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# INDOLEBUTYRIC ACID AND ZINC IN THE ROOTING OF ROOTSTOCK SEEDLINGS OF 'CRAVO' LEMON TREES IN HYDROPONICS

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**ABSTRACT**
It was intended to evaluate the influence of indolebutyric acid (IBA) and zinc upon the rooting and development of rootstock of 'Cravo' lemon tree transplants of bare root to the modified hydroponic system in the grafting stage. Plants of rootstocks were treated by immersion of their root system into the different solutions tested for 24 hours. The experiment was arranged in a completely randomized design with seven treatments composed of IBA doses of 50, 100 and 200 mg.$L^{-1}$ and Enervig® which contains in its formulation 33.92 g.$L^{-1}$ of Zn at 60, 90 and 120 ml.$L^{-1}$, and distilled water as a control with three replications and nine plants per plot, amounting to 189 plants , 15 cm tall. The rootstocks were transplanted to the tubes and taken to the modified hydroponic system, being evaluated (1) the development of the plants and (2) the time needed for the rootstocks to reach the grafting stage, considered ideal between 5 and 6 mm in diameter. The treatment with a solution of 100 mg.$L^{-1}$ of IBA proved superior to the others in the development of height and biomass yield and tended to provide greater stem diameter development. The grafting point was obtained, on average at 70 days after transplanting and the production of Rangpur lime trees in a hydroponic system may be recommended. When the root system of the 'Cravo' lemon tree was treated with 100 mg.$L^{-1}$ solution the IBA grafting point was obtained at 45 days after transplanting.

**KEYWORDS:** Citrus Crop. Propagation. Greenhouse.

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## INTRODUCTION

The Brazilian citrus crop is the most competitive in the world, with a production of 19,569,675 tons in 2019 for orange, lemon, and tangerine. The state of São Paulo is the main Brazilian production center, with an amount estimated harvest of 14,705,608 tons in 2019.[1]

Several factors are considered when establishing a citrus orchard, however it is a consensus that the choice of seedlings of certified quality is a predominant factor in citrus growing activity. Pompeu Júnior[2] emphasizes that the rootstocks affect several characteristics of the canopy varieties such as: vigor, production and their precocity, plant tolerance to adverse climates, besides conditions that give fruits with higher quality.

Among the production methods for high quality seedlings, hydroponic cultivation has been used in several other crops, especially forest species, passion fruit, strawberry, tobacco, besides the production of leafy vegetables, fruits and ornamental plants.[3] Recently, in the modified hydroponic system proposed by Faquin & Chalfun,[4] Oliveira[5] found that the production of citrus seedlings was technically feasible and Souza et al[6] working with pear trees and peach trees, aiming to obtain seedlings under the same condition, also considered feasible to use this technique, and obtained seedlings that could be retailed and that were according to the standards required (height and diameter established by Administrative Ruling No. 37, annex –IX) within a period of four months.

In the modified hydroponic system, rootstocks are prepared and transplanted from a bare root to the tubs, causing a certain amount of stress, reflecting directly on their development. The use of plant regulators, such as synthetic auxins, has been recommended according to Bassan et al.[7] who related the use of auxins to some studies performed with citrus plants and emphasized that these plant regulators may play a major role because they are directly related to root induction and development. The micronutrient zinc also plays an important role in rooting, since it activates tryptophan, which in turn is the precursor of auxin.[8]

Thus, the present study aimed at evaluating the influence of indolebutyric acid and zinc in the rooting and development of rootstocks of Rangpur lime trees transplanted with bare root to the modified hydroponic system until the grafting point.

