OLIVE CULTIVAR PROPAGATION BASED ON NEBULIZATION METHOD

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Abstract

The influence of IBA, NAA, and IAA acids on rooting percentage of olive green cuttings was studied by applying two concentrations in four terms, during three years (2007-2009) in Tirana olive research station. The cultivar chosen for this study was "I Bardhi Tiranes" (*Olea europaea L*). Seven treatments were tested: (i)Control (hydroalcoholic solution), (II) IAA 2000 ppm hydroalc. sol, (III) NAA 2000 ppm hydroalc. sol, (IV) IBA 2000 ppm hydroalc. sol, (V) IAA 5000 ppm hydroalc. sol, (VI) NAA 5000 ppm hydroalc. sol, (VII) IBA 5000 ppm hydroalc.sol. The breeding was carried out in four terms: (i) 5 March, (ii) 5 May, (iii) 5 September, (iv) 5 December. The highest rooting capacity resulted under the treatment of (iv) IBA 2000 ppm, 81.6% in May, and under the treatment (vii) IBA 5000 ppm 68.8% in March. The three hormones have displayed high rooting capacity under the treatment 5000 ppm, in March and December, while in May and September the treatments with 2000ppm. All the treatments with IBA, NAA and IAA, were distinguished both for the increase of rooting capacity and average number of roots. The treatments with Naphthyl acetic acid, (iii) and (vi), stimulated a higher root percentage compared to control and IAA. The effect of treatment (iii) NAA 2000 ppm was 55% in May, which is superior to the other three terms at 5000 ppm concentration. (ii)IAA 2000 ppm influence on the rooting was better than IAA 5000 ppm treatment (v) and the control, but worse compared to the effect of other acids. The first rooting period begins with spring awaking (bud's differentiation) and culminates in May, which coincides to intensive vegetative growth and cambium activity.

Keywords. Olive, *Olea europaea* L., Propagation, Nebulization, Hydroalcoholic, *In vivo* culture.

1. Introduction

"I Bardhi Tiranes" (BT) variety is the most widespread olive cultivar in Albania`s central regions. This cultivar is important to local farmers both economically and for its influence in the environment. Traditionally, this olive cultivar has been bred and propagated mainly by grafting, whereas green plant pieces method has been practiced during the five last years only [5]. Olive's breeding with in vivo and in vitro techniques has shortened the time needed for massive breeding of the selected material, by improving both the breeding coefficient and self - rooted plant health as they become more resistant to diseases [3]. The efficiency of nebulization breeding method is influenced by different factors, such as endogenous hormonal activity [4], the use of the exogenous hormonal stimuli [8] hormonal acid concentrations [2], the nature of the green piece and its positioning on the one-year sprig [4], the influence of breeding period [1].

Research for olive breeding has proved different rhizogen capacities from one variety to the other [3]. A lot of hormonal acids have been experimented with a lot of hydroalcoholic concentrations or talc and the results have changed according to the genotype used [4]. The study for "i Bardhi Tiranes" variety is important because it makes for almost 80% of the olive cultures in central Albania. The initial material used was a green micro particle, to study the effect the three hormones*: indole-3 butyric acid* (IBA), *Naphthyl acetic acid* (NAA), *indole acetic acid* (IAA), have in two extreme concentrations [2], and four periods of the cambium activity. The factor IBA 3000 ppm was experimented in Mach [7]. This research has not yet come to final conclusions on the effects that these hormonal acids have on different concentrations, in four different periods of the year.

