Performance of substrates in rooting capacity of olive tree cuttings

Desempenho de substratos na capacidade de enraizamento de estacas de oliveiras

Fabíola Villa^{1*}, Daniel Fernandes Silva², Paulo Dall'Oglio¹, Celio Potrich¹ e Fernanda Jaqueline Menegusso¹

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ABSTRACT

Plant cutting is a widely used propagation method for olive trees. Its viability depends on the capacity for the adventitious root formation of each species. Nevertheless, it is necessary to study the different characteristics of this process. The purpose of this study was to evaluate the influence of substrates on the rooting of semi hardwood stem cuttings of ten olive tree cultivars. Middle sized branches were collected from mother plants located in the Germplasm Bank of EPAMIG in Maria da Fé, MG, Brazil, and cuttings were prepared of approximately 12 cm in length, with 4-6 internodes, maintaining four leaves in the apical region. A completely randomized experimental design was used, with tree replications and 20 cuttings per plot. The experiment was conducted in greenhouses, in rooting plant beds containing a perlite and sand substrate with controlled intermittent water mist. After installation of the experiment, treatment was carried out with copper oxychloride solution at 3% to prevent possible fungus attacks. Evaluations were performed 70 days after cutting of the varieties. There was variation in rooting of olive tree cultivar cuttings. Better performance of sand was observed in the production of cuttings of olive tree cultivars Alto D'Ouro, JB1 and Galega which were also the cultivars that stood out among the genotypes studied.

KEYWORDS: *Olea europaea* L., propagation, cutting, cultivars.

RESUMO

A estaquia é um método de propagação muito utilizado para a oliveira. Sua viabilidade depende da capacidade de formação de raízes adventícias de cada espécie. Contudo é necessário estudar as

diferentes características desse processo. Objetivouse, no presente trabalho, avaliar a influência dos substratos no enraizamento de estacas semilenhosas de 10 cultivares de oliveira. Ramos medianos foram coletados de plantas matrizes localizadas no Banco de Germoplasma da EPAMIG, em Maria da Fé, MG, e preparadas as estacas com, aproximadamente, 12 cm de comprimento, com 4 a 6 internódios, mantendo na região apical quatro folhas. O delineamento utilizado foi o inteiramente casualizado, com 3 repetições e 20 estacas por parcela. O experimento foi conduzido em casas de vegetação, em canteiros de enraizamento contendo como substrato perlita e areia e nebulização intermitente controlada. Após a instalação do experimento, foi realizado tratamento com solução de oxicloreto de cobre a 3%, a fim de prevenir eventuais ataques de fungos. As avaliações foram realizadas 70 dias após a estaquia das variedades. Existe variação no enraizamento de estacas de cultivares de oliveira. Melhor desempenho da areia foi verificado na estaquia de cultivares de oliveira. Alto D'Ouro, JB1 e Galega que também foram as cultivares que mais se destacaram entre os genótipos estudados.

PALAVRAS-CHAVE: *Olea europaea* L., propagação, estaquia, cultivares.

INTRODUCTION

Brazil is an important country in the market scenario for table olives and olive oil, being the second and third largest importer of these products respectively (IOC 2012). Despite being a great consumer of these products, Brazil is totally dependent on importing them, which encourages national farmers to invest in the sector, generating a great demand for olive tree seedlings in the national market and consequently studies for the production of

*Autor para correspondência <fvilla2003@libero.it>

¹Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, PR, Brasil.

²Universidade Federal de Lavras, Lavras, MG, Brasil.

seedlings with high standards of quality.

Plant cutting is the most commonly used method in olive tree (*Olea europaea* L., Oleaceae) propagation. Nevertheless, the traditional system of propagation, that is with 60 cm hardwood stem cuttings rooted directly in the planting area, in addition to other drawbacks, presents the need for a great deal of material (VIEIRA NETO et al. 2008). The use of semi hardwood stem cuttings may overcome this drawback, but in this condition they present greater difficulty in rooting, which may be resolved with rooting conditions associated with growth regulators (OLIVEIRA et al. 2010).