## MATERIAL AND METHODS

The work was performed in the greenhouse of the Hydroponics sector of the Department of Soil Science at the Federal University of Lavras (UFLA), Municipality of Lavras, MG, at geographical coordinates "21° 13' 55" S and "44° 57' 43" W, and altitude of 925 m. The climate of the municipality is of the Cwb type, according to the Köppen classification. The structure, as well as the solution used in the experiment were proposed by Faquin & Chalfun.[4] Throughout the experiment, the tubs remained in their own supports under hydroponic conditions in the greenhouse, in shallow boxes in a leveled position called trays, where they received a nutrition solution proposed by

Faquin & Chalfun,[4] until the end of the evaluations. This tray is big enough to hold the tubs and, connected to a 1000-liter reservoir of nutrient solution with circulation triggered by a timer at 15-minute intervals and regulated by a motor pump connected to the reservoir. The excess nutrient solution of the trays returns to the reservoir by gravity through its own piping. Nutrient replacement in the reservoir nutrient solution is monitored throughout the electric conductivity, and its value is adjusted daily by adding stock solution of macro and micronutrients prepared according to the aforementioned authors. The pH of the nutritive solution was kept between 5.5 and 6.5. The nutrient solution was changed periodically, at every 30 days.

The rootstock plants of 'Cravo' lemon (*Citrus limonia* L. Osbeck) used were 15 cm tall, a commercial size known as 'cavalinho'. The plants were treated by immersion of their root system into the different solutions and tested for 24 hours. The experiment was distributed in an entirely randomized design with seven treatments, comprising Indole Butyric acid (IBA) at the doses of 50, 100 and 200 mg.$L^{-1}$, and Enervig®. The formula of this product contains 33.92 g.$L^{-1}$ of Zn. It was tested at the doses of 60, 90 and 120 ml.$L^{-1}$, and distilled water as control, with three replications and 9 plants per plot, a total of 189 plants. After treatment, the rootstocks were transplanted to the tubs and taken to the modified hydroponic system.

The diameter and height of the rootstocks was evaluated every fifteen days. The stem diameter was taken 10 cm from the neck, measured with a pachymeter. The time needed for the rootstocks to reach the grafting point was also determined and considered ideal between 5 and 6 mm in diameter, and the height of the plants was measured with a ruler taken from the base of the stem to the level of the substrate to the last apical bud.

When 60% of the rootstock had reached the grafting point, which occurred 90 days after planting, five plants were sampled per treatment and their dry mass was determined. The root system and the aerial part (stem and leaves) were separated and taken to the oven at 72 °C, for 72 hours, after which they were weighed separately to calculate the biomass distribution. The total dry mass was the total of the masses of roots + aerial part. The root/aerial part dry mass ratio was calculated by the division of the dry mass of the roots by the dry mass of the aerial part (stem + leaves).

The data were submitted to analysis of variance, applying the F and Tukey tests, at 5% probability, performed using the Sisvar application. A study of polynomial regression at the 5% level was done for the quantitative variables, using the R application.[10]

## RESULTS AND DISCUSSION

The height of the rootstocks of 'Cravo' lemon, according to the analysis of variance (Table 1), showed significant interaction between treatments and periods of evaluation. After deployment it was found that there was a significant difference between the treatments and periods.

The 'Cravo' lemon tree rootstocks grew in height and obtained data adjusted to the quadratic model in all treatments of the root system studied. Throughout the evaluation period, the rootstocks of 'Cravo' lemon submitted to treatment with a solution containing 120 ml.$L^{-1}$ of Enervig®, applied to

the root system, presented shorter plants and that their height grew until approximately the 38 cm. 80$^{th}$ day, reaching the maximum value of 38 cm (Figure 1). After this time, the height of the plants remained stable, showing less development in a longer period. The other treatments presented plants with heights considered equal and higher than those of the aforementioned treatment. After 45 days of evaluation, the rootstocks of the Rangpur lime submitted to the treatments with solution containing 60 and 90 ml.L$^{-1}$ of Enervig®, proved to be equal, but statistically inferior to the control.

**Table 1:** Summary of the analysis of variance for height and diameter of the rootstock stem of 'Cravo' lemon submitted to different root system treatments, cultivated in a hydroponic system. UFLA, Lavras-MG, 2013

| Source | Degree of freedom | Mean Squares | |
|---|---|---|---|
| | | Height | Diameter |
| Treatments | 6 | 795.397 | 0.063* |
| Period | 5 | 1366.746 | 0.079* |
| Period x Treatments | 30 | 11.635* | 0.001$^{ns}$ |
| **Coefficient of variation (%)** | | 11.03 | 10.96 |
| **General average** | | 46.59 | 0.50 |