2. Materials and Methods

The research was carried out at the Olive Station (Peze e vogel) during a three year period, 2007, 2008, and 2009. An apical meristem, supplied with two leaf pairs, was taken from the same centennial tree for three years. The three hormones were applied in two concentrations (2000 ppm dhe 5000 ppm) in four different periods (i) 5 March, (ii) 5 May, (iii) 5 September, (iv) 5 December. The same scheme was applied during all terms: (i) Control: hydroalcoholic solution, (II) IAA 2000 ppm hydroalc sol, (III) NAA 2000 ppm hydroalc. sol, (IV) IBA 2000 ppm hydroalc sol, (V) IAA 5000 ppm hydroalc. sol, (VI) NAA 5000 ppm hydroalc sol, (VII) IBA 5000 ppm hydroalc sol. The dip solution of each treatment was prepared in a pyrex container, in reduced light conditions, at a temperature 22-23^{0}C. In the hydoalcoholic concentrations: alcohol makes for 22% and H2O 78%. The pieces were sunk into the dip solution (basal part 1 cm), for 5 seconds. 200 cuttings were used for each treatment, (4 repetitions x 50 cuttings). A nebulization bank with dimensions 6.0m x 1.4m, perlite substrate, installed within a biological greenhouse (Figure 1). For 70 days the ambient temperature was kept at 20^{0}C during the day and 16^{0}C at night, whereas substrate temperature at 24^{0}C (± 1^{0}C). Temperature control was realized through a 5000 kkal furnace, two grundfos pumps, thermostats, flusostat and new control equipment. Air humidity was maintained at 85% by supplying water mist for each 15 Wh/m², 5 seconds [5]. This process was carried out under the functioning of light from an electronic leaf that surveys the percentage of humidity in the bank. Luminosity was 4500 lux. [9].

2.1. Evaluation of the indices

At the end of the rooting process we evaluated the following indices: (i) rooting percentage, (ii) number of first radices (grown over the root knot), (iii) the length of first roots (in cm) (Figure 1)

2.2. Statistic analysis

A convenient software (SAS/STAT) was modeled for 200 green cuttings experimented for each treatment. Distinguishing the differences among treatments was realized via the variance analysis for the Tukey-Kramer test, with a least significant difference (LSD) verified at P=0.005 [10].

3. Result and discussions

3. 1 The influence of hormonal acids on rooting percentage

The results displayed different rooting capacities of the olive green pieces, depending on the acid, concentration and period of application. The green apical pieces had better rooting capacity (81.6% and 68.8%) in the treatments containing IBA. The other treatments (NAA and IAA) are significantly different when referring to Tukey-Kramer (LSD= 2.123 HSD; P=0.05) [10]. At a lower level the rooting percentage without the use of hormonal stimulus was 5.8% - 16.2% and significantly different from the treatments with hormonal stimuli. (table. 1)

Figure 1: (from left to right: (i) the green piece supplied with two pairs of leaves., (ii) nebulizing bank., (iii) the bank with the green pieces during rooting., (iv) callus tissue with root meristemes., (v) the completed rooting process.

Tab 1. The effect of concentration of three hormones on rooting percentage. We noticed that hormones stimulated considerably different rooting percentages at the cv. "Bardhi i Tiranes".

Treatment	*March (%)*	*May (%)*	*September (%)*	*December (%)*
Control	13.5 **rs**	16.2 **q**	11.4 **t**	5.8 u
IAA 2000 PPM	31.3 **m**	41.1 **j**	29.6 **e**	12.4 st
NAA 2000 PPM	38.9 **k**	55.4 **f**	43.3 **c**	14.8 qr
IBA 2000 PPM	52.4 **g**	81.6 **a**	58.5 **e**	17.8 p
IA A 5000 PPM	36.6 **l**	38.0 **kl**	29.2 **e**	14.4 r
NAA 5000 PPM	41.3 **j**	43.0 **i**	47.4 **d**	19.3 p
IBA 5000 PPM	68.8 **b**	61.0 **d**	66.0 **c**	26.2 o

Levels not connected by the same letter are significantly different. The values of the percentages that have the same letter, do not have significant changes.

