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Propagation

Collection positions in the branch, humic substances and indolebutyric acid in 'Arbequina' olive minicuttings

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Abstract -Obtaining quality plantlets is essential for the expansion of olive growing in Brazil. Olive tree plantlets are produced by cuttings and the great obstacle for the production of plantlets on a large scale is the low rooting rate. In this sense, minicutting is a promising alternative, as it can optimize the collection of propagules and provide an improvement in the rooting index. The objective of this study was to verify the propagation of 'Arbequina' olive minicuttings collected in different positions of the branch and submitted to concentrations of humic substances with and without the use of indolebutyric acid (IBA). The experimental design was completely randomized, in a bifactorial scheme: collection positions in the branch (basal, median and apical) and concentrations of humic substances (SoloHumics®) with and without IBA (10 mL SoloHumics® + 0 mg L^{-1} IBA; 10 mL SoloHumics® + 1,000 mg L^{-1} IBA; 10 mL SoloHumics® + 2,000 mg L^{-1} IBA; and 10 mL SoloHumics® + 3,000 mg L^{-1} IBA). The 'Arbequina' olive tree can be propagated by basal and apical minicuttings submitted to the use of SoloHumics® + 1,000 mg L^{-1} of IBA and by medium minicuttings treated with SoloHumics® + 2,000 mg L^{-1} of IBA, due to the greater rooting potential.

Index terms: *Olea europaea* L.; vegetative propagation; rooting.

Posições de coleta no ramo, substâncias húmicas e ácido indolbutírico na miniestaquia de oliveira 'Arbequina'

Resumo-A qualidade das mudas é essencial para a expansão da olivicultura no Brasil. As mudas de oliveira são produzidas por estacas e o grande entrave para a produção de mudas em larga escala é a baixa taxa de enraizamento. Nesse sentido, a miniestaquia é uma alternativa promissora, pois pode otimizar a coleta de propágulos e propiciar melhoria no índice de enraizamento. O objetivo deste trabalho foi verificar a propagação de miniestacas de oliveira 'Arbequina' coletadas em diferentes posições do ramo e submetidas a concentrações de substâncias húmicas com e sem o uso de ácido indolbutírico (AIB). O delineamento experimental foi o inteiramente casualizado, em esquema bifatorial: posições de coleta no ramo (basal, mediana e apical) e concentrações de substâncias húmicas (SoloHumics®) com e sem AIB (10 mL SoloHumics® + 0 mg L^{-1} AIB; 10 mL SoloHumics® + 1.000 mg L^{-1} AIB; 10 mL SoloHumics® + 2.000 mg L^{-1} AIB; e 10 mL SoloHumics® + 3.000 mg L^{-1} AIB). A oliveira 'Arbequina' pode ser propagada por miniestacas basais e apicais submetidas ao uso de SoloHumics® + 1.000 mg L^{-1} de AIB e por miniestacas medianas tratadas com SoloHumics® + 2.000 mg L^{-1} de AIB, em função do maior potencial de enraizamento.

Termos para indexação: *Olea europaea* L.; propagação vegetativa; enraizamento.

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Introduction

The olive tree (*Olea europaea* L.) is a fruit tree belonging to the Oleaceae family. In the last two decades its cultivation has been encouraged in many countries of the South hemisphere, especially South America and Australia, due to the increase demand and the consumption of their products, the table olive and the olive oil (BODOIRA et al., 2016; TORRES et al., 2017).

For the expansion of the olive cultivation in Brazil, the choice of adapted cultivars is essential. Among these, the 'Arbequina' cultivar is the most used in the olive oil production in the Southern hemisphere (OLIVEIRA et al., 2012) and, in Brazil, is one of the most cultivated, due to the adaptation to the climate and soil of the producing regions. In addition, it is the preferred cultivar by the producers for the use in high-density orchards, due to the low vigor, high yield and extraordinary olive oil quality (SÁNCHEZ-ESTRADA and CUEVAS, 2018).

The olive plantlets are produced by cuttings and the great obstacle for plantlets production in large scale is the low rooting rate (OLIVEIRA et al., 2012; SILVA et al., 2012).

Among the options for propagation is the minicuttings, which emerged as a variation of the conventional cutting in species of the genus *Eucalyptus* (CASARIN et al., 2017). Among its advantages stand out the low cost, the need of small area for propagation, and the high yield per matrix plant due to the minicuttings small size (TIMM et al., 2017). In addition, the minicutting can promote improvements in the rooting indices.

Although it is a promising technique, the minicutting rooting can vary according to factors, such as, minicutting collection position in the branch, plant regulators type and concentration, cultivar, among others, and available information is scarce.

As for the minicuttings collection position in the branch, these can be basal, median and apical. The tissue chemical composition can vary, so cuttings from different branches tend to differ in terms of rooting potential (HARTMANN et al., 2011). In addition to this factor, the exogenous application of plant regulators has been one of the most studied techniques for the improvement in the rooting hormonal balance, with the indolebutyric acid (IBA) the most used auxin (INOCENTE et al., 2018). However, considering that restrictions are being imposed to the use of these regulators in plant production (WISZNIEWSKA et al., 2016), an alternative is the use of humic substances, which are the primary constituents of the soil organic matter, corresponding generically to the final product of the organic waste decomposition in the nature (MELO et al., 2016).

