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SUBSTRATE AND INDOLEBUTYRIC ACID IN THE ROOTING OF POMEGRANATE CUTTINGS COLLECTED IN TWO SEASONS

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ABSTRACT - The pomegranate (*Punica granatum* L.*)* presents potential for commercial exploitation as a fruit, ornamental or medicinal plant. However, for its usage in orchards, it is ideal that the seedlings come from vegetative propagation techniques. The aim of this work was to test the usage of indolebutyric acid (IBA) and substrates, at two harvesting times (before and after fruiting) in the cutting propagation of pomegranate. The work was carried out at UTFPR, *Campus* Dois Vizinhos. The experiments were installed in a randomized block design, in a 4 x 4 factorial scheme (IBA concentration x substrate), with four replications and ten cuttings per experimental unit. IAB was tested at concentrations 0; 1000; 2000 and 3000 mg L^{-1}. The used substrates were Red Latosol, medium textured sand, commercial substrate MecPlant® and a mixture of Red Latosol + sand + commercial (1:1:1, v/v). The cuttings were placed in beds in a screened protected environment, with 50% shading. Irrigation was performed three times a day for 30 min., by a micro sprinkler. After 60 days, rooting (%), cuttings with callus (%), number of primary leaf and root shoots, length of the three largest roots and percentage of dead cuttings were evaluated. Cutting presented itself as a potential technique to be adopted for pomegranate, using sand, with the use of IBA at concentrations between 1600 and 1750 mg L^{-1}. Cuttings must be collected before fruiting. However, rhizogenesis close to 50% was achieved at the time after such phenological stage.
Keywords: *Punic granatum* L., vegetative propagation, pomegranate.

SUBSTRATO E ÁCIDO INDOLBUTÍRICO NO ENRAIZAMENTO DE ESTACAS DE ROMÃZEIRO, COLETADAS EM DUAS ÉPOCAS

RESUMO - A romãzeira (*Punica granatum* L.), apresenta potencialidade para exploração comercial como planta frutífera, ornamental ou medicinal. No entanto, para seu uso em pomares ideal que as mudas sejam oriundas de técnicas da propagação vegetativa. O objetivo deste trabalho foi testar o uso de ácido indolbutírico (AIB) e substratos, em duas épocas de coleta (antes e após a frutificação) na propagação por estaquia do romãzeiro. Os trabalhos foram realizados na UTFPR, *Campus* Dois Vizinhos. Os experimentos foram instalados em delineamento blocos ao acaso, em esquema fatorial 4 x 4 (concentração de AIB x substrato), contendo quatro repetições e dez estacas por unidade experimental. O AIB foi testado nas concentrações 0; 1000; 2000 e 3000 mg L^{-1}. Os substratos utilizados foram Latossolo Vermelho, areia de textura média, substrato comercial MecPlant® e mistura de Latossolo Vermelho + areia + comercial (1:1:1, v/v). As estacas foram colocadas em canteiros em ambiente protegido tipo telado, com sombreamento de 50%. A irrigação foi realizada três vezes ao dia com funcionamento de 30 min., por microaspersão. Após 60 dias, avaliou-se o enraizamento (%), estacas com calo (%), número brotações primárias, de folhas e de radicelas, comprimento das três maiores radicelas e porcentagem estacas mortas. A estaquia apresentou-se técnica potencial a ser adotada para romãzeira, devendo-se para isso usar a areia, com uso de AIB nas concentrações entre 1600 e 1750 mg L^{-1}. As estacas devem ser coletadas antes da frutificação. Todavia, conseguiu-se rizogênese próximo a 50% na época após tal estádio fenológico.
Palavras-chave: *Punica granatum* L., propagação vegetativa, romã.

INTRODUCTION

A pomegranate tree (*Punica granatum L.*), belongs to the Punicacea family, which can be used as a fruit or ornamental plant. *In vitro* and *in vivo* scientific studies testing preparations of this plant have supported its antimicrobial and anti-inflammatory properties. (LEE et al., 2010; ISMAIL et al., 2012). These activities have the therapeutic potential of its fruit, of parts such as the bark, leaves and seeds (SOUSA et al., 2018). In Brazil, compared to other European countries, this species is little commercially explored, with little offer of

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the fruit for the modern market. This makes it a good commercial investment, due to the limited existing offer.

In any commercial planting, one must first think about the loss, and ideally, it should be considered as superior quality characteristics in terms of growth, development and phytosanitary aspects (HARTMANN et al., 2011).