Rooting of semi-hardwood stem cuttings varies among cultivars (THAKUR et al. 2016). In olive trees, auxin is a limiting factor in rooting (KURD et al. 2010), with indolebutyric acid (IBA) being the most commonly used. Treatment is normally performed for five seconds at the base of the cutting with a hydroalcoholic solution at the concentration of 2000 to 4000 mg L⁻¹ (PENSO et al. 2016), as OLIVEIRA et al. (2012), which used 3000 mg L⁻¹ for the rooting of promising semi-woody cuttings of olive tree for the Serra da Mantiqueira region, Brazil. A hormonal balance between synthetic growth promoters and inducers that may raise the auxin level in the tissue is necessary (KHAJEHPOUR et al. 2014).

In addition to hormonal treatment, other factors may affect rooting, as, for example, physiological conditions, age of the mother plant, genetic potential for rooting, healthiness, oxidation of phenolic compounds, and position of the cutting on the stem (AHMAD et al. 2014); as well as external or exogenous factors, such as temperature, light, humidity, substrate and time of harvest of the stem cutting (FACHINELLO et al. 2005).

According to LAZAJ et al. (2015), the best times are those that coincide with the end of the annual growth flush because as the leaves are at the maximum point of expansion, they are more efficient in use of light, and also in the provision of compounds necessary for the beginning of root production. External factors like temperature, light and humidity may be monitored in a controlled environment, such as in a greenhouse, and this is thus a recommended alternative for the olive tree. However, the results found in the literature are not very satisfactory, presenting very low rooting for some cultivars (OLIVEIRA et al. 2012).

The substrate used in the rooting of the stem

cuttings is also considered to be an important factor in the success of this stage. It must be clean, free of weeds and sufficiently firm and dense to maintain the cuttings in place during the rooting period (MOHAMED 2015). Various substrates may be used, such as perlite, sand, vermiculite or a mixture of them. The temperature of the substrate in the region of root formation should be maintained between 20°C and 24°C (MEHRI et al. 2013b). The environment around the cuttings should be very humid, from 80% to 90%, and with a mild temperature, which may be achieved through the use of intermittent water mist (CABALLERO & DEL RIO 2008).

With a view toward future studies and selecting superior genotypes adaptable to the conditions of the south of Minas Gerais State (Brazil), the purpose was to conduct the present experiment to study the rooting of semi-hardwood stem cuttings of ten cultivars of olive trees in two substrates: fine washed sand and perlite[®].

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at the Experimental Farm of EPAMIG (Empresa de Pesquisa Agropecuária de Minas Gerais, Brazil), in Maria da Fé, Minas Gerais, in november, 2010.

To carry out the experiment, adult plants from ten cultivars: 'Alto D'Ouro', 'Cornicabra', 'Frantoio', 'Galega', 'JB1', 'JB2', 'Mission', 'Penafiel SP', 'Picual' and 'Santa Catalina' were used from the collection maintained under normally recommended techniques at the EPAMIG unit (Maria da Fé, Brazil). Semi-hardwood stem cuttings were collected from the mid region around the treetop. Production of the respective cultivars is considered to be regular annual production and their fruits are used for both table olives and for olive oil.

The 0.5 mm diameter cuttings were prepared with a length of approximately 12 cm and four to six internodes, maintaining the same four leaves in the apical region. The treatment with hydroalcoholic solution with indolebutyric acid (IBA), at a concentration of 3000 mg L^{-1} , was performed by immersion of three centimeters of the base of the cuttings for five seconds in this solution.

After treatment with the plant growth regulator, the cuttings were placed in a greenhouse with automated intermittent mist, operating from 07h00 a.m. to 07h00 p.m., for ten seconds at intervals of ten minutes and humidity of around 80%. In the structure there were raised beds with dimensions of 1m height and 5 m length containing the fine washad sand and perlite. The cuttings were planted in the appropriate substrates with a spacing of 4 cm between rows and 3 cm between cuttings.

The substrates for rooting were irrigated before planting of the cuttings and a treatment was also performed with copper oxychloride solution at 3% for the purpose of preventing possible fungus attacks.

