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Time of treatment with IBA in Olive cultivars rooting

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Abstract-Phytoregulators such as indole butyric acid (IBA), have been used in the process of olive tree rooting in the form of concetrated hydroalcoholic solutions. The objective of this present study was to evaluate the efficiency of a low concentration of IBA (300 mg L⁻¹), diluted in a solution of only 10% of alcohol 70 °GL, in the rooting of four olive tree cultivars (Arbequina, Arbosana, Frantoio and Koroneiki), treated in different times of base immersion (0, 1, 2, 4 and 8 hours). The rooting was carried out in november 2015, in greenhouse with an irrigation system type intermittent mist chamber. The cuttings standard were 12 cm length and two pairs of leaves, planted in plastic tubings, containing carbonized rice husk as substrate. The experimental design was achieved in randomized blocks, in factorial arrangement of 4 x 5, with four replications and 12 cuttings per plot. After 70 days of rooting it was verified that the increase of treatment time affected the leaf retention, survival and rooting negativelly. Leaf retention presented positive relationship with survival and rooting. The treatment for one hour in a solution of IBA was effective in the cuttings rooting stimulus of cultivar Arbosana (66,7%). Cultivars Koroneiki and Arbequina presented low or null rooting whether or not the IBA was used.

Index terms: Olea europaea L., vegetative propagation, cuttings, indolbutyric acid.

Tempo de tratamento com AIB no enraizamento de estacas de cultivares de Oliveira

Resumo-Fitorreguladores, como o ácido indolbutírico (AIB), têm sido empregados no processo de estaquia da oliveira geralmente na forma de soluções hidroalcoólicas concentradas. O objetivo do presente estudo foi avaliar a eficiência de uma baixa concentração de AIB (300 mg L⁻¹), diluído em solução com apenas 10% de álcool a 70 ºGL, no enraizamento de estacas de quatro cultivares de oliveira (Arbequina, Arbosana, Frantoio e Koroneiki), tratadas por diferentes tempos de imersão da base (0; 1; 2; 4 e 8 horas). A estaquia foi realizada em novembro de 2015, em estufa com sistema de irrigação do tipo nebulização intermitente. As estacas foram padronizadas com tamanho de 12 cm de comprimento e dois pares de folhas, plantadas em tubetes plásticos contendo casca de arroz carbonizada como substrato. O delineamento experimental foi em blocos casualizados, no esquema fatorial 4 x 5, com quatro repetições e 12 estacas por parcela. Após 70 dias de estaquia, foi verificado que o aumento do tempo de tratamento afetou negativamente a retenção foliar, a sobrevivência e o enraizamento. A retenção foliar apresentou relação positiva com a sobrevivência e o enraizamento. O tratamento por 1 hora em solução de AIB foi eficiente no estímulo do enraizamento das estacas da cultivar Arbosana (66,7%). As cultivares Koroneiki e Arbequina apresentaram baixo ou nulo enraizamento, independentemente do uso ou não de AIB. Termos para indexação: Olea europaea L., propagação vegetativa, estaquia, ácido indolbutírico.

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Introduction

In Brazil, olive tree growth is still a recent rural activity in expansion (OLIVEIRA et al., 2009), where this country is one of the greatest importers of olive tree products of South America, and, Argentina, one of the greatest suppliers, besides Spain and Portugal (VIEIRA NETO et al., 2008). The consumption and imports increase turn the Brazilian market promising for this activity (MESQUITA et al., 2006). Furthermore, growth traditional areas in the world are limited to the existing crops, with no significant perspective of increase, making olive trees to gain space in South American countries (EMBRAPA, 2005).

In order to reach success in fruit production it is necessary, among other factors, high quality seedlings, with no phytosanitary problems, which present good development when they are combined with adquate handling. The techniques used for spreading olive trees are grafting and rooting. This last one shows lower cost and time to obtain seedling, demanding less qualified labor. But, so that the technique is feasible, according to what Fachinello et al. (2005) emphasize, it demands that the cultivar shows high capacity to form roots, taking into consideration that its root system and later development in the area of production show good quality.

Exogenous application of phytoregulators has been one of the most studied techniques for the improvement of the hormonal balance in rooting, where the indole butyric acid (IBA) is the most used auxin. Several researches developed towards olive trees rooting have used concentrated hydroalcohol solutions of IBA (SEBASTIANI et al., 2002; OLIVEIRA et al., 2003; OLIVEIRA et al., 2009; PEIXES et al., 2011; DENAXA et al., 2012; SILVA et al., 2012), therefore, the results found vary a lot according to the dosage, the cultivar, the period of rooting, the substrate and the type of cutting, among other factors.