Such substances present bio-stimulating effect, very similar to the auxins (BALDOTTO and BALDOTTO, 2013), besides they are nutrient sources for the plants, providing carbon, nitrogen, phosphorus and sulfur (SILVA et al., 2015).

Based on these aspects, the objective of this study was to verify the propagation of Arbequina olive minicuttings, collected in different branches and subjected to SoloHumics® concentrations with and without IBA use.

Material and methods

The experiment was conducted from October to December 2019, in a greenhouse with controlled temperature (25ºC±2ºC), belonging to the Plant Science Department of the Eliseu Maciel Agronomy Falculty (FAEM) of the Pelotas Federal University (UFPel), located in Capão do Leão – RS, Brazil.

The experimental design was completely randomized in a bifactorial scheme 3 x 4, with four replications and 15 minicuttings per experimental unit. The treatment factors were the minicuttings collection position in the branch (basal, median and apical) and humic substances concentration (SoloHumics®) with and without indolebutyric acid (IBA) (10 mL SoloHumics® + 0 mg L^{-1} IBA; 10 mL SoloHumics® + 1,000 mg L^{-1} IBA; 10 mL SoloHumics® + 2,000 mg L^{-1} IBA; and 10 mL SoloHumics® + 3,000 mg L^{-1} IBA).

The propagative material was derived from olive tree clonal minigarden (*Olea europaea* L.) Arbequina cultivar two years old produced by minicutting and kept in 30L pots containing Beifort S10 B® substrate, in the UFPel vegetative house. The branches used were from the matrix plants second pruning, performed in the spring (October 15), with the interval between the first and the second pruning of 150 days.

The SoloHumics® commercial product was donated by the SoloHumics® fertilizers industry, and is composed of humic substances obtained from the turf extraction, containing: humic acid (25%), Fulvic acid (5%) and organic matter (60%).

The branches, of about 12 to 15 cm in length, were collected from the matrix plants in the morning and provisionally arranged in a container with water, to avoid dehydration. Subsequently, to obtain the different types of minicuttings, the branches were divided into three segments: basal, median and apical. Then, minicuttings were prepared and standardized from 3 to 4 cm, with the basis in bezel, containing two leaves cut to the middle and with diameters of 4, 3 and 2 mm for basal, median and apical cuttings respectively (Figure 1). With the aid of a scalpel, a surface lesion was performed in the minicuttings' base with about 0.3 cm and then the bases were immersed in SoloHumics® at 10 mL concentration (containing 2,500 mg L^{-1} of humic substances) for 90 minutes, following methodology described by Ritter (2019). Subsequently, all the minicuttings were treated with or without IBA (according to the treatment), with their base immersed in the solution for 10 seconds.

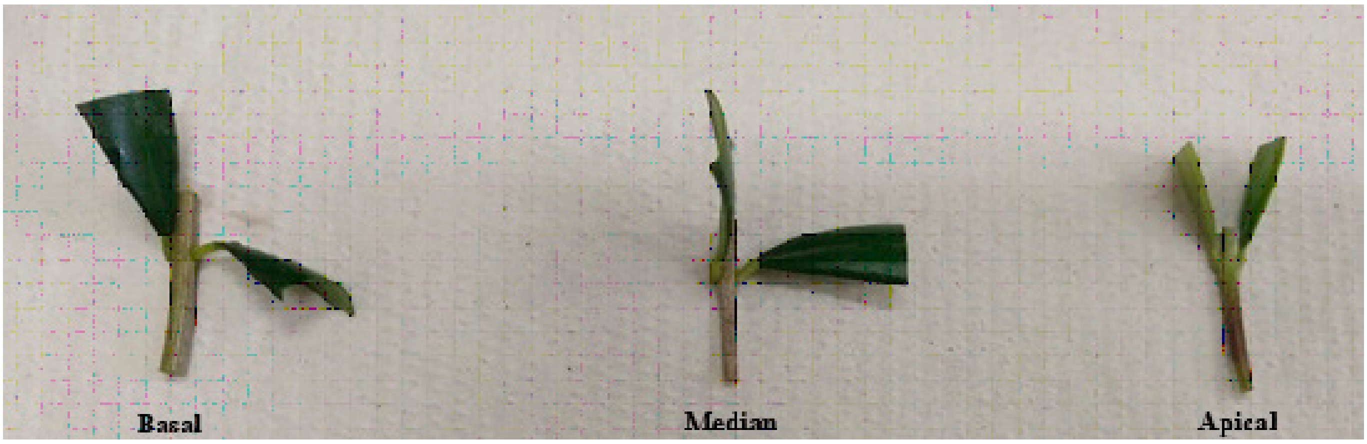

Figure 1. Basal, median and apical ‘Arbequina’ olive tree minicuttings. UFPel, Pelotas/RS, 2019.

