# OLIVE MULTIPLICATION IN DIFFERENT PHASES OF MERISTEMATIC DEVELOPMENT

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The object of this research is propagation through green macro explants derived from the apical segment of the sprig in the variety "Kaninjot, Mixan, Bllanic and Himara. The parts were treated with Indole-3 Butyric Acid, 1g$l^{-1}$, 3g$l^{-1}$, 5g$l^{-1}$ and Control in four phases of the meristematic development; February (½ asleep), May (active), September (active) and December (asleep).
Thermal regimes 24ºC/18ºC (±1 ºC), the mist technique was applied according to Photo synthetically Active Radiation 5 sec/11-13 k.kal/cm².
After 70 days, the percentage of rooting of studied cultivars varies from 23 to 90%. The two IBA dosages have not affected rooting in the same way within each period. IBA at 3 g $l^{-1}$ and 5g $l^{-1}$ gave high rooting percentages for Kaninjot and Mixan cultivars. At high cambial activity, the IBA concentration of 3g $l^{-1}$ 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 concentration of 1g$l^{-1}$, had weak reactions and was inefficient. 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. Defoliation has varied between 8.3 and 14.1%. The presence of the leaves has been a stimulating factor for rooting ($r^2$=0.93). The number of roots has increased in parallel with the increase of concentration, ($r^2$=072).

**Keywords.** Olive, cultivars., mist-propagation, explants, hidrooalcoolic, culture.

## 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 *(Hartmann, H.T. 1952)* . exogenous hormonal stimulants (*Leva et all.1999*). hormonal acid concentrations (*Bartolini et all. 1989)* the nature of the green cutting and its place in the spring the time applied for propagation, etc. *Caballero JM. 1983*. A lot of researches for the propagation of the olive varieties have proved different rooting capacities from one variety to the other, *Fabri A. 1980*. 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 4 main olive cultivars in Albania. in the typical vegetation phases: February, May, September and December

## Materials and methods

***Vegetative material*:** Green cuttings of the four olive cultivars (*Kaninjot, Bllanic, Himara and Mixan*), are tested for their rooting capacity with the "mist-propagation" method, in correlation

with three concentrations of indol-3 butyrik acid (AIB) and (Control), during four stages of their meristem tic activity.
Rooting period has been every month on the fifth day. (February, May, September, December). The green cuttings 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 1 $g\ l^{-1}$, 3 $g\ l^{-1}$ and 5 $g\ l^{-1}$. A Control treatment has been considered and applied as comparative. In every hydro alcoholic concentration, the percentage of alcohol is 24% and $H_2O$ 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 6000 lux. Solar integrator module has been set to be automatic, G*onda L et all 2006*.
**Statistical analysis:** It has been carried out by means of software modeled for 100 green cuttings for Rooting percentage (rooted cuttings in %), defoliation percentage estimated for each cultivar in %). The Variance Analysis for Tukey-Kramer test, (P= 0.05) has recognized and certified the differences of each treatment. (*Jmp.Sas/Stat***.***2008)*

## 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. An important cause of the fallen leaves were the infections of *Cycloconium oleaginum* and the physiological state. 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, *Hartmann, H.T 2002* & *Ciampi, C. et all.1958* & *Bouillenne, R et all 1933*.
***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 3$g\ l^{-1}$, but also for 5$g\ l^{-1}$, whereas for other varieties there has been either one dose or the other with regard to only one typical rooting term. The Kaninjot cv has had an extremely high rooting percentage in both concentrations: with IBA 5$g\ l^{-1}$ (*78.6% in February and 72% in September*). But also with IBA 3$g\ l^{-1}$ it rooted (71-0% in February, 89.0% in May, and 57.0% in September). A high rooting percentage has also resulted for cv. Mixan. with IBA 5$g\ l^{-1}$ *55.3% in February in may and 56% in September while with IBA* 3$g\ l^{-1}$*57% in may* ) . Whereas the cultivars; *Bllanic and Himara*, have displayed low rooting percentage (*Table-1*). 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 JM.1983.*

