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Presence of leaves and use of IBA in adventitious rooting, chlorophyll fluorescence, and root ontogeny of 'Fepagro C 13' and 'Trifoliata' cuttings

Presença de folhas e uso de AIB no enraizamento adventício, fluorescência da clorofila e ontogenia radicular em estacas de 'Fepagro C 13' e 'Trifoliata'

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Highlights

Leaves influence rooting in 'Fepagro C 13' and 'Trifoliata' cuttings.
The use of IBA influences the photochemical efficiency of leaves of cuttings.
'Fepagro C 13' and 'Trifoliata' do not have an anatomical barrier to rooting.

Abstract

Cutting is proposed as an alternative method for the propagation of citrus rootstocks to obtain clonal material especially in varieties with a low rate of polyembryony and possibly reduce the seedling formation period. The aim of this study was to examine the influence of leaves and the application of indolebutyric acid (IBA) on adventitious rooting and chlorophyll fluorescence as well as to characterize root ontogeny in 'Fepagro C 13' and 'Trifoliata' citrus rootstock cuttings. Semi-hardwood cuttings were collected in December 2018 and subjected to the following treatments: presence or absence of leaves, with or without application of IBA (4000 mg L^{-1}). Over 92 days in a greenhouse environment, leaf abscission percentage, cutting mortality, rooting percentage, and number of roots per cutting were evaluated. Additionally, initial (Fo), maximum (Fm), and variable (Fv) chlorophyll fluorescence, photochemical efficiency (Fv/Fm), and Fv/Fo ratio were analyzed. Tissue samples from the base of the cutting were collected for the anatomical study. The presence of leaves on 'Fepagro C 13' and 'Trifoliata' cuttings is essential for adventitious rooting. The use of auxin influences the photochemical efficiency of leaves on the cuttings. In 'Trifoliata' cuttings, adventitious roots originate from the vascular cambium.

Key words: Photochemical efficiency. Rootstock. Vascular cambium. Vegetative propagation.

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Resumo

A estaquia é proposta como método alternativo para a propagação de porta-enxertos de citros, visando a garantia de obtenção de material clonal, especialmente em variedades com baixa taxa poliembriônica, e uma possível redução no período para a obtenção das mudas. O objetivo deste estudo foi avaliar a influência das folhas e da aplicação de ácido indolbutírico (AIB) sobre o enraizamento adventício, fluorescência da clorofila e caracterizar a ontogenia radicular em estacas dos porta-enxertos citrangeiro 'Fepagro C 13', e 'Trifoliata'. Estacas semilenhosas foram coletadas em dezembro de 2018 e submetidas aos tratamentos: presença ou ausência de folhas com aplicação ou não de AIB (4000 mg L^{-1}). Ao longo de 92 dias em casa de vegetação foram avaliados os percentuais de abscisão foliar, mortalidade de estacas, percentual de enraizamento e número de raízes por estaca. Também os dados da fluorescência inicial (Fo), máxima (Fm) e variável (Fv) da clorofila, a eficiência fotoquímica (Fv/Fm) e a razão Fv/Fo foram avaliados. Amostras de tecido da base da estaca foram coletadas para a realização do estudo anatômico. A presença das folhas nas estacas de 'Fepagro C 13' e 'Trifoliata' é essencial para o enraizamento adventício. O uso de auxina influencia a eficiência fotoquímica nas folhas das estacas. Em estacas de 'Trifoliata' as raízes adventícias originam-se a partir do câmbio vascular.

Palavras-chave: Câmbio vascular. Eficiência fotoquímica. Porta-enxertos. Propagação vegetativa.

Introduction

Propagation by cuttings is an alternative method for the production of citrus rootstock seedlings, which are traditionally propagated through apomictic seeds (Souza & Schafer, 2018). This method is aimed at ensuring the production of clonal material, especially in varieties with a low rate of polyembryony, and possibly reducing the seedling development period (Oliveira et al., 2014; Sarmiento et al., 2016), particularly under subtropical conditions, characteristic of the southern region of Brazil. Additionally, cuttings can be an alternative in view of the lower availability of seeds on the market due to the occurrence of greening (*Candidatus Liberibacter* spp.), which negatively impacts the physical and physiological quality of rootstock seeds. Plants infected by the bacterium produce fruits with reduced weight and size, whose seeds exhibit lower viability and germination rates and seedling emergence potential (Morelli, 2016).

Several factors influence the adventitious rooting of cuttings in woody species, including inherent characteristics of the parent plant and environmental aspects. These factors also include the endogenous content of phytohormones and carbohydrates, the nutritional and water status of the parent plant, the type of cutting, the ontogenetic and physiological age of the material, the collection period, temperature, humidity, incidence of light, among others (Li et al., 2009; Pijut et al., 2011).