The minicuttings were put in transparent and articulated plastic packaging Sanpack® (235x169x100mm) perforated in the base, containing expanded mean vermiculite, previously moistened with 500 mL of distilled water. Then, a preventive phytosanitary treatment of the minicuttings was performed with Captana fungicide (3 g L^{-1}) using a spray mist with capacity for 500 mL and after, they were kept in greenhouse. During the experiment, every 15, days they were watered with the use of spray mist with capacity for 1,500 mL, using about 80 mL of distilled water per packaging, which were kept with lid (closed), in order to form a wet microenvironment to avoid tissue dehydration.

After 60 days the percentage of minicuttings survival and foliar retention; the number of leaves; the percentage, number and length of sprouts; the rooting percentage; the percentage of minicuttings with calluses and root, with calluses and without root, besides the number, length and dry matter mass of roots were evaluated. For the evaluations of the sprouts length and roots length variables, it was used graduated ruler and the results were expressed in centimeters (cm). For root drying, the Quimis® oven (Q317M) at 60ºC was used until constant weight (approximately 3 days). After the drying, the samples were weighed in precision scale, obtaining their respective dry matter masses expressed in grams (g).

The data were analyzed for normality by the Shapiro Wilk test; homoscedasticity by the Hartley test; and, the waste independence by graphical analysis, and they evidenced that data transformation is not necessary. Subsequently, the data were submitted to variance analysis by the F test ($p \leq 0.05$). After finding statistical significance, the effects of the minicutting positions in the branch were compared by the Waller-Duncan test ($p \leq 0.05$).

The effects of the SoloHumics® + IBA concentrations were evaluated by regression models ($p \leq 0.05$), as follows: $y = y_o + ax$; $y = y_o + ax + bx^2$; $y = yo + ax + bx^2 + cx^3$. The model selection was based on the low residue, low *p*-value, and high R2 and R2 adj.

Results and discussion

For the variables such as, survival percentage; foliar retention; number of leaves; percentage, number and length of sprouts it was verified significance for the interaction among the treatment factors tested (Table 1, Figures 2 and 3).

Regarding the survival percentage, when using concentration of 1,000 mg L^{-1} of IBA, no difference was observed among the minicuttings collection positions in the branch. While in those in which SoloHumics® + IBA in concentrations, 2,000 and 3,000 there were differences for all minicuttings collection positions in the tested branch, and the apical minicuttings presented the lowest percentage of survival in both IBA concentrations (Table 1).

Corroborating with this, Freire et al. (2020), in an experiment conducted with *Apuleia leiocarpa* minicuttings collected in different portions of the branch (apical, median and basal) with different IBA concentrations (0, 1,000, 2,000 and 4,000 mg kg^{-1}) they observed that the minicuttings originate from the apical portion had lower percentage of survival in all IBA concentrations. Such fact may be related to the material lignification, because according to Hartmann et al. (2011) cuttings collected from apical positions of the branch present lower degree of lignification and therefore are more sensitive to dehydration.

Table 1. Survival percentage; foliar retention; number of leaves; percentage, number and length (cm) of sprouts; roots number, length (cm) and dry matter mass of 'Arbequina' olive tree according to minicuttings collection positions in the branch in different SoloHumics® + IBA concentrations. UFPel, Pelotas/RS, 2019.

Minicuttings collection positions in the branch	SoloHumics® + IBA concentrations (mg L^{-1})			
	0	1,000	2,000	3,000
	Survival (%)			
Basal	100.00 a[1/]	97.5 a	82.50 b	80.00 a
Median	97.50 a	97.5 a	92.50 a	70.00 b
Apical	77.50 b	97.5 a	73.33 c	55.00 c
	Foliar retention (%)			
Basal	100.00 a	100.00 a	87.50 a	78.13 a
Median	100.00 a	100.00 a	94.44 a	79.46 a
Apical	100.00 a	100.00 a	93.65 a	63.33 b
	Number of leaves			
Basal	2.30 a	2.13 b	1.48 b	1.34 a
Median	2.20 a	2.08 b	2.16 a	1.38 a
Apical	2.04 a	2.43 a	1.72 ab	1.00 a
	Sprouts (%)			
Basal	27.50 a	7.50 a	0.00 b	0.00 a
Median	23.06 a	5.00 a	16.11 a	0.00 a
Apical	12.60 a	17.78 a	17.41 a	5.00 a
	Number of sprouts			
Basal	0.30 a	0.10 a	0.00 b	0.00 a
Median	0.25 a	0.05 a	0.22 a	0.00 a
Apical	0.16 a	0.20 a	0.17 a	0.05 a
	Length of sprouts (cm)			
Basal	0.10 a	0.03 a	0.00 b	0.00 a
Median	0.06 ab	0.03 a	0.03 ab	0.00 a
Apical	0.03 b	0.05 a	0.05 a	0.03 a
	Roots number			
Basal	0.65 b	2.00 ab	1.93 b	1.43 a
Median	1.40 a	1.51 b	2.90 a	2.31 a
Apical	1.19 a	2.72 a	1.89 b	1.53 a
	Roots length (cm)			
Basal	0.77 b	1.89 a	1.55 b	1.46 a
Median	1.49 a	1.64 a	2.54 a	1.98 a
Apical	1.57 a	1.50 a	2.05 ab	1.40 a
	Roots dry matter mass (g)			
Basal	0.02 b	0.09 a	0.05 b	0.05 ab
Median	0.06 a	0.06 b	0.13 a	0.06 a
Apical	0.03 b	0.07 ab	0.04 b	0.03 b

[1/]Means accompanied by the same letter in the column do not differ by the Waller-Duncan test ($p \leq 0.05$) comparing the minicuttings collection positions in the branch at each concentration of SoloHumics® + IBA.