The effect of treatments on rooting percentage was verified with Tukey-Kramer and All Pears, (LSD = 2.23HSD, P=0.05). We have expressed the effects of the acids IBA, NAA, IAA in the rooting percentage of the green pieces "I bardhi Tiranes" cv in two concentrations and four different moments of the yearly cycle. In **May**: (Treatment 1-7) 16.2, 41.1, 55.4, 81.6, 38.0, 43.0 and 61.0; treatment 4, IBA 2000 ppm (81.1%) rooting percentage inversely correlated with the hormonal concentration. In March: the rooting percentage increases proportionally with the increase of concentration. 13.5, 31.3, 38.9, 52.4, 36.6, 41.3, 68.8 (treatments 1-7). In September the percentages were: 11.4, 29.6, 43.3, 58.5, 29.2, 47.4, 66.0 (treatment nr.7-66.0%). Treatments in December**:** 5.8, 12.4, 14.8, 17.8, 14.4, 19.3, 26.2; treatment nr.7 (26.2%)

The weather conditions influenced changes on C/N ratio of *the green cutting*, with a surplus of nitrogen at some moments and reduction in others. The green pieces during vegetative growth were accompanied by an increase in the level of the auxinic endogenous level. Auxins, are the key to cell differentiation, thus their reduction at some moments was reflected in the rooting percentage [1, 8].

***Indole-3 butyric acid* (IBA)** had higher rooting percentage compared to NAA and IAA.The concentration IBA2000 ppm in May resulted in 81.6% of green pieces with differentiated roots, whereas the concentration IBA5000 ppm showed better results in March (68.8%), and in December (26.2%%). The treatments with IBA stimulated higher rooting percentage during all the periods compared to NAA and IAA, thus making its use more economic**.**

Naphthylacetic acid,(NAA) stimulated higher rooting percentage compared with control and IAA with statistically verified differences, Tukey-Kramer (LSD=2.11 HSD). Rooting percentage (55.0%), in concentration 2000 ppm was in May, whereas in the other terms there was 5000 ppm superior concentration.

Indole acetic acid, (IAA) is the natural substance, sensitive to the degrading enzymes in the presence of light. Its influence over rooting was higher compared to the control, but lower compared to IBA and NAA.

As far as callus formation in the basal part of the piece is concerned, the best formations have coincided with the hormones, especially with IBA and NAA. Callus formed by the cells of cortex parenchyma and phloem on both sides of the sclerencimatic ring, seems like a mass of a spongy tissue**.** In its interior, cell groups are formed beside the vascular cambium and the phloem parenchyma, (rooting strands) which grow in the interior and exterior to form the vascular tissues that are in contact with the xylem [2].

3. 2 The influence of the rooting period.

Rooting percentage has been significantly influenced by the rooting season (P≤ 0.01, figure 2).

The higher rooting percentage compared to control was obtained through three hormones (IBA,NAA, IAA). The average treatment results in each term showed that the green pieces on 5th of May rooted 8.5% more than on the 5th of March, 7.7% more than on the 5th of September and 35.9% than on the 5th of December, and it was proved that the most appropriate time is in May.

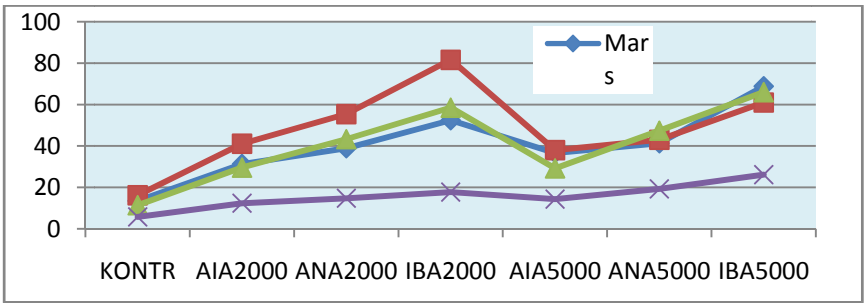

Figure 2: Rooting percentage of the ‘‘I Bardhi i Tiranes’’ variety in four periods of the year and for seven treatments.

