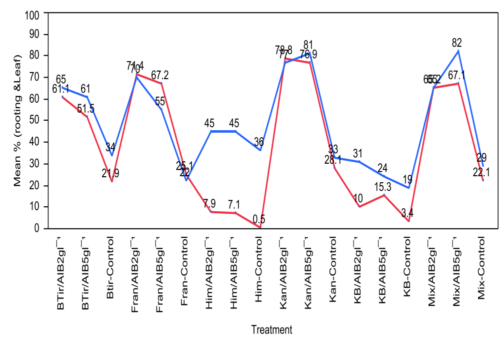


Figure-3 Regression orthogonal for propability of interdependence and distribution of points in realized regression of percentage leaves persistente by rooting (%) (the averages of six genotypes of olive). In these plots, points that are not on the border of credibility, (P=0.05) are not reliable.

Figure 4. Graphic for distribution of rooting (%) and the percentage of leaves resistance, about variety, period and the concentration of IBA, analyzed the averages of six genotypes of olive.

***In Tab 1. The effect of both IBA concentrations on rooting percentage*;** and the relation to the six cultivars seems to have stimulated considerably different concentrations. Through the variance of the values (lsd 2.23 HSD, Alpha=0.05), it resulted as follows: **In February**; Kan/IBA5gl^{-1}, displays the highest rooting percentage of 95.9%, with a dominant position compared to other treatments. Whereas the treatment Kan/IBA2gl^{-1}, 83.8% as well as the treatment Mix/IBA5gl^{-1}

85.9%, have a high rooting percentage without differences. **In May** the treatment IBA2gl^{-1} display a value of 89% and the treatment Fran IBA2gl^{-1}, 80.0% displayed a higher rooting value compared to treatments with IBA5gl^{-1} and the Control. **In September**: there are the treatments with IBA2gl^{-1}, which have a higher rooting percentage as well as a more favorable and dominant position than IBA5gl^{-1}. **In December**: All treatments displayed lower rooting capacities compared to the other terms. The use of the IBA2gl^{-1} in any cultivar displayed dominance compared to the IBA2gl^{-1} concentration.

The interconnections between the time of propagation and the hormone in different concentrations, displayed strong correlative relations and have a dominant position for the period of February and December with concentration IBA5gl^{-1} (r= 0.673) and May/September with concentration IBA2gl^{-1} (r=0.908), i.e.: when there is intensive vegetative flux there is higher rooting percentage, *Bartolini et al 1989.*

In any case the difference between the percentages achieved with the dose 2gl^{-1} and 5gl^{-1} is not significant except for Kan-S, Mix, and Fran cv. But if we focus the analysis towards the differences between the varieties, it can be noticed that the Kani-S cv, has given the highest rooting percentage for the two doses of the IBA applied (92.6% and 95.9%), followed by Mix cv. (85.9% and 82.0%). The Fran and BT cv, have manifested average results with significant changes (*lsd 1.23*) with the other cultivars. Only KB and Him cv, displayed really low percentages (less than 40%), in both concentrations and in the four application periods for rooting. The really high percentage of the callogenesis in Him cv and KB cv has been considerably different and opposite to the low rooting percentage achieved in these two varieties, *Ismaili 2008.*

The cultivars have had a better promontory rooting activity during the period of spring – summer compared to winter, and in correlation with the concentrations it seems that the endogenous equilibriums are different for each cultivar. More efficient are the cultivars IBA2gl^{-1} when there is vegetative growth and active cambium activity; on the contrary when the cambial activity is low high concentrations of the IBA have resulted in better results. There have been several cases (*Vegetative Flux*), when the IBA in high concentrations has resulted limiting or toxic, *Leva et all.1999.*

The effect of leaves resistance. The leaves have had an important role in the life persistence of the cuttings and rooting emission, because they constitute the only source of nutrition during their stay in the nebulisation bank. Generally it has resulted that all the cuttings which have emerged roots have preserved the leaves, whereas those which have dropped the leaves do not have differentiated roots, *Bartolini et al 1989.* After root emission there was a low percentage of cuttings which dropped the leaves and this phenomenon was a pathogens cause, or because of excessive humidity, *Caballero 1983.*

Table 3 and graph. 4, display the importance of leaves resistance for the process of rhizogenesis, which give the necessary energy for the formation of roots. Most of the cuttings preserve the totality of their leaves depending on the applied concentration of the cultivars. For example

Kaninjot cv in both IBA concentrations, has had cuttings with 4 leaves, 87%, and 75% whereas the cultivars with a low rooting percentage have been associated with high defoliation percentage. The Kan-S cv displays the lowest percentage of cuttings mortality, whereas the KB cv and Him cv the highest percentage. In the concentration IBA5gl^{-1} about 50% of the green cuttings have preserved the number of leaves 100%. The most exaggerated foliage has corresponded to the control.