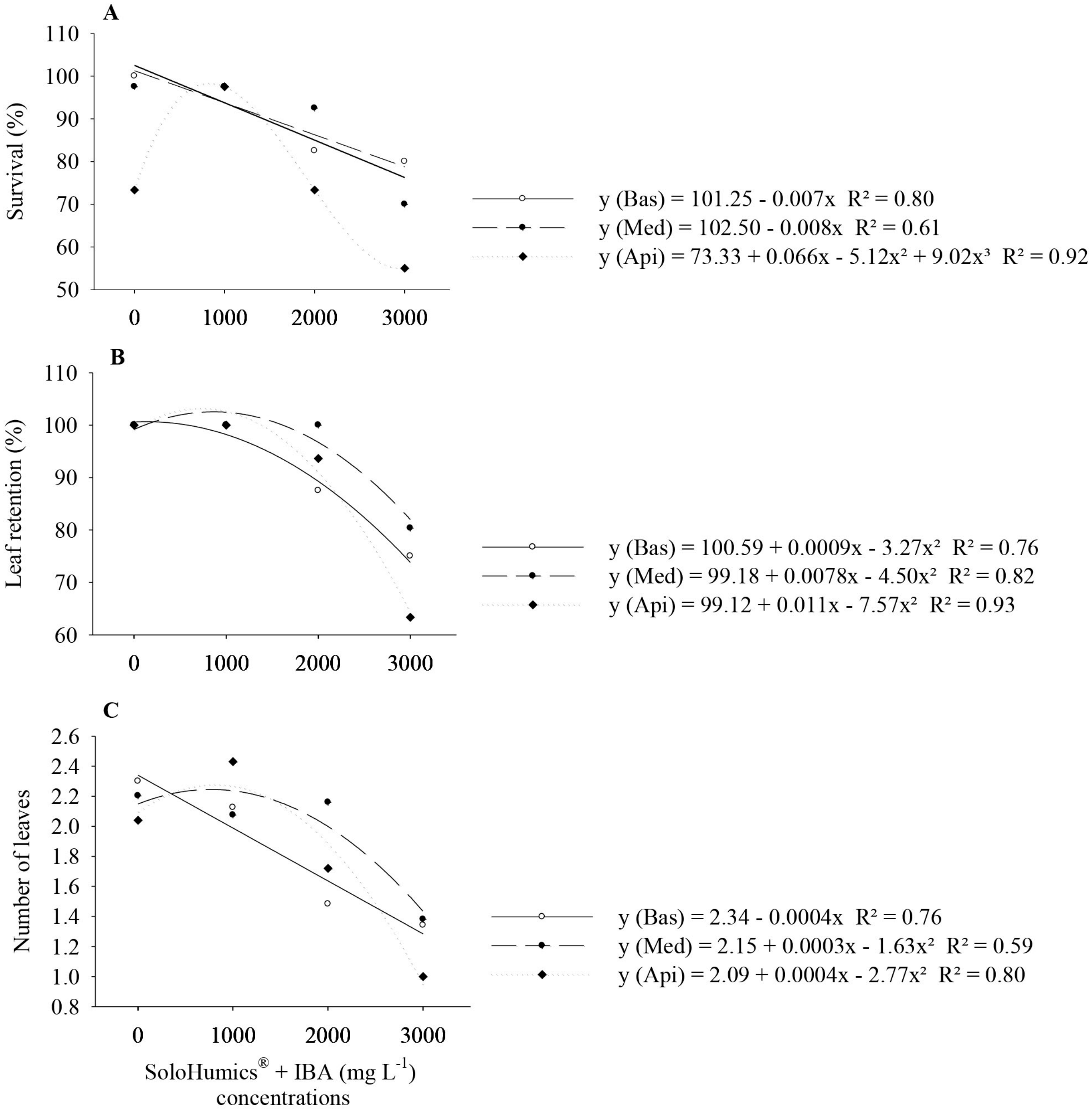

Figure 2. Survival percentage (A); foliar retention (B) and, number of leaves (C) of ‘Arbequina’ olive tree minicuttings according to the minicuttings collection positions in the branch in different concentrations of SoloHumics® + IBA. UFPel, Pelotas/RS, 2019.