A randomized block experimental design (DBC) was used in a double ten factorial (ten olive tree cultivars) x two (substrates of fine washed sand and perlita[®]), with four replications and 20 cuttings per experimental unit.

Evaluations were performed 70 days after planting of the cuttings. For indication of survival of the cuttings, the following characteristics were noted: percentage of callused and rooted cuttings, number and average length (cm) of roots.

The collected data was analyzed statistically using the SISVAR system of analysis of variance for balanced data (FERREIRA 2011), with comparison of mean values being made by the Scott-Knott test at 5% significance for the levels of the qualitative factors.

RESULTS AND DISCUSSION

According to analysis of variance, a significant effect of substrate x cultivars was verified for the number of rooted and callused cuttings (Table 1).

It may be observed that there was a difference in rooting (Figure 1) and callus formation among the cultivars studied, with Alto D'Ouro, JB1 and Galega presenting the greatest number of cuttings rooted, and JB2 the greatest number of cuttings callused in the sand substrate. In relation to the substrate used, the number of rooted and callused cuttings was greater in the beds that contained sand. The difference of rooting among olive tree cultivars has already been reported and, among other factors, is due to genetic differences and the vigor of the mother plant (PIO et al. 2005). The Frantoio and Mission cultivars, according to reports from authors, have little to no rooting of material arising from the cutting process (RALLO 2005).

The greatest number of callused cuttings was observed for JB2 in beds that contained sand as a substrate (Table 1), and an inverse proportional relationship was observed with the number of rooted cuttings. ULLAH et al. (2012) also observed this inverse effect between the percentages of cuttings rooted and callused in different olive tree cultivars.

The presence of calluses at the base of the cuttings has an inverse relationship with root formation, in spite of being independent events; the requirements for each occurrence are similar (HAN et al. 2009). For FACHINELLO et al. (2005) there is no direct relationship between callus formation and rooting, an affirmation that is corroborated by PEIXE et al. (2007), which says that in species of difficult rooting, callus formation is frequent, but its development is not directly related to the formation of adventitious roots.

Callus formation can be important in the rooting of olive cuttings according to the cultivar to be rooted, since an anatomical study of the rooting of olive cuttings Galega vulgar. PEIXE et al. (2007) verified that there is a great correlation between the formation of callus and rooting of the cuttings, but for Cobrançosa cultivar the adventitious roots were formed in tissues close to the vascular exchange and there was no strong relation with the presence of callus.

In the present work the cuttings of the Alto D'ouro, JB1 and Galega cultivars presented that better rooting did not present the best callus formation, whereas JB2 that presented low rooting in sand had a high callus formation. These results prove the opposite relationship between these factors for these cultivars. There were also cultivars that had indifferent behavior presenting both low rooting and low callus index, such as Mission and Frantoio.

In relation to the rooting percentages, high rates were registered for the Alto D'Ouro, JB1 and Galega cultivars (Table 2). A large variation in rooting percentage of the cuttings (from 30% to 90%) was observed, corroborating CABALLERO & del RIO (2008), upon working with the suitability of olive tree cultivars, finding results varying from 0 to 89%.

MEHRI et al. (2013a), working with rooting of olive tree cuttings under intermittent mist chamber conditions and maintenance of controlled environmental temperatures and substrate verified better results when perlite or a mixture with vermiculite were used as rooting mediums of Spanish cultivars.

The results found in this study are divergent from those presented by some authors (PENSO et al. 2016, OLIVEIRA et al. 2012).

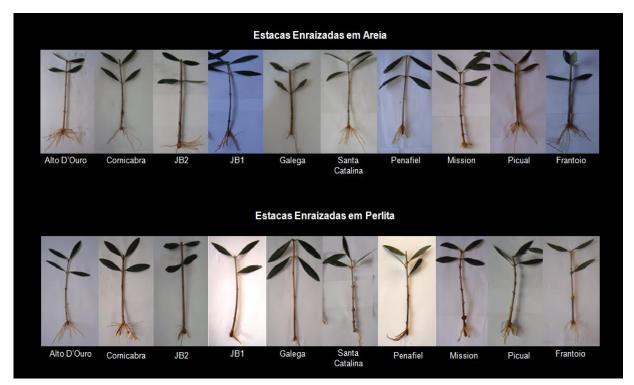
Table 1 - Table 1 - Number of olive tree variety of cuttings rooted and callused. Unioeste, Campus MarechalCândido Rondon, PR, Brazil, 2017.