The objective of this research was to evaluate the efficiency of an IBA concentration that was considered as low (300 mg L⁻¹), in the rootings of four olive trees cultivars submitted to different times of base immersion.

Material and Methods

The experiment was achieved in the College of Agronomy and Veterinary Medicine (FAMV) of Passo Fundo University (UPF), in Passo Fundo, RS, at 28°15'S and 52°24'W, altitude of 680 m, in the Central Plateau of Rio Grande do Sul. The cuttings were collected from olive trees of the orchard located in the Agronomy Extension and Reasearch Center (Cepagro) of FAMV, at UPF Campus I. The orchard was implanted with 7 x 7 m spacing in September 2012, where the plants were not irrigated. The rooting was achieved in November 2015 and kept in greenhouse with an irrigation system type intermittent mist chamber, controled by *timer*, being wet at every 8 minutes per 10 seconds, is such a manner to keep the cuttings wet. The cuttings were standardized in 12 cm length, with bisel cut in their base, keeping two pairs of whole leaves in their superior tips. Lesions of 1.5 cm in opposite sides (bark scrapping) were made to show the exchange and improve the IBA solution absorption. When preparing the IBA solution, the pure product (bland Sigma) was diluted in ethylic alcohol 70 °GL (10% total volume) together with distilled water.

The cuttings of Arbequina, Arbosana, Frantoio and Koroneiki cultivars were submitted to treatments with no phytoregulators and base immersion for 1, 2, 4 and 8 hours in 300 mg L⁻¹ IBA. The experimental design was in randomized blocks, factorial arrangements of 4 x 5, with four replications and 12 cuttings per plot. The cuttings were planted in plastic tubings of 140 cm³ containing carbonized rice husk.

The evaluation was performed 70 days after rooting, determining the percentage of leaf retention, survival and rooting, number of roots per cutting and the length of the largest roots. The data were submitted to the analysis of variance and the differences between the averages compared by the Scott-Knott test at 5% of error probability, using the Assistat statistical program.

Results and Discussion

Cultivars Arbosana and Koroneiki presented the largest percentages of leaf retention and survival (Table 1), whereas for Arbequina, a significant fall of leaves and death was observed, where only 5.4% of live cuttings remained. The increase of the treatment time with IBA badly affected leaf retention as well as survival. There was no significant difference between the witness cuttings (with no IBA) and submitted to treatment for one hour, thus when they were kept for 2, 4 or 8 hours in contact with 300 mg L^{-1} solution of IBA, there was increase of the leaf fall and death, where times were not different between themselves.

Positive relationship between the leaf retention capacity and cuttings must be emphasized, thus the leaves help the supply of photoasimilates and energy for the maintenance of the methabolism, besides being carbonized auxins and co-factors that are important so that rooting to occurs. Donatti (2008) emphasizes that the olive tree cuttings are sensible to humidity loss, leading to a high level of stress that appears some weeks after the material preparation, showing symptoms like the leaves loss and necrosis in the cuttings base. Oliveira et al. (2009) verified that, after 65 days of the cv. Ascolano 315 rooting, from the four leaves kept on the cuttings, 1 leaf in average remained, determining around 60% death, which could reach 100% if there was no effect by the wet chamber.

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Cultivars	Leaf retention (%)	Survival (%)
Koroneiki	49.8 a	60.4 a
Arbosana	50.3 a	62.1 a
Frantoio	37.3 b	50.8 b
Arbequina	6.0 c	5.4 c
Times of treatment with IBA		
Witness	47.8 a	57.3 a
1 h	43.4 a	52.1 a
2 h	32.2 b	39.6 b
4 h	31.1 b	39.6 b
8 h	24.9 b	34.9 b
CV (%)	34.96	27.84

Table 1 – Leaf retention and cutting survival of four olive tree cultivars submitted to different times of base
immersion in 300 mg L ⁻¹ of IBA. Passo Fundo. RS. 2016

Averages followed by the same small letter in the column do not differ by the Scott-Knott test at 5% error probability.

The treatment with a low concentration solution of IBA showed to be efficient towards the stimulus to rooting only for cv. Arbosana (66.7%) cuttings, when they were treated for 1 h (Table 2). In longer treatments there was reduction of rooting, which kept between 14.6% and 31.2%, not differentiating from the witness (22.9%). In the treatment in which the IBA was not used, cv. Frantoio did not differentiate from Arbosana, with the same 22.9% rooting. Treated for 1 h (33.3%) it did not differentiate from the witness, but it was inferior to Arbosana, and in longer treatments there was damage to rooting (from 4.2% to 16.7%). Cultivars Koroneiki and Arbequina presented low or null rooting, whether IBA was used or not, varying from 0.0% to 14.6%, where cv. Arbequina was an indicator of the low rate of survival.