In this respect, the presence of leaves and the application of synthetic auxin are factors that can influence root formation. Leaves act as a source of endogenous auxin and photoassimilates for root development. However, depending on the species and greenhouse conditions, they can also favor the dehydration of the cuttings. In contrast, the exogenous application of auxin can stimulate and increase the formation of adventitious roots, and the response to this application varies widely depending on the species and the type and concentration of auxin used (Hartmann et al., 2017).

From the collection of propagation material and production, including the period in the greenhouse until rooting, cuttings are exposed to environmental changes such as in temperature, luminosity, and humidity, which can affect their photosynthetic apparatus. As a result, alterations take place in the transfer of electrons between photosystems I and II, photochemical complexes in the thylakoid membrane of chloroplasts responsible for absorbing light energy in photosynthesis and thus acting on the root formation process. These changes and their effects can be evaluated based on the fluorescence of chlorophyll a. The technique makes it possible to evaluate the photochemical efficiency of photosystem II (PSII) and the overall physiological state of plants (Baker & Rosenqvst, 2004) in a non-destructive and fast manner to detect changes in the photosynthetic activity of leaves in response to several types of stress (Martinazzo et al., 2012).

Studies have related the site of origin of root primordia to the rooting capacity of species and suggested that, in easy-to-root species, root primordia originate in the cambium or adjacent cells, whereas in difficult-to-root species, root primordia develop from the dedifferentiation of parenchymatic cortical cells in response to the injury generated in the making of the cuttings (Steffens & Rasmussen, 2016; Hartmann et al., 2017).

In Rio Grande do Sul, Brazil, ‘Trifoliata’ (*Poncirus trifoliata* (L.) Raf) is the main rootstock used, which is employed exclusively on 74% of citrus farms in the state (Sulzbach et al., 2016). This preference is because, in addition to producing fruits with excellent qualitative traits, it exhibits high resistance to cold, to diseases such as tristeza and gummosis, and to the nematode *Tylenchulus semipenetrans,* and is tolerant to citrus sudden death. The ‘Fepagro C 13’ rootstock, a hybrid between ‘Pêra’ orange (*Citrus sinensis* (L.) Osb.) and *P. trifoliata,* is an important option for diversifying the genetic base in orchards, especially under the soil-climatic conditions of the southern region of Brazil. Obtained in 1956 by researcher Carlos M. M. Dornelles, it was registered as a cultivar in 2007 (Gonzatto et al., 2018). The rootstock induces the formation of medium-sized, early-producing plants with great longevity. It also induces the production of good-quality, late-maturing fruits. It is tolerant to tristeza, xyloporosis, and citrus sudden death and resistant to *Elsinoe fawcettii* and *T. semipenetrans* and its resistance to gummosis is considered medium. It is also highly tolerant to frost, but not very tolerant to drought and waterlogging, which makes it recommended for sandy soils (Oliveira et al., 2008).

Given the foregoing, this study was developed to evaluate the influence of leaves and the application of synthetic auxin on adventitious rooting and metabolic responses of semi-hardwood cuttings of citrus rootstocks ‘Fepagro C 13’ and ‘Trifoliata’. Additionally, the study proposes to characterize root formation in the different rootstocks, observing stem anatomy and root ontogeny.

Materials and Methods

The study was carried out from December 2018 to March 2019 on the premises of the Department of Horticulture and Forestry (DHS) at the Faculty of Agronomy of the Federal University of Rio Grande do Sul (UFRGS), located in Porto Alegre - RS, Brazil (30º29' S and 51º06' W).

Semi-hardwood branches (approximately 1 m in length) of citrus rootstocks 'Fepagro C 13' and 'Trifoliata' were collected from the Citrus Germplasm Collection at the Experimental Agronomic Station (EEA-UFRGS, 30°05'22" S, 51°39'08" W), in the municipality of Eldorado do Sul - RS, Brazil (Km 146, BR 290). After collection, the branches were moistened, packed in polyethylene bags, and transported to the experimental site. Cuttings nine to twelve centimeters in length were made from the apical portion of the branches. At the base of the cuttings, using a scalpel blade, two longitudinal and opposite sections (approximately 1 cm long) were made and the phloem was removed to increase the area of exposure of the vascular cambium.

The treatments consisted of the presence or absence of leaves and the application or non-application of synthetic auxin on the cuttings. In the cuttings whose leaves were maintained, two mature leaves were kept in their upper portion. Cuttings subjected to auxin application were submerged in a solution of indolebutyric acid (IBA) at a concentration of 4000 mg L^{-1} for 10 s. The solution was obtained by dissolving indole-3-butyric acid ($C_{12}H_{13}NO_2$) AR in 0.5M KOH.