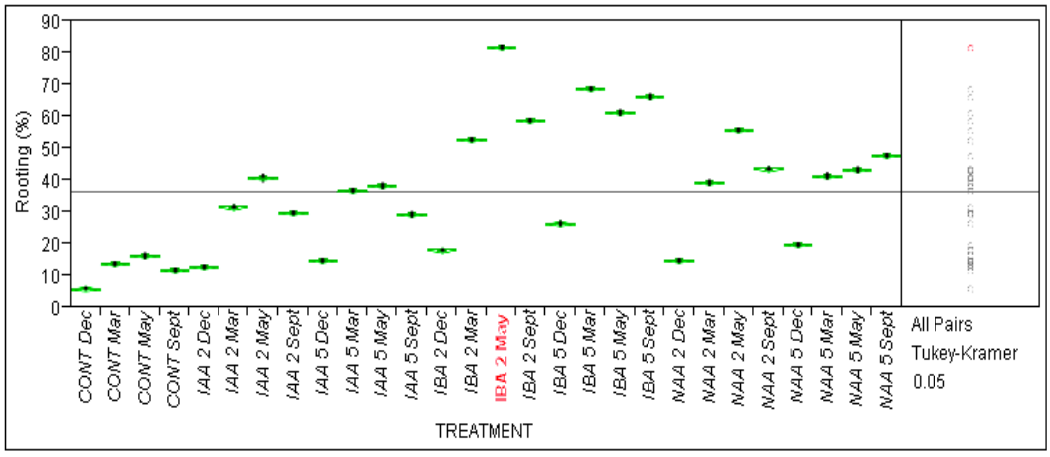

Figure 3. The analysis of variance, Tukey-Kramer & all pairs tests for seven treatments in four terms (March, May, September, December) for the rooting percentage of the “I bardhi Tiranes” variety (Means Comparisons for all pairs were done by using Tukey-Kramer HSD LSD=2.23, p=0.05).

The percentage of rooting presented graphically show that in May treatments nr 1, 2, 3, 4, 5, expressed had higher values than in any other experimented time, except for treatments 6 and 7, which make for treatments with higher concentrations of the hormones, with higher level of toxicity. In December, the treatments had inferior rooting values whereas in March and September they stimulated rooting percentage without significant changes among each other Tukey-kramer, LSD=2.231HSD P=0.05 (Figure 3).Rooting percentage in March, analysed in Tukey-Kramer (LSD=2.23HSD, p=0.05), treatment 7 (68.8%) had considerable changes with treatment 4 (52,4). Treatments; 2,3,5 had values with no significant changes. Treatment 1 (13,5%) had lower value, and with significant changes compared to the others. In May: Treatment 4, IBA 2000 ppm (81.1%) displayed significant changes with other treatments,(p=0.05). Rooting percentage increased with the decrease of the hormonal concentration. In September**:** treatment 7, (IBA5000) had higher percentage, treatments 2 and 5 had no changes, whereas the control had inferior values. In December: treatment nr.7 (26.2%) had the highest value with significant changes compared to the other treatments which displayed inferior values.

These differences were the result of the cambium activity at different development stages, which was more evident in May. Cambium development was stimulated by temperature increase from the minimum active temperature up to 32^0C, while above 35^0C its activity decreased. The variety i Bardhi i Tiranes (BT) undergoes two phases with powerful cambium development, (i) March-May and (ii) September. [7].

Lower concentrations of growth stimulators generally had a stronger influence in May and September, whereas higher concentrations were more effective in February and December.

Cellular rooting ability in December was inhibited by increased amount of oxides and the reduction of the enzymes that counter the inhibitors. Despite the high dosage of the IBA, IAA and NAA absorbed and transported to the phloem lack of enzymatic activators to synthesize the exogenous auxinic complexes caused low rooting percentage at this period of the year [8, 9].