Seedlings of any species can be obtained through seeds (natural process) and by vegetative parts, the latter being advantageous for providing uniform plants with a shorter juvenile period, which is desirable to be used in orchards. Among the possible techniques to use for asexual propagation, one of the most advantageous is cutting. However, there is no established protocol for obtaining clones using the cutting technique.

In one of the few studies carried out with the pomegranate cutting propagation, Freitas et al. (2017) verified that the results were influenced by the origin of the plant material and by the substrate, with better responses for the seedlings production with the Barreiras material (CE) and with Tropstrato® substrate. Batista et al. (2011) recommended the use of Plantmax® substrate for the production of pomegranate seedlings by cuttings. However, commercial substrates can increase seedling production costs. The substrate is the mean that provides structural support to seedlings through the root proliferation that also supply the need for oxygen, water and nutrients (VALLONE, 2010). In the case of cuttings, the substrate can interfere with both the rooting and the quality of the seedlings produced, which makes it an important factor to be determined.

In addition, there are other factors that influence the results of rhizogenesis, such as cuttings collection time and the need for exogenous application of auxin.

As for the timing of cuttings collection, this can interfere on their rooting, as they are related to the condition of the internal carbon/nitrogen balance, neither of which can be favorable or not for rhizogenesis, as well as, for the speed of metabolic reactions, which will culminate in fast or slow cell differentiation and subsequent root cell formation, in addition to tissue hydration.

Auxins interfere in the establishment of rhizogenesis more quickly with the indolebutyric acid (IBA) being more used, as it has lower phytotoxicity (PEÑA et al., 2012), compared to other auxins. However, depending on the endogenous concentration of auxins in tissues, their use can be harmful. Fochesato et al. (2006) by evaluating the obligation of IBA in cuttings of sorghum (*Laurus nobilis* L.) obtained 4000 mg L^{-1} phytotoxicity in the cuttings, which generated a lower number of formed roots. The same was reported by Franzon et al. (2004) in herbaceous cuttings of feijoa (*Acca sellowiana* Berg) using doses higher than 4000 mg L^{-1} of IBA. Therefore, such a test with pomegranate is important, especially if related to the time of collection.

The objective of this work was to analyze the feasibility of adopting the propagation technique by cuttings for pomegranate, seeking to test the best time of collection, substrate and concentration of indolebutyric acid (IBA).

MATERIAL AND METHODS

The works have been carried out at the Teaching and Research Unit Nursery for the Production of Horticultural Seedlings, at the Federal Technological University of Paraná (UTFPR), *Campus* Dois Vizinhos (PR). The cuttings were collected from matrix pomegranate trees existing on the premises of the aforementioned institution, in two periods, each period constituting an experiment. The first time was carried out with collection before fruiting (September) and the second, after fruiting (March). At each time, the experiment was installed in a randomized block design, in a 4 x 4 factorial scheme (IBA concentrations x substrate), with four replications of 10 cuttings per plot. The woody cuttings were prepared with 10 cm in length and 10 mm in diameter.

In the cuttings, two superficial lesions were made in the basal part, on opposite sides, removing a portion of the bark. On this basis, after injuries, IBA solutions were applied, varying in concentrations, which were 0; 1000; 2000 and 3000 mg L^{-1}. Afterwards, the cuttings were vertically buried up to 2/3 of their length in the substrates Red Latosol, medium textured sand, commercial substrate MecPlant® and a mixture between Red Latosol + sand + commercial substrate (1:1:1, v/v). The cuttings were placed in beds (3 x 2 x 0.3 m) and irrigation was performed three times a day for 30 min., by a micro sprinkler. This material was kept in a protected, screened environment, with 50% shading.

After 60 days, the percentages of rooting and cuttings with callus, number of primary shoots, leaves and adventitious roots, length of primary adventitious root and the three largest (cm), and the percentage of dead cuttings were evaluated. The data were subjected to analysis of variance and, for those that were significant, an average comparison was performed using the Duncan test (α = 0.05) for a qualitative factor and regression analysis for a quantitative factor, using the Sanest computer program (ZONTA; MACHADO, 1984). Rooting percentage data were transformed, according to *arc sine* $\sqrt{x/100}$, according to Lilliefors' normality test. The remaining data did not undergo transformation.