The underlined results in *table-1*, show that the persistence of rooting in 9 weeks is indispensable to achieve the ability of the green cuttings to root. Whereas the green cuttings of the Him cv and KB, despite having preserved the leaves, have undergone modifications of the callus quality, degeneration in a spongy and hyperplastic form which have been followed by low rooting percentage.

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, *Figure-3 and graphic- 4.*

The values presented graphically in *fig-1 and 2*, express the rhizogenic average percentage of the cultivars. It is noticed that they change considerably under the influence of the season when they take the material for propagation. In the diagram it is obvious that the natural percentage values begin in February, a period when vegetation begins, and are weakened later by fifty up to the end of the vegetative cycle in December. *Fiorino et al. 1980.* The results showed that vegetative growth of the olive has influenced on the rooting percentage. The best rooting results are presented with the time as soon as vegetative growth becomes intensive, and coincide with the moments when vegetative growth is inhibited, the values of rhizogenesis are limited, thus undergoing a considerable decrease which coincides to the phase that trees mature their fruit. This phenophase corresponds when the inhibitors, especially the phenols are with the highest concentrations, *Leva et al. 1999.*

The beginning of vegetative growth happens during the 10 last days of February, which corresponds to a daily average growth of 0.08 mm/day, whereas in March 0.7 mm/day. The intensive rhythms of growth have resulted in May and June (1.9 and 2.4 mm/day), in September 0.9-1 mm/days and in December zero. High rooting percentage is noticed during the period of intensive vegetative growth. The rhythms of vegetative growth begin, become intensive and are gradually reduced until they reach point zero during winter hibernation. Although a slight increase is noticed in March 0.7 mm/day rooting results are high, because the level of the natural promoters for rooting begins to become active. The two concentrations of IBA and their relation to the phonological season show the efficiency of concentration IBA2gl^{-1}, when the endogenous capacity is maximal (May-September) and concentration IBA2gl^{-1} is more efficient when the promoters fail, or are in minimal quantity, The correlations in the *Figure-3* have displayed the connection between rooting percentage and defoliation percentage, $r^2=0.93$, meanwhile between the rooting percentage and numbers of roots has strong links $r^2=0.81$, but the relationship between of numbers of leaves and roots is weak $r^2=0.79$. 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.

Figure-3, for the Probability of interdependence of N. leaves By Rooting (%), turns out that the rooting percentage increase goes in parallel with the number of leaves on green cuttings $r^2=0.93$ and number of roots by rooting (%), again in the walking connection and parallel growth $r^2=0.79$.

The intense vegetative growth, the IBA concentration and Genotypes as well as the relation among these is strong (Genotypes/May/IBA2gl^{-1}) and (Genotypes/Shkurt/Shtator/IBA5gl^{-1}). It displays a higher rooting percentage (alpha= 0.05), which statistically is important because tf <tk. (t>2). The treatments simultaneously (14, 19, 20) have values of tf>tk, (2.19, 2.17, 2.43), thus in this way it is proved that the apical parts stimulated with IBA, 2gl^{-1} and 5gl^{-1} in May have a positive influence on the rooting percentage. Whereas all the treatments in December gave unimportant results because the value tf<tk,(1.66<2), and in this case the hypothesis of this phase at any part and at any concentration is not proved statistically.

Based on three-years averages, the distribution of values were rooting percentage of probability between 0.05 and 0.01, making no statistically significant average below 32%. Threshold lognormal=*0.69*. **Figura-1.,** Multivariate Principal Components/Factor Analysis. Principal coordinates analysis is based on 18 main treatments (genotype + treatment). PC1 and PC2 possess 99% of the total variation. Treatments were distributed in four representative positive and negative space of PC1 and PC2.

Conclusion

The process of rhizogenesis of the green cuttings of the olive is a complicated phenomenon which aims at the formation of a new plant capable of growing independently.

The experiments carried out so far, prove the existence of a correlation among the cultivars, the concentrations and period of time applied for rooting. The olive “with nebulisation” displayed an obvious sensibility considering ripeness of the cuttings in all cultivars.

In February and May there was a higher rooting percentage more than 50%, thus making it convincing that the nebulisation method is efficient. The cultivar Kryps Berati, had an average rooting percentage in May, whereas in the other terms it displayed a low rooting percentage. The cultivar Himara displayed low rooting percentage in all the four experimented terms.

The dose of IBA; 2gl^{-1} and 5gl^{-1}, stimulated high rooting percentages, but also in correlation with the period of treatment.

The doses of IBA ($2gl^{-1}$ and $5gl^{-1}$) were efficient in correlation with the period when they were used, thus giving a maximal increase of the rooting percentage and leaves resistance up to the end of the rooting process.

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