The low rooting of the cv. Arbequina cultivars was also observed by Oliveira et al. (2003), who obtained 27.5% of the rooted cuttings, and Ribeiro (2010), who obtained rates inferior to 8.6%. In turn, Denaxa et al. (2012) observed that the percentage of Arbequina rooting was higher in the summer (76%), followed by the October rooting (40%) and spring (37%). This season variation was associated to changes in carbohydrates concentrations in the cuttings base, enhancing the importance of the nutritional state of the matrix-plant. By considering that, in this present study, rooting was achieved in the latest November (spring), it is possible to state that the low rooting may be related to the physiological condition of the matrix-plant and cutting. Cv. Frantoio did not show rooting indexes superior to 33.3%, although Sebastiani et al. (2002) consider ease of rooting as for this cultivar when it is superior to 50%. Possible influences of the juvenile degree of these matrix plants, which were three years old, can also be considered. Some species can show strict reltionship between the greatest juvenile degree and the increase of the rooting rate.

Table 2 –Rooting of fourolive tree cultivars cuttings submitted to different times of base immersion in 300 mg L^{-1} of AIB. Passo Fundo, RS, 2016

		Ro	ooting (%)		
Cultivars		Time of in	mmersion in I	BA	
	Witness	1 h	2 h	4 h	8 h
Arbosana	22.9 Ba	66.7 Aa	31.2 Ba	31.2 Ba	14.6 Ba
Frantoio	22.9 Aa	33.3 Ab	4.2 Bb	16.7 Bb	10.4 Ba
Koroneiki	2.1 Ab	4.2 Ac	14.6 Ab	14.6 Ab	4.2 Aa
Arbequina	12.5 Ab	2.1 Ac	0.0 Ab	4.2 Ab	0.0 Aa
$\overline{\mathrm{CV}(\%)}$	67 49				

Averages followed by the same small letter in the column do not differ by the Scott-Knott test at 5% error probability.

The reduction of the leaf retention and survival of the cuttings by the treatment with IBA (Table 1), and rooting in cvs. Arbosana and Frantoio (Table 2), with longer treatments (from 2 to 8 h), show the possibility that the phytoregulator provokes an effect of their phytotoxity or stress. One of the indicators of stress is the enzyme activity of peroxidase. Pisa and Lima (2003) mention that the enzyme activity can be decisive in the plants adaptation feasibility, where there was an increase because of chemical, physical or biological stress. The possible negative influence of IBA was mentioned by Hausman (1993), studying the poplar rooting in vitro, when verfying significant increases in the peroxidase activity in the presence of the exogenous auxin. Zanol et al. (1997), when studying the use of IBA in rooting in vitro of the apple tree rootstock 'Marubakaido', verified that the absence of IBA showed low increase in the enzyme activity, but when it was present, the values were higher.

Alterations in the activity of regulatory enzymes of different biochemical processes, as well as cellular levels of varied metabolites during rooting, have shown the involvement of peroxidase, AIA-oxidase and phenolic compounds in the process of root formation in cuttings (MAYER, 1986; CABONI et al., 1997; SEBASTIANI et al., 2002; SEBASTIANI E TOGNETTI, 2004; PEIXES et al., 2011). Peixes et al. (2011) emphasize that, although auxins are widely used in the induction of rooting, little is known about their specific action or interaction with other endogenous compounds. Testing dosages of IBA, they identified larger concentrations of AIA free in the tissues of cv. Cobrançosa, with ease of rooting (70%). After the treatment for 20 seconds in a solution of 3.500 mg L⁻¹ of IBA, the levels of AIA reduced after 2 hours for cv. Galega Vulgar, which show difficulty of rooting (10%), and only from 6 hours for cultivar Cobrançosa, which show ease of rooting. The peroxidase activity did no show significant differences between cultivars, but there was significant differences with relation to the poliphenoloxidases activities and AIA-oxidase.

Fachinello et al. (2005) state that the increase of endogenous auxin concentrations used in the cutting provokes stimulating effect of roots to a maximum value, from which any increase can lead to an inhibitory effect. The appropriate content of exogenous auxin depends on the species or cultivar and on the auxin concentration in the tissue. Therefore, the treatment with a low concentration solution in periods that are longer than the ones used in the rooting protocols of semi-hardwood cuttings, tested in this present study, may cause changes in concentrations of the endogenous AIA and IBA, and in the interaction of thsese substances with other compounds used in the rooting process, acting negatively in the rhizogenesis process of some cultivars and in determined times of treatment.