RESULTS AND DISCUSSION

Experiment 1: first collection

The results were significant for the substrate factor in the variable percentages of rooted and dead cuttings (Table 1) and for the length of adventitious roots, and for the three largest adventitious roots (Table 2). In the other variables this factor had no significant effect. Cuttings collected before fructification showed higher rooting with sand (91.03%), which also had lower mortality (9.37%). This greater survival was statistically similar to commercial and mixed substrates. The superiority of sand in both variables is believed to be due to its physical characteristics based on its good porosity and lower water retention. According to Tognon; Petry (2012), sand has the advantages of being used as a substrate in the propagation by cuttings due to its drainage capacity being fast. Thus, this material does not allow the accumulation of water at the stake base, which may be favorable for the formation of adventitious rhizogenesis.

TABLE 1 - Rooting percentage of dead pomegranate cuttings, according to the type of substrate used, in the collection performed before fruiting.

Substrates	Rooted cuttings (%)	Dead cuttings (%)
Red Latossol	69.62 b*	22.50 a*
Medium textured sand	91.03 a	9.37 b
Mecplant®	74.40 b	18.75 ab
Red Latossol + sand + Mecplant® commercial substrate Mix	76.44 b	13.75 ab
CV(%)	24.93	8.31

*Averages followed by the same lowercase letter in the column do not differ from each other by Duncan's Test, at 5% probability of error.

TABLE 2 - Length of the three largest adventitious roots and adventitious roots (cm) of pomegranate cuttings, according to the substrate used, in the collection carried out before fruiting.

Substrates	Length of the three largest adventitious roots (cm)	Lenght of the adventitious roots (cm)
Red Latossol	9.48 c*	6.22 b
Medium textured sand	18.90 a	11.61 a
Mecplant®	15.42 ab	6.99 b
Red Latossol + sand + Mecplant® commercial substrate Mix	12.15 bc	6.61 b
CV (%)	25.47	24.75

*Averages followed by the same lowercase letter in the column do not differ from each other by Duncan's Test, at 5% probability of error.

Pinto; Moura (2021) recommended that the substrate used for propagation by cuttings to be extremely porous, with low water retention capacity, characteristics presented by sand. Furthermore, sand also provided greater length of adventitious roots and, together with commercial sand, the three largest adventitious roots. This may be a consequence of the sand being chemically inert, causing the cuttings to direct their post-rhizogenesis reserves to greater elongation, thus aiming to seek for nutrients and, in the case of commercial substrate, for perhaps having a higher content of organic matter in its composition, which is advantageous for the growth of the cutting in a balanced way, between the aerial part and the root system.

As for the IBA usage, there was a significant effect of this factor on the percentage of cuttings with calluses (Figure 1), dead (Figure 2), on the length of the three largest adventitious roots (Figure 3) and on the number of adventitious roots (Figure 4).

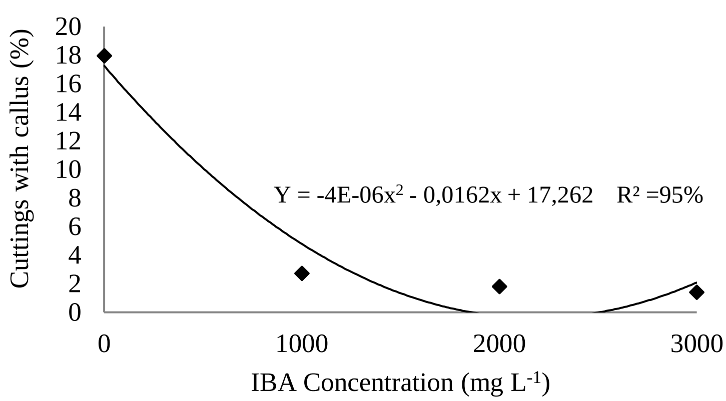

FIGURE 1 - Calogenesis percentage (%) in pomegranate cuttings, collected before fruiting, as a function of IBA concentrations.

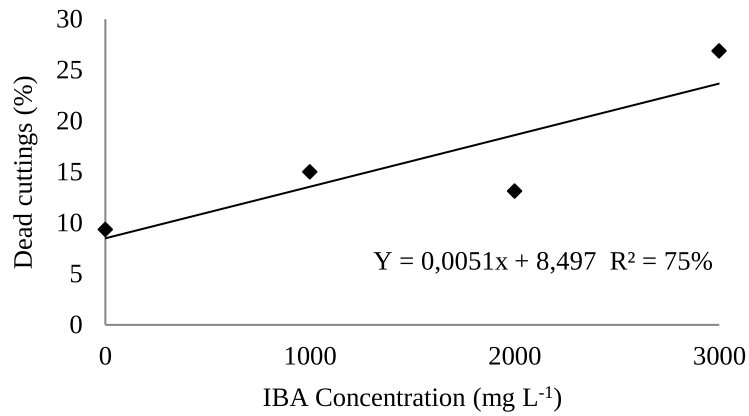

FIGURE 2 - Dead cuttings percentage (%) of pomegranate, collected before fruiting, as a function of IBA concentrations.