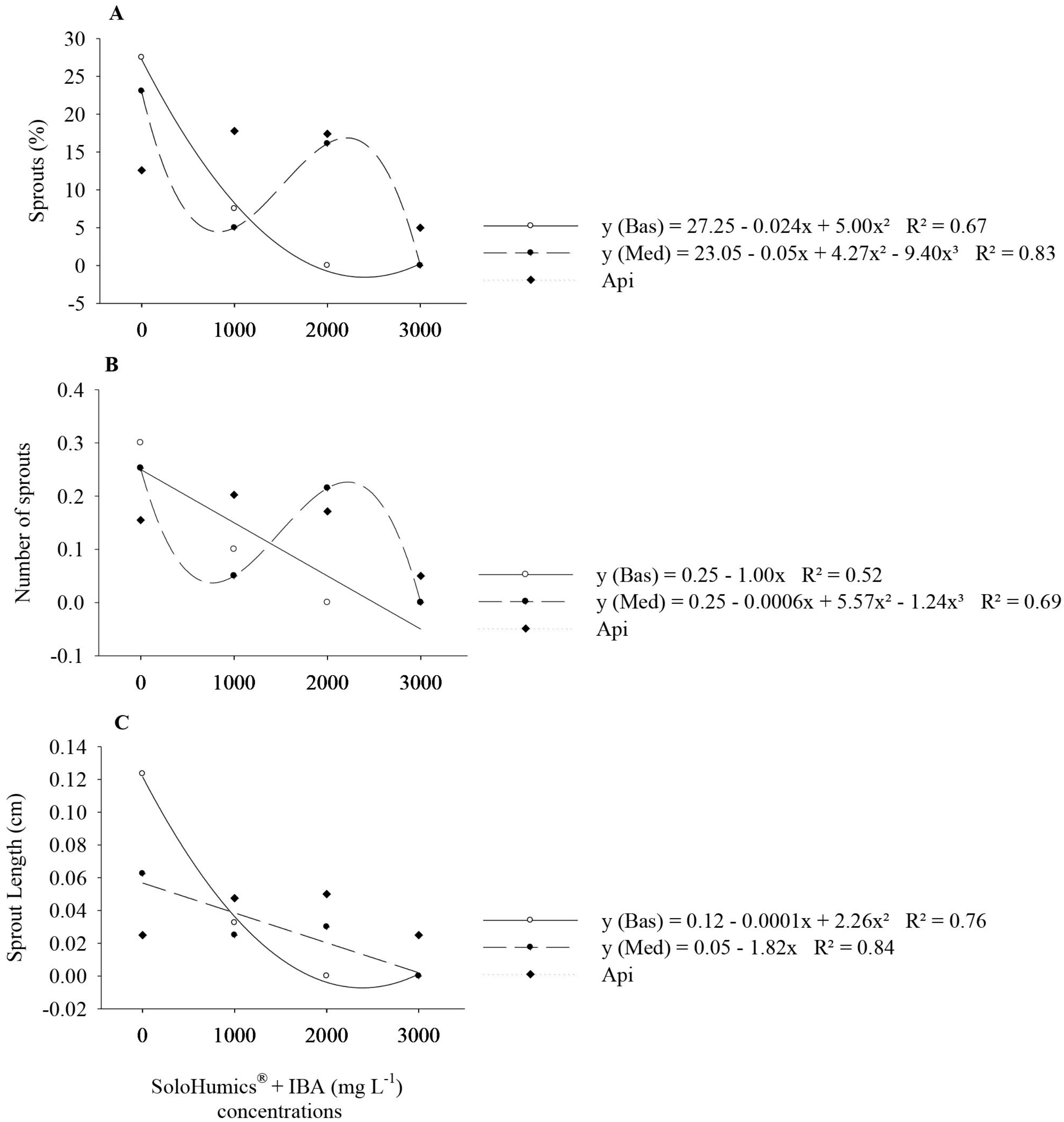

Figure 3. Percentage (A); number (B) and, sprouts length (C) of ‘Arbequina’ olive minicuttings according to the minicuttings collection positions in the branch in different concentrations of SoloHumics® + IBA. UFPel, Pelotas/RS, 2019.

In the comparison among the concentrations of SoloHumics® + IBA, the survival percentage was adjusted to the linear regression model for basal and median positions, and to the cubic polynomial regression model for apical minicuttings. The highest percentages of survival, foliar retention and number of leaves occurred with the use of SoloHumics® + 0 and 1,000 mg L^{-1} IBA concentrations in the basal and apical minicuttings and decreased in all the collection positions in the branch with concentration of 3,000 mg L^{-1} IBA (Figure 2).

Similar results were obtained by Moreira et al. (2017) with 'Barnea' olive minicuttings, where using different concentrations of IBA (0, 1,000, 2,000 and 3,000 mg L^{-1}), recorded decreases in the survival percentage. Assessing these results is possible to infer that the plant regulator has caused stress, affecting the survival percentage and the minicuttings foliar retention.

For percentage, number and length of sprouts variables, the highest means for basal and median minicuttings were obtained when only the SoloHumics® was used. For the apical minicuttings the highest means were verified with 1,000 mg L^{-1} IBA, (Table 1) and, for all positions there were decreases when we used 3,000 mg L^{-1} IBA (Figure 3). The reduction in the percentage, number and length of sprouts for this treatment can be related to the imbalance between auxins and cytokines, promoted by the high concentration of auxin used, because the excess of auxin can inhibit the sprout (TAIZ; ZEIGER, 2013).