*Significant at 5% probability by the F test

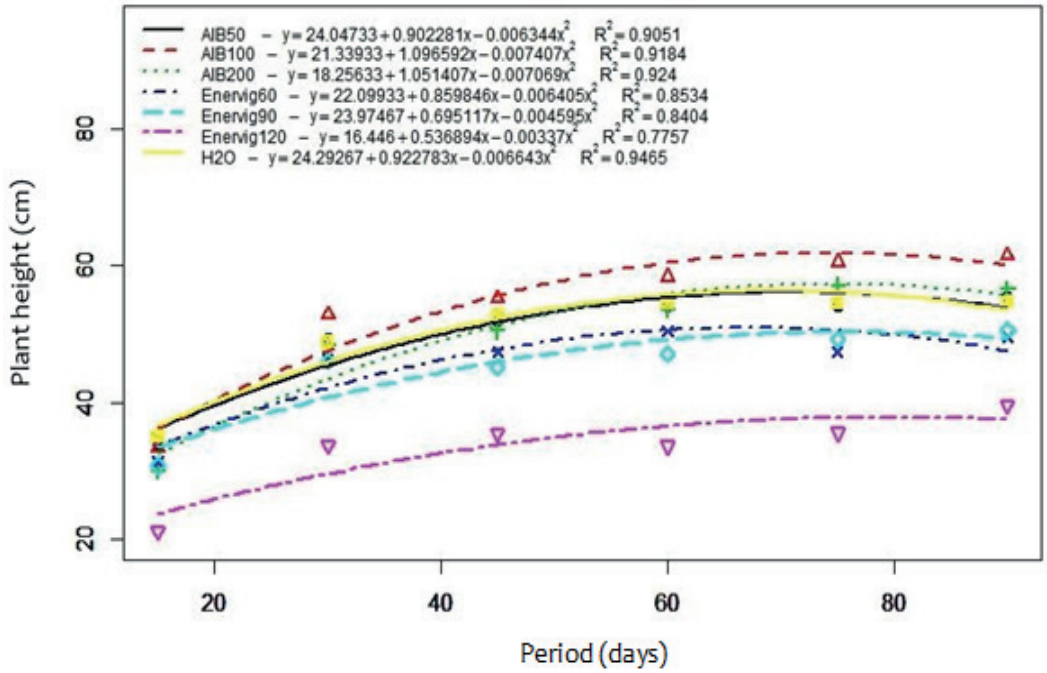


**Figure 1:** Height of 'Cravo' lemon tree rootstock submitted to different root system treatments, cultivated in a hydroponic system. UFLA, Lavras-MG, 2013.

The highest plants were obtained when the treatment with 100 mg.L$^{-1}$ of IBA was applied. Plants subject to this treatment grew in height until approximately the 74$^{th}$ day, reaching a maximum of 62 cm (Figure 1). After this time, the plant height remained stable, showing greater development in less time. The data are different from Davoglio Junior et al.[11]

who evaluated the root system and the vegetative development of citrus plants in different environments and concluded that the treatments used did not have a significant effect on the plant height. Souza et al.[12] evaluated the synergistic effect of mycorrhization of the 'Carrizo' citrange and concluded that the positive action of the synthetic auxin can only be confirmed when associated with the action of micorrhizzic fungi.

For the stem diameter, the interaction between treatments and periods of evaluation was not significant (Table 1). Thus, the two factors were studied independently.

The smaller diameters of the rootstock stem of Rangpur lime tree were obtained when the root system was treated with a solution containing 120 ml.L$^{-1}$ of Enervig®, and this treatment was inferior to the others (Table 2). The rootstocks of Rangpur lime trees submitted to treatments with solutions containing 60 and 99 ml.L$^{-1}$ of Enervig®, proved to be equal but were inferior to the control.

**Table 2:** Diameter of the rootstock stem of 'Cravo' lemon tree submitted to different root system treatments, cultivated in a hydroponic system. UFLA, Lavras-MG, 2013

| Treatments | | Diameter of Stem (cm) |
|---|---|---|
| H2O | Control | 0,50 a |
| 50 | mg.L$^{-1}$ AIB | 0,50 a |
| 100 | | 0,53 a |
| 200 | | 0,50 a |
| 60 | ml.L$^{-1}$ Enervig® | 0,46 b |
| 90 | | 0,46 b |
| 120 | | 0,35 c |
| CV (%) | | 10,96 |
| General average | | 0,47 |

Means followed by the same letters are not different through the Tukey test at 5% probability.