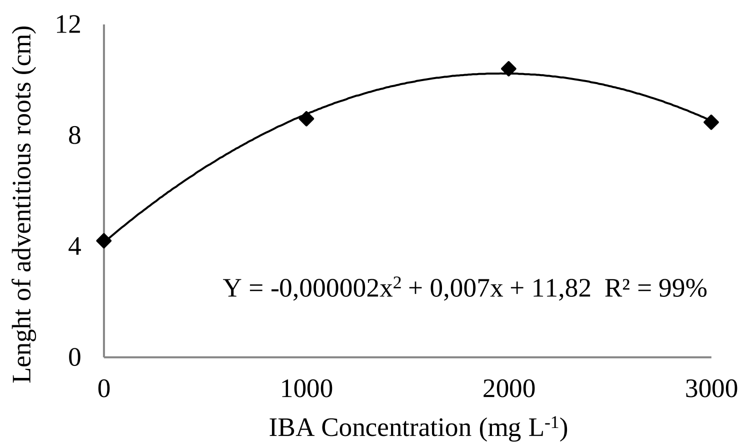

FIGURE 3 - Length of adventitious roots (cm) of pomegranate cuttings, collected before fruiting, as a function of IBA concentrations.

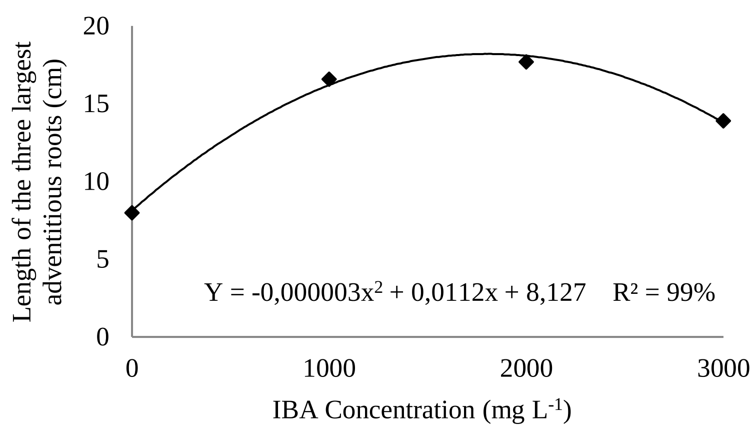

FIGURE 4 - Length of the three largest adventitious roots (cm) of pomegranate cuttings, collected before fruiting, as a function of IBA concentrations.

It was found that the percentage of cuttings with callus presented a decreasing quadratic behavior when the concentration of IBA used was increased (Figure 1). The same was not observed for obtaining rooted cuttings, which were not influenced by the tested IBA concentrations. Even though they are phenomena that occur in isolation, callus formation and adventitious rooting are influenced, in most cases, by the same factors, and the appearance of callus may be an indicative parameter of the appearance after the adventitious roots, as it indicates the occurrence of cell differentiation. In some cases, there was even an inverse effect between the percentage of rooted cuttings and those with calluses, as demonstrated in “Araçá” tree by Nachtigall et al. (1994). In the present experiment there was not this same behavior, being only observed greater callus formation in the absence of IBA.

Regarding the percentage of dead cuttings (Figure 2), an increasing linear behavior was obtained with the increase in IBA concentrations.

Such behavior may be due to the toxicity appearance in the cutting tissue by this auxin. Every species, when receiving different concentrations of IBA at the base of its cuttings respond to it, normally, in a linear way for rooting or for mortality, being this variation in response to the existing endogenous concentration. Thus, it is necessary to identify for each species the one that provides the best responses, regardless of the factors under study. Silva et al. (2009) highlighted that when using higher concentrations of auxins in the asexual propagation of “camu-camu”, they also obtained a greater number of dead cuttings with an increase in these concentrations. Betanin (2010) when applying IBA in cuttings of “corticeira-da-serra” verified a positive effect of this on the survival of the cuttings when they were treated with 3000 mg L^{-1}.