The presence of leaves and new sprouts is important in the production of photoassimilated due to the increase of the photosynthetic area, which can influence the minicuttings rooting performance, changing the auxin levels and carbohydrate reserves. However, Oliveira et al. (2003) working with cuttings with different nodes numbers and olive leaves from 'Arbequina' and 'Picual' cultivars found that the difference in the number of leaves provoked little influence on their roots formation.

In this sense, it is possible to observe that in the present study the basal and median minicuttings that presented the highest sprouting percentage, 27.50% and 23.06% respectively, with the use of SoloHumics®, also presented the lowest roots number and length when compared to the other concentrations of SoloHumics® + IBA.

According to Wei et al. (2019), the high proportion of cytokine regarding the auxin promotes sprouts formation, while the reverse provides the roots formation. Therefore, in the present study it is possible to infer that the highest number of sprouts and lower number of roots in the minicuttings treated only with SoloHumics® is related to the concentration of endogenous cytokine.

For the variable rooting percentage there was significance for the treatments, but not for the interaction among them (Table 2, Figure 4). For calluses percentage with root was verified significance only for minicuttings collection positions in the branch (Table 2) and for calluses percentage without root there was no statistical significance in any of the treatments. For root, number, length and dry matter mass, it was verified significance for the interaction among the treatment factors tested (Table 1, Figures 5 and 6).

Table 2. Percentage of calluses with root and rooting in 'Arbequina' olives trees according to the minicuttings collection position in the branch. UFPel, Pelotas/RS, 2019.

Variables	Minicuttings collection position in the branch		
	Basal	Median	Apical
Rooting (%)	58.40 a[1/]	72.34 a	60.83 a
Calluses with root (%)	54.96 a	64.50 a	60.50 a

[1/]Medium accompanied by the same letter in the line do not differ between themselves by the Waller-Duncan test (p≤0.05) comparing the minicuttings collection positions in the branch.

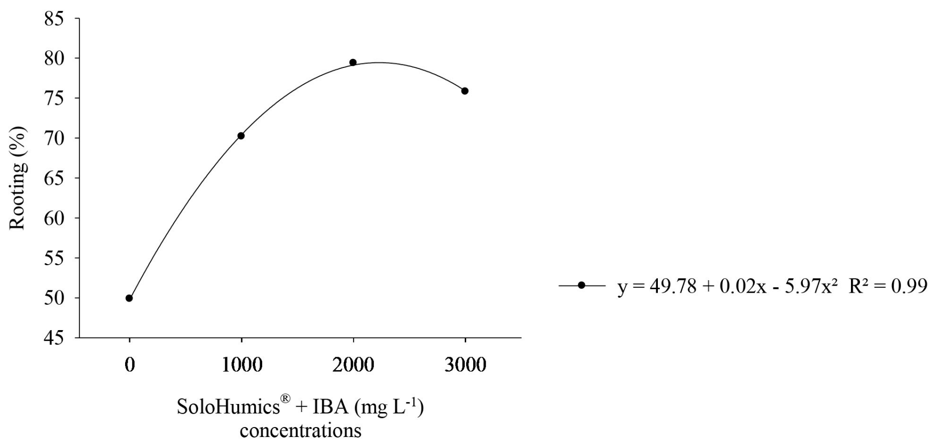

Figure 4. Percentage of minicuttings rooting in 'Arbequina 'olive trees in different concentrations of SoloHumics® + IBA. UFPel, Pelotas/RS, 2019.