There was no significant difference between the IBA based treatments and the control (Table 2), but when the treatment was applied with 100 mg.L$^{-1}$ of IBA, the rootstocks showed a tendency to superiority for the stem diameter and based on the results shown in Table 3. Then, it can be inferred that, in general, this treatment provided a trend for greater development of the 'Cravo' lemon tree rootstocks.

These results agree with those of Souza et al.[12] that achieved a significant difference for the diameter of the neck of the plants evaluated when the treatment with IBA was associated with the action of the mycorrhizal fungi in Carriszo citrange. They emphasized the possibility of performing the grafts earlier and thus shortening the seedling production period. In general, the model that best represented the behavior of plant diameter in the different period of evaluation was linear, with an higher adjustment ($R^2$= 97.19%) and there was an addition of approximately 0.00216 cm for every day of evaluation (Figure 2).

It was found that the graft point, 5 mm, taken 10 cm from the plant neck, was obtained on average 70 days after the root system of 'Cravo' lemon was treated. However, the treatment with 100 mg.L$^{-1}$ of IBA allowed obtaining plants that were appropriate for grafting, with a minimum stem diameter of 5 mm at 45 DAT (days after transplanting) (Figure 3).

This result was different from those obtained by Serrano et al.[14] and Grassi Filho et al.[15]who, analyzing the rootstock production of 'Cravo' lemon trees, and obtained an

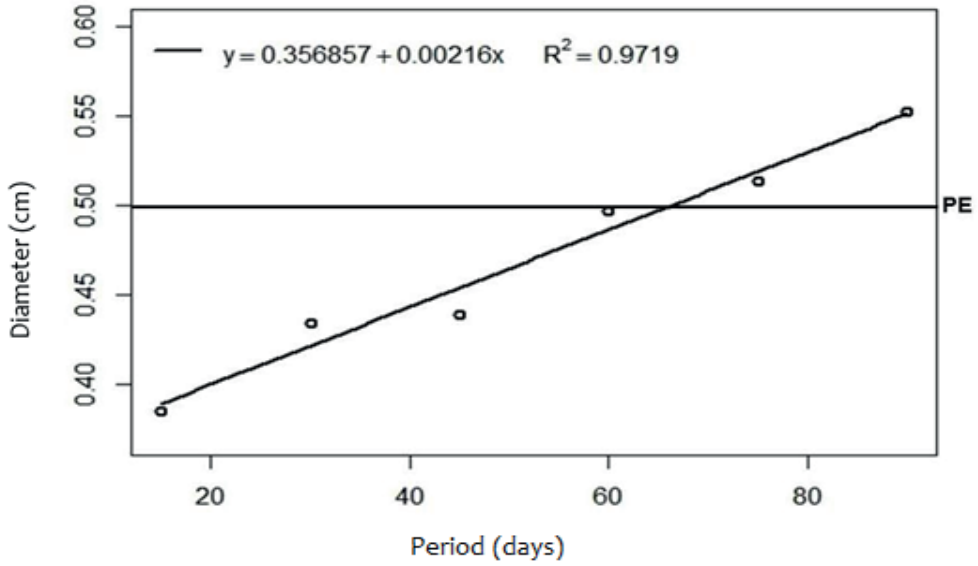


**Figure 2:** Mean diameter of the rootstock stem of 'Cravo' lemon trees submitted to different root system treatments. DAT. Days after transplantation. PE: Grafting point. UFLA, Lavras-MG, 2013.