Substrates	Sand	Perlite®	Sand	Perlite®
Cultivars	Number of cuttings rooted		Number of cuttings callu	
Alto D'Ouro	3.599 aA*	2.353 aB	2.134 bA	1.344 bB
JB1	3.456 aA	1.739 aB	0.707 dA	1.184 bA
Galega	3.159 aA	1.689 aB	0.926 cA	0.966 bA
Penafiel SP	2.518 bA	1.346 aB	1.435 cA	0.707 bA
Santa Catalina	2.218 bA	1.689 aA	1.507 cA	2.034 aA
Cornicabra	2.207 bA	2.076 aA	1.966 bA	2.076 aA
Picual	1.978 bA	1.184 aA	1.652 cA	1.635 aA
JB2	1.633 cA	1.798 aA	3.252 aA	1.931 aB
Frantoio	1.465 cA	1.184 aA	2.218 bA	2.129 aA
Mission	1.217 cA	1.217 aA	1.919 bA	1.643 aA

Tabela 1 - Número de estacas enraizadas e calejadas de cultivares de oliveira. Unioeste, Campus Marechal Cândido Rondon, PR, 2017.

*Mean values followed by the same small letter in the column and capital letter in the row do not differ among themselves by the Scott-Knott test at 5% probability.

*Médias seguidas pela mesma letra minúscula na coluna e maiúscula na linha não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro.



- Figure 1 Differences of rooting in cuttings of 10 olive tree cultivars in beds containing sand and perlite[®]. Unioeste, Campus Marechal Cândido Rondon, PR, Brazil, 2017.
- Figura 1 Diferenças de enraizamento em estacas de 10 cultivares de oliveiras, em bancadas contendo areia e perlita[®]. Unioeste, Campus Marechal Cândido Rondon, PR, 2017.

Table 2 - Average	percentage	of olive tre	e cuttings	rooted	and	callused.	Unioeste,	Campus	Marechal	Cândido
Rondon,	PR, Brazil,	2017.								

Substrates	Sand	Perlite®	Sand	Perlite [®]
Cultivars	Percentage of cuttings rooted		Percentage of cuttings cal	
Alto D'Ouro	90 aA*	59 aB	53 bA	34 bB
JB1	86 aA	43 aB	18 dA	30 bA
Galega	79 aA	42 aB	23 bA	24 bA
Penafiel SP	63 bA	34 aB	36 cA	18 bA
Santa Catalina	55 bA	42 aA	38 cA	51 aA
Cornicabra	55 bA	52 aA	49 bA	52 aA
Picual	49 bA	30 aA	41 cA	41 aA
JB2	41 cA	45 aA	81 aA	48 aB
Frantoio	37 cA	30 aA	55 bA	53 aA
Mission	30 cA	30 aA	48 bA	41 aA

Tabela 2 - Percentagem média de estacas enraizadas e calejadas de cultivares de oliveira. Unioeste, Campus Marechal Cândido Rondon, PR, 2017.

*Mean values followed by the same small letter in the column and capital letter in the row do not differ among themselves by the Scott-Knott test at 5% probability of error.

*Médias seguidas pela mesma letra minúscula na coluna e maiúscula na linha não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro.

OLIVEIRA et al. (2012) verified rooting percentage ranging from 1 to 77% in different substrates and cultivars considered promising for the Brazilian region of Serra da Mantiqueira, PENSO et al. (2016) further reports that the percentage of rooting can vary widely within the same cultivar in response to environmental and intrinsic conditions of the plant matrix, such as in rooting of Koroneiki cultivar. This difference of results clearly shows the great variety existing in rooting potential among different olive tree cultivars, using different types of substrates.