*Tabela-1. The effect of both IBA concentrations and the period on rooting percentage, defoliation percentage and number of roots.*

| PERIOD | FEBRUARY | | | MAY | | | SEPTEMBER | | | DECEMBER | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **TREATMENT** | Rooting % | **N° leaves** | **N° Roots** | Rooting % | **N° leaves** | **N°. Roots** | Rooting % | **N° Leaves** | **N° Roots** | Rooting % | **N° Leaves** | **N° Roots** |
| Kaninjot/ Control | 35.0 klmn | 2.1 fghi | 3.2 ef ghij | 31.0 mno | 1.86 ijk | 4.3 bc defgh | 29.0 no | 1.86 ijk | 3.3 ef ghij | 19.0 p | 2.0 hij | 2.33 g hijkl |
| Bllanic/ Control | 0.0 w | 0.33 nopq | 0.0 l | 4.0 stuvw | 0.96 lmnop | 2.33 g hijkl | 0.0 w | 0.13 q | 0.0 l | 0.0 w | 0.46 mnopqr | 0.0 l |
| Himara/ Control | 0.0 w | 1.16 klm | 0.0 l | 2.0 uvw | 1.06 lmn | 1.33 ijkl | 0.0 w | 0.26 opq | 0.0 l | 0.0 w | 0.23 pq | 0.0 l |
| Mixan /Control | 27.0 o | 2.4 bcfghi | 4.0 bc defg | 29.0 no | 2.23 efghi | 4.6 bc defg | 11.0 qrs | 1.2 klm | 2.6 fg hijk | 11.0 qrs | 1.03 lmno | 2.66 f ghijk |
| Kaninjot/ IBA 1$gl^{-1}$ | 47.0 ghi | 2.0 hij | 5.33 abcde | 49.0 fgh | 2.53 bcfghi | 4.66 b cdefg | 49.6 fgh | 2.53 bcfghi | 4.3 b cdefgh | 38.3 jkl | 2.0 hij | 3.0 ef ghij |
| Bllanic/ IBA 1$gl^{-1}$ | 7.3 rstuv | 0.66 mnopq | 2.33 g hijkl | 9.6 rst | 1.2 klm | 2.66 f ghijk | 1.6 vw | 0.3 nopq | 1.0 jkl | 0.0 w | 0.26 opq | 0.0 l |
| Himara/ IBA1$gl^{-1}$ | 4.3 stuvw | 0.46 mnopqr | 3.0 ef ghij | 11.0 qrs | 1.06 lmn | 2.66 f ghijk | 0.0 w | 0.2 pq | 0.33 kl | 0.0 w | 0.36 Nopqr | 0.0 l |
| Mixan/ IBA 1 $gl^{-1}$ | 39.0 ghij | 2.1 ghij | 6.3 abc | 51.0 efg | 2.4 cdfghi | 4.3 bc defgh | 41.0 hij | 2.46 bcfghi | 3.6 de fghi | 32.0 lmno | 2.2 ghijl | 3.3 ef ghij |
| Kaninjot/ IBA 3$gl^{-1}$ | 71.0 bc | 3.03 abcd | 6.0 abcd | 89.0 a | 3.43 a | 6.66 ab | 57.0 de | 2.66 bcdefg | 4.0 cd efgh | 41.0 ijk | 1.33 jkl | 3.6 de fghi |
| Bllanic /IBA 3$gl^{-1}$ | 6.0 rstuvw | 0.33 nopq | 3.3 ef ghij | 13.0 pqr | 0.76 mnopq | 3.3 ef ghij | 2.0 uvw | 0.56 mnopq | 2.33 g hijkl | 0.0 w | 0.46 mnopqr | 0.0 l |
| Himara/ IBA3$gl^{-1}$ | 4.0 stuvw | 0.3 nopq | 2.33 g hijkl | 13.0 pqr | 0.66 mnopq | 3.0 ef ghij | 5.0 stuvw | 0.73 mnopq | 1.33 ijkl | 0.0 w | 0.13 q | 0.0 l |
| Mixan/ IBA 3 $gl^{-1}$ | 50.0 efgh | 2.3 defghi | 2.66 f ghijk | 57.0 de | 2.9 abcdef | 7.3 a | 43.3 efgh | 2.83 abcdefg | 5.3 abcde | 38.0 jklm | 2.73 abcdefg | 3.6 de fghi |
| Kaninjot/ IBA 5$gl^{-1}$ | 78.6 b | 3.2 ab | 6.33 abc | 68.0 cb | 3.03 abcd | 6.33 abc | 72.0 bc | 3.16 abc | 4.3 bc defgh | 46.0ghi | 2.8 abcdefg | 3.6de fghi |
| Bllanic/ IBA 5$gl^{-1}$ | 7.0 rstuvw | 0.93 lmnop | 2.33 g hijkl | 7.0 rstuvw | 0.86 mnopq | 2.33 g hijkl | 3.0 tuvw | 0.73 mnopq | 2.33 g hijkl | 0.0 w | 0.2 pq | 0.0 l |
| Himara/ IBA5$gl^{-1}$ | 9.0 rstu | 0.6 mnopq | 3.3 ef ghij | 17.0 pq | 0.96 lmnop | 4.3 bc defgh | 1.0 vw | 0.73 mnopq | 2.0 hijkl | 0.0 w | 0.26 opq | 0.0 l |
| Mixan/ IBA 5 $gl^{-1}$ | 55.3 ef | 3.0 abcde | 5.33 abcde | 52.0 efg | 2.56 bcfghi | 6.0 abcd | 56.0 def | 2.9 abcdef | 5.0 ab cdef | 35.0 klmn | 2.03 hij | 3.3 ef ghij |