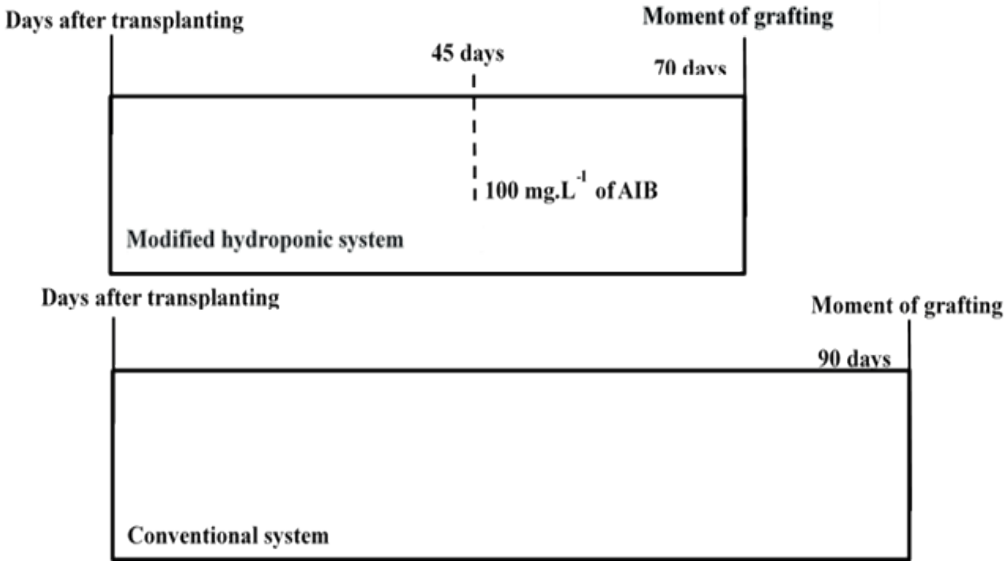


**Figure 3:** Chronology (days after transplantation) of the citric rootstock production in a Modified Hydroponic System (UFLA) and a Conventional one[13]. UFLA, Lavras-MG, 2013.

average grafting point at 154 and 150 DAT, respectively. In studies on pear trees and peach trees, under the similar conditions as herein, Souza et al.[16] managed to anticipate to 163 and 148 DAT, respectively, the time needed to obtain the rootstocks of these species.

When the dry mass of the roots wasanalyzed and the relationship between this variable and the dry mass of the aerial part, it was found that there was no statistical it was found that there was no statistical difference among the treatments applied to rootstocks of 'Cravo' lemon trees (Table 3),

and, independently of the treatment used on their root system, re-rooting was not compromised by cultivation in a modified hydroponic system. For the total dry mass of the plants, the treatment with a solution of 100 mg.L$^{-1}$ of IBA was superior, but not statistically different from the other doses of this auxin, as well as of the control. In an experiment that investigated whether applying IBA would have an effect on the plant-arbuscular mycorrhizae symbiosis, Souza et al.[12] found that the doses of IBA did not change the dry weight of the aerial part and the roots of the non-mycorrhizal plants.

**Table 4:** Dry Mass of Root (g) (DMroot) and aerial part (DMap), Total Dry Mass (g) (DMtotal) and Ratio of Dry Mass of Root to aerial part (DMroot/DMap) of 'Cravo' lemon tree, submitted to different root system treatments, cultivated in a hydroponic system. UFLA, Lavras-MG, 2013

| Treatments | | DMroot* | DMap | DMtotal | DMroot*/ DMap |
|---|---|---|---|---|---|
| H2O | Control | 2,17 a | 34,54 ab | 40,45 abc | 0,17 a |
| 50 | mg.L$^{-1}$ AIB | 2,89 a | 34,71 ab | 43,14 abc | 0,24 a |
| 100 | | 3,38 a | 46,16 a | 58,06 a | 0,25 a |
| 200 | | 2,95 a | 38,15 ab | 46,90 ab | 0,23 a |
| 60 | mg.L$^{-1}$ Enervig® | 2,08 a | 28,59 bc | 33,04 bc | 0,16 a |
| 90 | | 2,47 a | 28,64 bc | 34,80 bc | 0,21 a |
| 120 | | 1,89 a | 18,00 c | 21,63 c | 0,21 a |
| CV (%) | | 24,75 | 18,07 | 20,36 | 35,13 |
| General average | | 2,55 | 32,68 | 39,72 | 0,21 |

Means followed by the same letters in the columns are not different through the Tukey test at 5% probability. * The data concerning the Dry Mass of Root were transformed by √x.