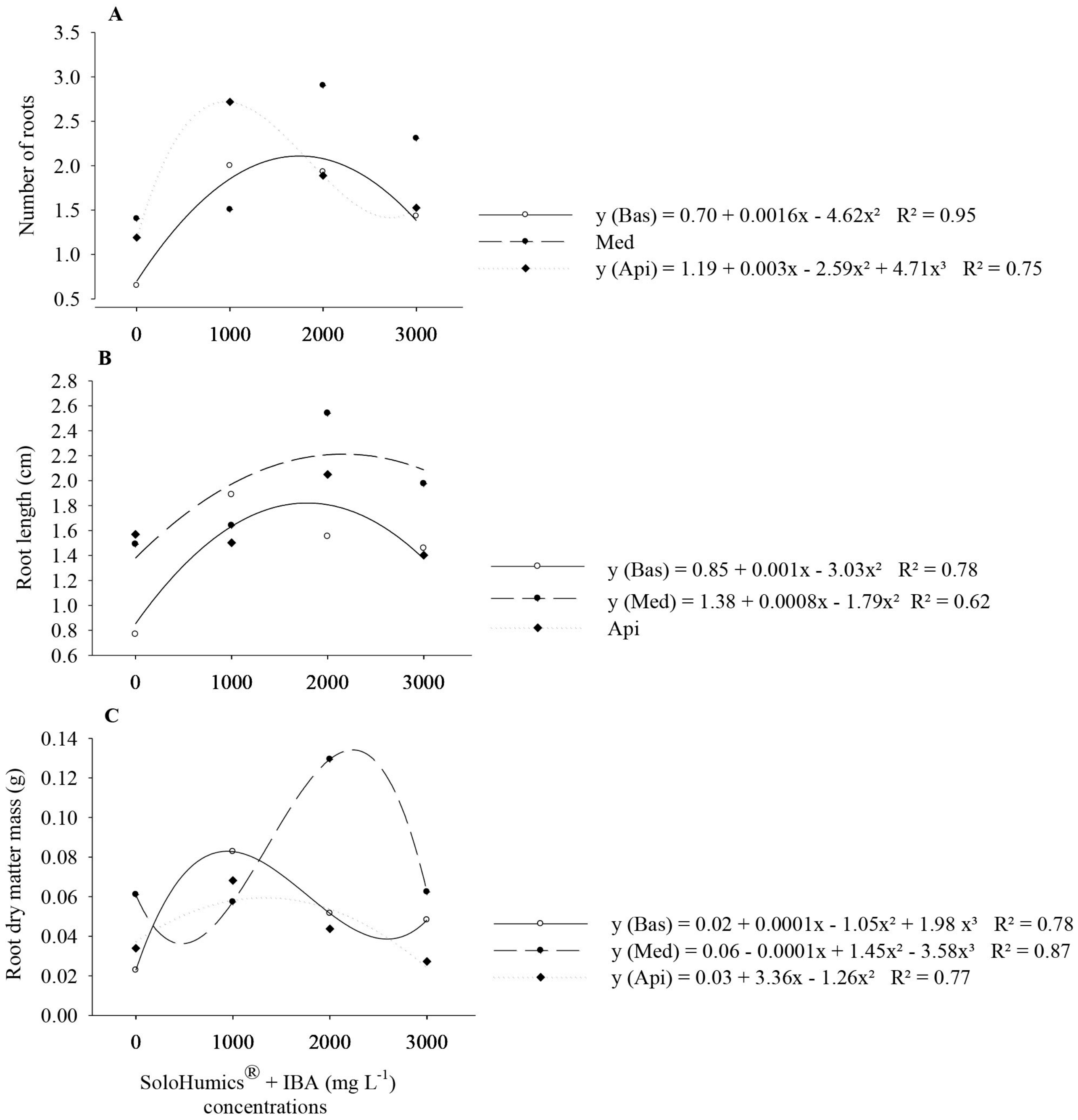

Figure 5. Root number (A); length (B) and, dry matter mass (C) in 'Arbequina' olive minicuttings according to the minicuttings collection positions in the branch in different concentrations of SoloHumics® + IBA. UFPel, Pelotas/RS, 2019.

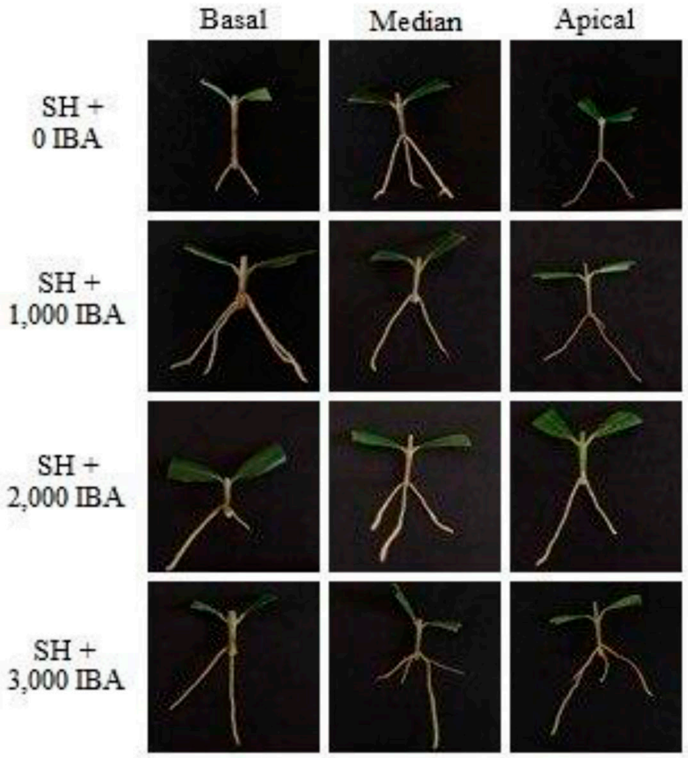

Figure 6. Basal, medians and apical 'Arbequina' olive minicuttings at 60 days of cultivation with SoloHumics® (SH) + 0, 1,000, 2,000 and 3,000 mg L^{-1} IBA. UFPel, Pelotas/RS, 2019.

Regarding the percentage of minicuttings with calluses and rooted there was no statistical difference between the minicuttings collection positions in the branch; however, the highest means were obtained in median cuttings, 64.50 % and 72.34 % respectively (Table 2).