For average number of roots per cutting and average length of roots per cutting, there was statistical difference among the treatments of each isolated factor (Table 3). Difference in the number of roots for the cultivars studied may be observed, with the best results verified in cuttings from Alto D'Ouro, Galega and Penafiel cultivars. The results were significant only among the cultivars.

The number of roots of the cuttings studied here differed from the studies of some authors (OLIVEIRA et al. 2009), possibly through the effects of the substrates used, phenological stages of the mother plants and period of collection of the cuttings, this latter factor being directly connected to the flux and mobilization of reserve substances (carbohydrates) that affect root production (DIAS et al. 2011).

For average length of the roots, there was no statistical difference among the cultivars, being significant results only for substrates according to Table 3.

In Table 4, greater average length of roots in the sand substrate is observed. In addition to sustaining the cuttings, the substrate has an influence on availability of water and oxygen in the rooting medium, exercising a positive effect on the physiological process of rooting (RELLÁN-ÁLVAREZ et al. 2016). This condition was evident when better results were verified in the sand, with greater water retention capacity, but also with sufficient porosity for occurrence of good drainage.

The use of sand as a substrate for growing fruit bearing species has shown positive results because, in addition to having very low ion exchange capacity, it is considered to be an easily managed substrate (ISFENDIYAROGLU et al. 2009). The greatest limitation for use of sand as a substrate is the difficulty of handling due to excessive weight, especially when wet (OLIVEIRA et al. 2012).

Future studies are necessary in relation to the use of other types of substrates, time periods for removal of cuttings, cultivars adapted to Brazilian conditions Table 3 - Average number and length of roots per cutting of olive tree varieties. Unioeste, Campus MarechalCândido Rondon, PR, Brazil, 2017.

Tabela 3 - Número médio de raízes por	estacas de cultivares de oliveira.	Unioeste, Campus Marechal Cândido)
Rondon, PR, 2017.			

Varieties	Number of roots	Average length of the roots (cm)		
Alto D'Ouro	3.110 a*	2.176 a*		
Galega	2.786 a	1.633 a		
Penafiel	2.377 a	1.575 a		
JB1	2.178 b	1.727 a		
Cornicabra	2.117 b	1.796 a		
Frantoio	1.804 b	1.657 a		
JB2	1.662 b	1.754 a		
Santa Catalina	1.656 b	1.821 a		
Picual	1.570 b	1.683 a		
Mission	1.450 b	1.324 a		

*Mean values followed by the same small letter in the column do not differ among themselves by the Scott-Knott test at 5% probability of error.

*Médias seguidas pela mesma letra minúscula na coluna não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro.

- Table 4 Substrates used in rooting of olive tree varieties cuttings. Unioeste, Campus Marechal Cândido Rondon, PR, Brazil, 2017.
- Tabela 4 Substratos usados no enraizamento de estacas de cultivares de oliveira. Unioeste, Campus Marechal Cândido Rondon, PR, 2017.

Substrates	Average length of roots (cm)		
Sand	1.820a		
Perlite [®]	1.609b		

*Mean values followed by the same small letter in the column do not differ among themselves by the Scott-Knott test at 5% probability of error.

*Médias seguidas pela mesma letra minúscula na coluna não diferem entre si pelo teste de Scott-Knott, a 5% de probabilidade de erro.

and propagation techniques, as, for example, grafting, which may be used in future studies with some cultivars that prove to have difficult rooting by the cutting method.

In addition to the possibility of use of various commercial substrates found on the market, such as perlite[®], the nursery operator should have other substrate options, mainly easily purchased components, such as sand, with this component being able to provide gains in the seedling production process, leading to formation of quality seedlings and furthermore promoting utilization of components easily available to the nursery operator, which may lead to a decrease in the final cost of the seedling.

CONCLUSION

There is variation in the rooting of olive tree cultivar cuttings.

Better performance using sand was verified in the cutting process of Alto D'Ouro, JB1, Galega and Penafiel SP olive tree cultivars.

Alto D'Ouro, JB1 and Galega stood out among the cultivars studied.

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