The highest values for the variable dry mass of the aerial part were obtained when the root system of the 'Cravo' lemon tree was treated with a solution of 100 mg.L$^{-1}$ of IBA. This treatment statistically surpassed the doses of Enervig®, but proved to be equivalent to the other doses of IBA and the control (Table 3). The results ascribed to the use of IBA are different from those obtained by Bassan et al.[7] who concluded that IBA did not show a significant effect on any of the variables studied.

The use of Enervig® based solution at a concentration of 120 ml.L$^{-1}$ presented the worst results to produce biomass in 'Cravo' lemon tree rootstock. This may be attributed to the excessive induction of synthesis of endogenous auxin, indole acetic acid (IAA), since zinc is a catalyst of tryptophan synthesis which is a precursor of IAA, causing an inhibitory effect on the root induction. Paes et al.[17] analyzing the effect of synthetic auxins on the rooting of the ornamental specie and using the Ouro Flora Enraizador® product whose composition is 4% zinc sulfate as additional treatment did not recommend using it because of high mortality and reduced percentage of plant rooting.

## CONCLUSIONS

The treatment of the root system of the 'Cravo' lemon tree with a solution of 100 mg.L$^{-1}$ of IBA was superior to the others as to the development of height and the production of biomass and tended to provide a greater development of the stem diameter. Thus, it can be used to produce this rootstock.

The grafting point was obtained, on average, 70 days after transplanting, and the production of the 'Cravo' lemon plants in a

hydroponic system can be recommended. When the root system of the 'Cravo' lemon plant was treated with a solution of 100 mg.L$^{-1}$ of IBA the grafting point was obtained 45 days after transplantation.

## ÁCIDO INDOLBUTÍRICO E ZINCO NO ENRAIZAMENTO DE MUDAS DE PORTA-ENXERTOS DE LIMOEIRO 'CRAVO' EM HIDROPONIA

**RESUMO**

No sistema hidropônico modificado os porta-enxertos são preparados e repicados de raiz nua para os tubetes, podendo ocasionar estresse. O uso de fitorreguladores, bem como, de cofatores de enraizamento, a exemplo de auxinas sintéticas e do zinco, tem sido recomendado, podendo reduzir esse estresse, auxiliando no enraizamento. Objetivou-se, com o presente trabalho, avaliar a influência do Ácido Indol Butírico do Zinco no reenraizamento e desenvolvimento de porta-enxertos de Limoeiro 'Cravo' repicados com raiz nua para o sistema hidropônico modificado até o ponto de enxertia. Foram utilizadas plantas de porta-enxertos de Limoeiro 'Cravo' (*Citrus limona* L. Osbeck) com 15 cm de altura. As plantas foram tratadas através de imersão do sistema radicular destas nas diferentes soluções testadas durante 24 horas. O experimento foi disposto em delineamento inteiramente casualizado, com sete tratamentos, composto do Ácido Indol Butírico (AIB) nas doses de 50, 100 e 200 mg.L$^{-1}$, e do produto Enervig®, que contém em sua formulação 33,92 g.L$^{-1}$ de Zn, nas doses de 60, 90 e 120 ml.L$^{-1}$) e água destilada, como testemunha, com três repetições e 9 plantas por parcela, totalizando 189 plantas. A seguir, os porta-enxertos foram repicados para os tubetes e levados ao sistema hidropônico modificado. Foram avaliados: (1) o desenvolvimento das plantas e (2) o tempo necessário para os porta-enxertos atingirem o ponto de enxertia, considerado ideal entre 5 e 6 mm de diâmetro. O tratamento do sistema radicular de limoeiro 'Cravo' com solução de 100 mg.L$^{-1}$ de AIB foi superior aos demais no desenvolvimento em altura e na produção de biomassa e tendeu a proporcionar um maior desenvolvimento em diâmetro de caule. Assim, pode ser empregado na produção desse porta-enxerto. O ponto de enxertia foi obtido, em média, aos 70 dias após a repicagem, podendo ser recomendada a produção de limoeiro 'Cravo' em sistema hidroponia. Quando se tratou o sistema radicular de limoeiro 'Cravo' com solução de 100 mg.L$^{-1}$ de AIB o ponto de enxertia foi obtido aos 45 dias após a repicagem.

**PALAVRAS-CHAVE:** Citricultura. Propagação. Ambiente Protegido.

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