For the greatest exchange exposure, stimulus to dvision cell and to make the absorption of substances that improve rooting (FACHINELLO et al., 2005) easier, the cuttings were submitted to lesion in opposite sides of the basal segment. Some cuttings presented necrosis in their base that could have probably been caused by the period they remained immersed in solutions of IBA (from 1 to 8 h). Oliveira et al. (2009) say that the substances used to dilute the growth regulator, when in low concentration, do not allow total dilution of IBA, but when in high concentration and depending on the species, may cause necrosis in of the base tissue, damaging rooting. Ethyl alcohol 70 °GL for the dilution of IBA solution was used in this research, therefore, the proportion of alcohol:water (v:v) of the final solution was reduced from 50%, as it is normally achieved in concentrated solutions, for 10% of alcohol. Despite trying to reduce the solution content of alcohol, the possibility of occuring damages to the process of healing the lesion can not be discarded.

The number of roots formed when the IBA was used was low (averages from 0.8 to 6.8 roots per cutting) and there was no difference between the cultivars (Table 3). Cultivar Arbosana, in all immersion treatments in IBA, presented cuttings with greater number of roots, between 21.2 and 25.7, where there was no difference from Frantoio when it was treated for 1 h (18.1 roots) and for 4 h (16.5 roots). The cuttings of cvs. Koroneiki and Arbequina, for all treatments with IBA, presented low formation of roots (from 0.0 to 5.9 roots per cutting), where there was no difference from Frantoio in treatments of 2 h and 8 h (9.3 roots). Silva et al. (2012), when evaluating the cuttings rooting of 35 olive tree cultivars, obtained as best results 9.6 to 1.0 roots per cutting, since for Arbequina there was an average formation of 2.4 to 6.0 roots, according to the period.

The stimulus of IBA in the formation of greater number of roots in cultivares Arbosana and Frantoio was also observed by Pio et al. (2005) in cultivar Grapollo, obtaining a larger number of roots with the raise of the concentration of IBA, where 3.000 mg L⁻¹ favored the greatest number of roots per cutting, average of 7.4 roots in cuttings with two pairs of leaves, and 5.5 roots in cuttings with one pair of leaves and without leaves. These results were inferior to the ones found in this study for cultivars Arbosana and Frantoio, when treated with IBA, however, they were superior to cultivares Arbequina and Koroneiki. Oliveira et al. (2009) also verified increase in the number of roots by increasing the dosage of IBA to 3.000 mg L⁻¹, in treatments for 5 seconds.

The average length of the three longest roots per cutting, with no IBA treatment, was low (from 0.6 to 2.4 cm) and it was not different among the cultivars (Table 3). Cultivar Arbosana showed the longest roots, with the treatment of 4 h immersion in IBA (7.7 cm), where the other treatments did not show any difference among themselves. The longest roots in cultivar Frantoio were obtained in treatments of 1 h and 4 h. Koroneiki and Arbequina were the cultivars that showed the shortest roots, not showing any difference between themselves and, also, as for the treatments for 2 h and 8 h of cv. Frantoio. Therefore, only cultivars Arbosana and Frantoio showed greater increase of roots using IBA. This stimulus to the roots growing was also observed by Oliveira et al. (2003) and Pio et al. (2005), with 3.000 mg L⁻¹ IBA.

_	Number of roots per cutting					
Cultivars	Time of immersion in IBA					
	Witness	1 h	2 h	4 h	8 h	
Arbosana	4.0 Ba	24.1 Aa	25.7 Aa	24.6 Aa	21.2 Aa	
Frantoio	6.8 Ba	18.1 Aa	9.3 Bb	16.5 Aa	9.3 Bb	
Koroneiki	0.8 Aa	2.8 Ab	3.0 Ab	5.9 Ab	3.0 Ab	
Arbequina	4.0 Aa	3.5 Ab	0.0 Ab	2.3 Ab	0.0 Ab	
CV (%)	68.09					
		Length of the three largest roots (cm)				
Arbosana	2.4 Ba	5.4 Ba	4.6 Ba	7.7 Aa	3.8 Ba	
Frantoio	2.4 Ba	4.9 Aa	1.2 Bb	3.7 Ab	2.5 Ba	
Koroneiki	0.6 Aa	1.0 Ab	1.2 Ab	2.5 Ab	0.9 Ab	
Arbequina	0.9 Aa	0.6 Ab	0.0 Ab	0.3 Ac	0.0 Ab	
CV (%)	64.67					

Table 3 – Number of roots and average of the three largest roots in the cuttings of four olive tree cultivars submitted to different times of base immersion in 300 mg L^{-1} of IBA. Passo Fundo, RS, 2016

Averages followed by the same capital letter line, and small letter in the column, do not differ by Scott-Knott test at 5% error probability.

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