Levels not connected by same letter are significantly different. **Rooting %. lsd.1.12hsd. N° of Roots –lsd. 1.03hsd and N° of Leaves – lsd.1.31 hsd. (*P=0.05*).**

**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 1.12 HSD, Alpha=0.05*), it resulted as follows: **In February**; *Kaninjot IBA 5*$g\ l^{-1}$, displays the highest rooting percentage of 78.6%, with a dominant position compared to other treatments. Whereas the treatment *Kaninjot IBA 3* $g\ l^{-1}$ 71% as well as the treatment. **In May** the treatment, Kaninjot IBA3 $g\ l^{-1}$ display a value of 89% displayed a higher rooting value compared to treatments with IBA 5 $g\ l^{-1}$ and the Control. Two varieties: Bllanic dhe Himara have manifested lower rooting ability compared with Kaninjot and Mixan variety. **In September**:

there are the treatments with IBA 3 $g\ l^{-1}$, which have a higher rooting percentage as well as a more favorable and dominant position than IBA 5 $g\ l^{-1}$. **In December**: All treatments displayed lower rooting capacities compared to the other terms. The use of the AIB 5 $g\ l^{-1}$ in any cultivar displayed dominance compared to the 3 $g\ l^{-1}$ and 1 $g\ l^{-1}$ concentration.

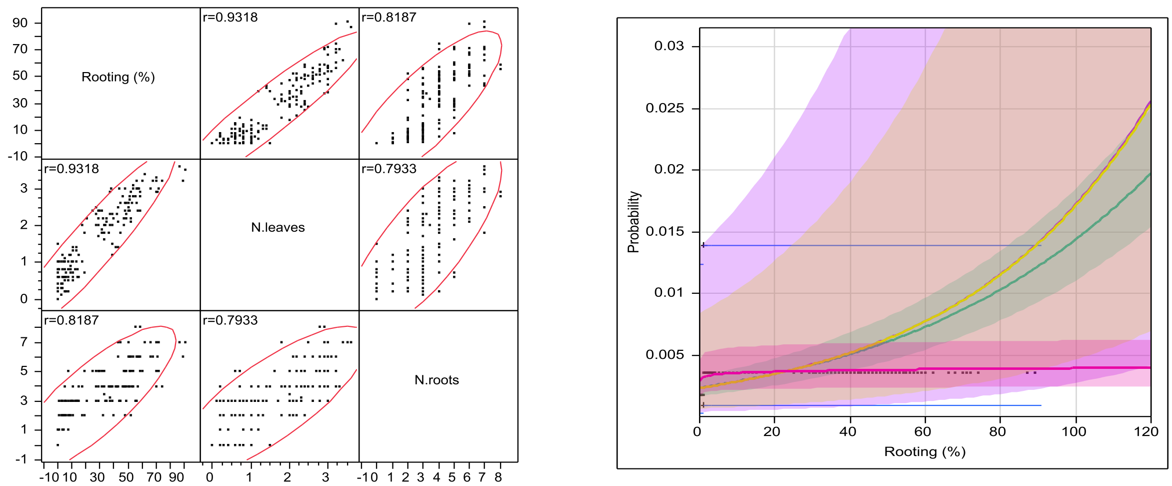
The interconnections between the time of propagation and the hormone in different concentrations, displayed strong correlative relations and have a dominant position for the period of February and December with concentration 5$g\ l^{-1}$ (*r= 0.712*) and May/September with concentration 3 $g\ l^{-1}$ (r=0.908), i.e.: when there is intensive vegetative flux there is higher rooting percentage.