Anatomical patterns of adventitious root formation can be divided into direct and indirect development. In the direct pattern is recommended the occurrence of competent cells, which, after the induction, initiate cellular divisions in a polar pattern of primordial differentiation. In contrast, in the indirect pattern, there is involvement of an initial non-competent state, in which the cells are unable to respond to inductive stimuli; after the induction, indirect cell divisions occur with calluses formation in the basal region, prior to primordial differentiation (HARTMANN et al., 2011; ALMEIDA et al., 2017).

According to Hartmann et al. (2011) for some species, calluses development can be a precursor of root formation. In the present study, in most of the rooted minicuttings, the presence of calluses was verified, thus demonstrating indirect pattern of adventitious root formation.

High percentage of calluses was also verified by Silva et al. (2012) working with semi-woody cuttings of 'Arbequina' olive tree collected in two seasons (April and August) and treated with 3,000 mg L^{-1} IBA, where the rooting percentage was 26 and 24%, and the calluses percentage of 32 and 37% respectively, for April and August. Despite the highest percentage of calluses relative to rooting, the authors state that some olive tree cultivars need longer time for cellular differentiation and, consequently, adventitious root formation after the calluses formation process.

On the other hand, Denaxa et al. (2019), in an experiment with 'Arbequina' subapical cuttings with concentration of 2,000 mg L^{-1} of IBA, observed that most of the cuttings rooted without calluses formation, while few formed calluses. Fachinello et al. (2005) can explained such fact, they state that there is no direct relationship between calluses formation and cuttings rooting and reiterate that although they are independent phenomena, in most cases, calluses formation and the appearance of adventitious roots are influenced by the same factors and can occur simultaneously.

Corroborating with this information, Denaxa et al. (2019) state that the adventitious root formation of 'Arbequina' olive tree can be affected by auxin interaction, enzymatic activities and anatomical structure.

For rooting percentage, there was adjustment to the quadratic polynomial regression model, and the results were increasing up to the concentration of 2,000 mg L^{-1} IBA. With the use of 3,000 mg L^{-1} IBA, the rooting percentage was of 75.81% (Figure 4).

It is important to highlight that, even with the reduction in the values when using 3,000 mg L^{-1} IBA+ SoloHumics® the results related to root development in all minicuttings collection positions in the branch were superior to those obtained by Casarin et al. (2018). These authors in an experiment conducted in the spring with 'Arbequina' olive cuttings collected from a clonal minigarden in conventional cultivation system (matrices kept in black plastic bags with Carolina Soil® substrate) found that when using the concentration of 3,000 mg L^{-1} the rooting percentage was of 8% in the first year and of 49% in the second year.

This increase in rooting percentage can be associated with the use of SoloHumics® + IBA, because when only the SoloHumics® was used the rooting percentage was of 49.87% (Figure 4).

Oliveira et al. (2010), testing 0 and 3,000 mg L^{-1} of IBA, associated with the use of different concentrations of organic fertilizers in the soil in 'Ascolano 315' olive cuttings verified that when used only the organic fertilizer the results were not satisfactory. However, as the fertilizer concentrations increased also increased the rooting percentage, and when the fertilizer was used together with 3,000 mg L^{-1} of IBA there were increments in root formation.

Median minicuttings presented higher roots number, length and dry matter mass with the use of SoloHumics® + 2,000 mg L^{-1} IBA, while in the basal and apical minicuttings the highest number of roots was recorded when these were treated with SoloHumics® + 1,000 mg L^{-1} IBA (Table 1).

With the use of SoloHumics® + 3,000 mg L^{-1} IBA, there were decreases for the variables such as, roots number, length and dry matter mass in all minicuttings collection positions in the branch. Moreira et al. (2017), observed the highest number of roots with the use of 2,000 mg L^{-1} of IBA, for 'Barnea' olive minicuttings, and reduction with the increase of the concentration to 3,000 mg L^{-1}. Frölech et al. (2020) verified an increase in the number of roots in 'Maria da Fé' olive minicuttings with the use of 1,000 mg L^{-1} of IBA, and reduction in the concentration of 2,000 mg L^{-1}. These results show the different responses regarding the rhizogenic potential in olive cultivars and IBA concentrations used.

Timm et al. (2015) state that the appropriate content of exogenous auxin for rooting stimulus depends on the concentration existing in the tissue, and that the increase of the exogenous auxin concentration applied in minicuttings causes a root-stimulating effect up to a maximum value, from which any auxin addition has inhibitory effect.

Despite the differences in the aerial and root development of the minicuttings collected in different positions in the branch, the results demonstrated to be possible to use basal, medians and apical minicuttings, which represents greater use of the collected branches in the matrix plant. In addition, SoloHumics® + 1,000 mg L^{-1} IBA, for basal and apical minicuttings and SoloHumics® + 2,000 mg L^{-1} IBA, for median minicuttings can be used due to the highest rooting potential.

Conclusion

The 'Arbequina' olive tree can be propagated by basal and apical minicuttings submitted to the use of SoloHumics® + 1,000 mg L^{-1} of IBA and by median minicuttings submitted to the use of SoloHumics® + 2,000 mg L^{-1} of IBA.

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