Concentration 2000 ppm resulted more efficient when the trees had a powerful cambial flux, and vice versa, low cambial activity in high concentrations gave better results [4]. It seems that the good rooting period begins with the spring awakening of the tree (bud differentiation) and culminates in May, coinciding with the intensive vegetative growth and increased cambial activity. This phenomenon is noticed in the lack of hormones (Control) which show the following rooting capacities: 13.5% in March, 16.2% in May, 11.4% in September and 5.8% in December. The higher percentage observed in May might be explained with the presence of higher amounts of endogenous auxines in the plant's tissue as well as the really active cambial activity [4].

The initial phase of the sclerencimatic activity observed in March makes the three hormones at concentration 5000 ppm more efficient as they increase the rooting capacity by 8% more than the concentration 2000 ppm, the latter stimulating a better rooting capacity in May (12%). After bud blossoming and sprig growth the following phenomena were observed: sprig growth, inflorescence and fruit formation were accompanied by the carbohydrates and hormones augmentation, which result in better rooting. [8].

Tab. 4, The number of the first roots and their average length expressed in centimeters.

Treatment	*March*		*May*		*September*		*December*	
	N.of roots	Cm	N.of roots	Cm	N.of roots	Cm	N.of roots	Cm
Control	3.6	5.2	3.8	4.9	2.7	5.1	1.4	3.1
*I*AA 2000ppm	4.1	5.5	4.4	5.6	3.3	4.9	2.1	2.3
NAA 2000 ppm	5.4	4.7	5.9	5.1	4.0	5.7	3.0	2.2
IBA 2000 ppm	6.7	4.9	6.8	5.5	6.1	5.6	4.2	3.1
IAA 5000ppm	5.7	4.8	5.4	4.8	4,2	4.7	3.3	3.1
NAA 5000 ppm	6.6	5.5	6.7	5.4	4.9	5.2	3.7	3.4
IBA 5000 ppm	8.0	6.3	8.2	5.4	5.8	4.6	4.4	3.1

Treatments changes have also played a certain role for the number of roots by making possible a rooting system with 4-5 primary root branches. Statistically significant changes are observed between the control and other treatments (IAA and NAA). In table *4,* we noticed that the root dimensions are slightly different but these differences are statistically non significant which means that this index is not a derivative of hormonal treatment [4, 7]. The radicles differentiated in the cortex tissue were more abundant with the application of high dosage of hormones. The variability is a result of hormone permeation in the

phloem, enzymatic activity and the formation of callus tissue, and consequently showing the differentiation of the adventive radicles from the meristem cells**.** [1]. The three-year research concluded that IBA had higher rooting effects while NAA and IBA yielded similar results, but with significant changes between them (LSD=3.234 HSD). It also affected the number of roots by creating rooting systems with 4-5 primary roots. These changes were statistically significant between the control and other treatments (IAA and NAA).

4. Conclusions

Growth hormones influenced the rooting capacity of olive cultivar "I bardhi i Tiranes". This capacity varied in accordance with the hormone concentrations and seasons of the year.. IBA hormone at concentration 2000 ppm was more active when cambium activity was higher (May and September) and less active when cambium activity was lower (February and December). In February and December the hormones at concentration 5000 ppm were more efficient.

By analyzing the impact of two concentrations during all periods of the year we concluded that the three hormones under applied in this study stimulated more the rooting capacity at concentration 5000 ppm (5.3 %) and at concentration 2000 ppm (33.2%) than the Control. Indole Butyric Acid was more efficient in both concentrations: 65.4% in May and 55.3% in March.

The use of synthetic acids showed a possibility for the increase of the economic value of the method, based on the percentage of rooting and its quality (number of roots).

The best rooting period coincides with the spring awakening (bud differentiation) and culminates in May during the intensive vegetative growth.

5. References

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