As for the length of adventitious roots and the three largest adventitious roots (Figures 3 and 4, respectively), a quadratic behavior was observed in both, with maximum points at concentrations of 1616.67 and 1750 mg L^{-1} of IBA, respectively. This demonstrates the positive action of the use of IBA on the root growth of pomegranate cuttings, and should be taken care so that the concentrations tested do not cause tissue toxicity.

Experiment 2: second collection

According to the results obtained at this time of collection, there was only a significant effect with the AIB factor for the number of leaves and sprouts (Figures 5 and 6, respectively). In both variables there was a linear effect with an increase in the concentration of AIB, assuming that this auxin provided greater stimulus for cell division and subsequent differentiation at the apex of the cutting causing such behaviors to occur. Thus, it is believed that such response at the apex caused damage to the stimulus for greater adventitious rhizogenesis in pomegranate cuttings.

In order to differentiate, and for the formation of adventitious roots it is necessary to have a favorable C/N ratio. It is assumed that, with greater sprouting responses and number of leaves with increased IBA concentration, there was a greater consumption of carbon reserves needed to obtain of such adventitious roots, the fact that at the lowest concentrations of AIB were not sufficient to stimulate such a process of differentiation and subsequent rhizogenesis, explaining these results. In addition, it is believed that because the collection was carried out after fruiting, the cuttings at that time had a lower C:N ratio, not being in a favorable condition for greater rhizogenesis, which can be confirmed by the rooting average obtained at this time (52.35%) compared to experiment 1 (78.42%). For the variables length of the primary adventitious root and the three largest adventitious roots, percentage of callus and number of adventitious roots, there was no significant effect for substrate interaction x IBA concentration and for each isolated factor.

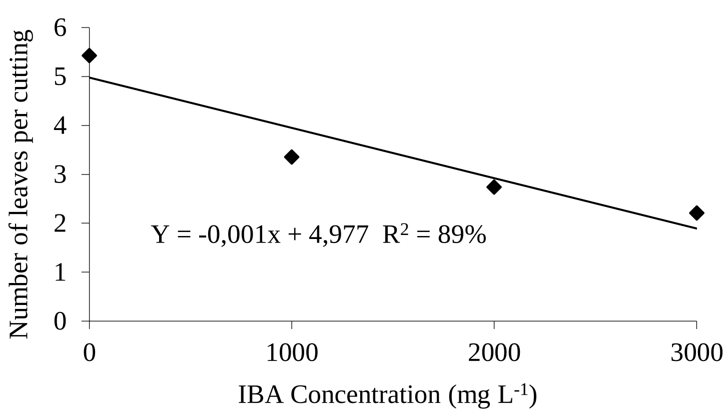

FIGURE 5 - Number of leaves per pomegranate cuttings according to the concentration of IBA used, when they were collected after fruiting.

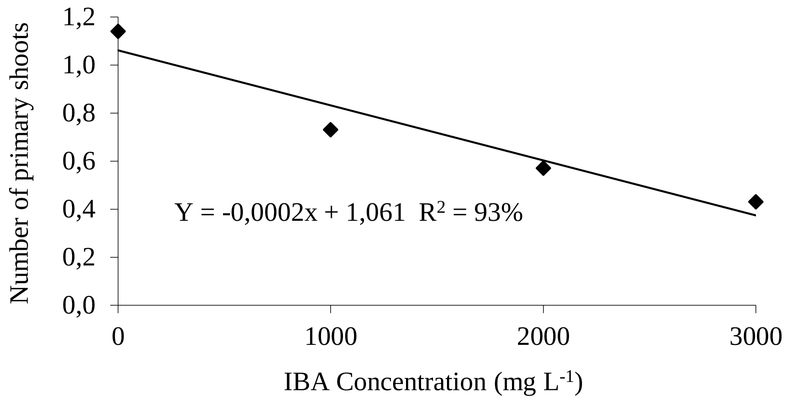

FIGURE 6 - Number of primary shoots of pomegranate cuttings according to IBA concentration when they were collected after fruiting.

In general, when analyzing the results obtained in this study, it was possible to check that the pomegranate tree can be propagated by cuttings, but in the future, trying to improve its rhizogenesis, in an attempt to obtain averages, close to 100% of rooting, other factors such as rooting, substrate heating, parent plant nutrition, other types of auxins, etc, could be tested.

CONCLUSIONS

Cutting presented itself as a potential technique to be adopted for pomegranate, using sand, and the usage of IBA at concentrations between 1600 and 1750 mg L^{-1}.

Cuttings must be collected before fruiting.

However, rhizogenesis close to 50% was achieved at the time after such phenological stage.

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