In any case the difference between the percentages achieved with the dose 3 $g\ l^{-1}$ and 5 $g\ l^{-1}$ is not significant except for Kaninjot cv and Mixan cv. But if we focus the analysis towards the differences between the varieties, it can be noticed that the Kaninjot cv has given the highest rooting percentage for the three doses of the IBA applied (*89%*), followed by Mixan cv. (*55.3*). The treatment 1 $g\ l^{-1}$ have manifested average results with significant changes, with the other treatments. Only Bllanic cv and Himara cv, displayed really low percentages (less than 20%), in both concentrations and in the four application periods for rooting. The really high percentage of the callogenesis in *Himara cv and Blanic cv*, has been considerably different and opposite to the low rooting percentage achieved in these two varieties, (*Ismaili H. et all. 2011).*

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 3 $g\ l^{-1}$ and 1 $g\ l^{-1}$ 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. *Fabbri, A. 1980.*

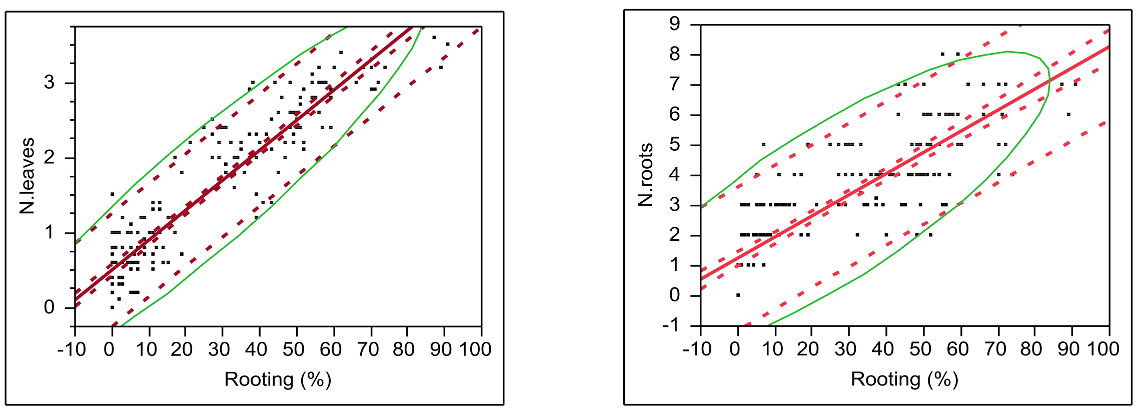
***The effect of leaves resistance.*** The leaves have had an important role in the life persistence of the cuttings and rooting emission, because they constitute the only source of nutrition during their stay in the nebulisation bank. Generally it has resulted that all the cuttings which have emerged roots have preserved the leaves, whereas those which have dropped the leaves do not have differentiated roots, *Bartolini et all. 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, *Fernandes S. et all. 2004***.**

*Table 1 and figura-3a,* 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 Kaninjot cv displays the lowest percentage of cuttings mortality, whereas the Bllanic cv and Himara cv, the highest percentage. In the concentration 5 $g\ l^{-1}$ about 50% of the green cuttings have preserved the number of leaves 100%. The most exaggerated foliage has corresponded to the control.




**(a)** **(b)**

**Figura-2, (a)** Multivariate Pairwise Correlations estimated by REML method for rooting (%), N.leaves, n.roots and treatments. (b) Regression orthogonal for distribution of rooting (%) for different propability, about variety, period and the concentration of IBA, analyzed the averages of four genotypes of olive.




**(a)** **(b)**

**Figura-3 (a)** Regression orthogonal for propability of interdependence and distribution of points in realized regression of number.leaves by rooting (%) and **(b)** number roots by rooting (%), (the averages of four genotypes of olive). In these plots, points that are not on the border of credibility, (P=0.005) are not reliable.

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 Himara cv and Bllanic, 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, *Fabbri, A. et all. 2004*.

The correlations 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. Based on three-years averages, the distribution of values were rooting percentage of probability

between 0.005 and 0.001, making no statistically significant average below 32%. *lambda=0.1 table-1 and Figure-2ab.*
*Ne figuren-3 (a.b)* Probability of interdependence Bivariate Fit of N. leaves By Rooting (%), turns out that the rooting percentage increase goes in parallel with the number of leaves on green cuttings ($r^2$=0.0.93) and number of roots by rooting (%), again in the walking connection and parallel growth ($r^2$=0.79).
The values presented graphically in fig.7, 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.

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. All. 1999.*
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 three concentrations of IBA and their relation to the phonological season show the efficiency of concentration 3 $g\ l^{-1}$, when the endogenous capacity is maximal (May-September) and concentration 5 $g\ l^{-1}$ is more efficient when the promoters fail, or are in minimal quantity.

## 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 Bllanic, 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; 3 $gl^{-1}$ and 5 $g\ l^{-1}$ stimulated high rooting percentages, but also in correlation with the period of treatment.

The doses of IBA 3 $g\ l^{-1}$ and 5 $g\ l^{-1}$, 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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