After the treatments were applied, the cuttings were placed in expanded polystyrene trays (12 cm high × 5 cm wide) filled with carbonized rice husk substrate and transferred to a greenhouse with intermittent mist irrigation (15 s of misting at 4-min intervals during the day (07h00 to 19h00) and 15-min intervals during the night (16h00 to 07h00)). The total time the cuttings spent in the greenhouse was 92 days. Throughout this period, the average temperature in the rooting environment was 28.7 ºC and relative humidity varied between 70 and 91%.

The experiment was laid out in a randomized block design with four replications in a triple 2^3 factorial arrangement (two rootstocks, presence/absence of leaves, with/without auxin application). Each plot consisted of 18 cuttings, totaling 576 cuttings in the trial.

Rooting variables

During the greenhouse period (at 25, 46, 61, 78, and 92 days after staking), the leaf abscission percentage (number of cuttings with leaf fall), mortality (number of dead cuttings), and rooting percentage (number of cuttings with roots ≥ 1.0 cm) of the cuttings and the average number of roots formed per cutting were measured. On each date, three cuttings of each treatment were evaluated per block, totaling 12 cuttings per treatment; and at the end of the experiment six cuttings were evaluated, totaling 24 cuttings per treatment.

Chlorophyll fluorescence

Chlorophyll fluorescence was evaluated using a fluorimeter (OS1-FL Modulated Fluorometer, Opti-Sciences Inc.). The following variables were evaluated: initial fluorescence (Fo); maximum fluorescence

(Fm); variable fluorescence (Fv), calculated as the difference between Fm and Fo; maximum photochemical quantum efficiency of photosystem II (Fv/Fm); and Fv/Fo ratio. These evaluations were performed on the parent plants, in the field, the day before cuttings were collected (period zero) and at the beginning (1st day), during (50 days), and end (83 days) of the greenhouse period. The evaluations were carried out on four random mature leaves per block, totaling 16 leaves per treatment. The measurements were performed between 02h00 and 04h00 under dark-adapted conditions.

Anatomical evaluation

On each evaluation date (25, 46, 61, 78, and 92 days after staking), sample fragments (1-2 mm thick) were collected from the base of the cuttings of the two rootstocks with the presence of leaves and application of auxin. The samples were fixed in McDowell and Trump (1976) solution (composition: 1% glutaraldehyde, 4% formaldehyde in 0.1M phosphate buffer; pH 7.2) and dehydrated in a series of solutions with increasing concentration of ethyl alcohol (10, 20, 30, 40, 50, 60, 70, 80, 90, 100%). Subsequently, the samples were impregnated in plastic resin with a low melting point to make the blocks for sectioning in a Leica rotary microtome. Sections (4-6 µm thick) were stained in 0.05% Toluidine Blue (O'Brien & McCully, 1981) and observed under light microscopy. The images were analyzed and recorded using a Leica DMR microscope with an AxionCam HRC Zeiss digital camera and the image capture program Axionvision SE 64 v. 4.9.1; and the Leica M165 FC stereomicroscope with a Leica DFC 500 digital camera and LAS software version 4.1. Anatomical traits of the rootstock stem and the region of root formation (ontogenesis) were observed and a descriptive analysis was performed.

Statistical analysis

Rooting variables data were subjected to analysis of variance and means were compared by Tukey's test at 5% probability, with the percentages of leaf abscission, mortality, and rooting of the cuttings transformed by the equation $\sqrt{(x+1)}$. When significant differences were detected for the variables, a polynomial regression analysis was undertaken for the different evaluation dates. Fluorescence data were subjected to analysis of variance and compared by Tukey's test at 5% probability. Statistical analyses were performed using the Sisvar (vs. 5.6) (Ferreira, 2011) and Minitab® (vs. 19) statistical programs.

Results and Discussion

Rooting variables

Only the rootstock factor affected leaf abscission in the cuttings, unlike auxin application (Table 1). *Poncirus trifoliata* cuttings showed greater leaf abscission, which reached 50% at the end of the rooting period and was higher than that observed in 'Fepagro C 13' cuttings, which reached 22%. During the greenhouse period, the cuttings of both rootstocks showed a linear increase in leaf abscission. For 'Fepagro C 13', the highest abscission rate was observed between 61 and 78 days after staking (8%), whereas for 'Trifoliata' it occurred mainly up to 46 days (13%) (Figure 1A).

Table 1

Analysis of variance for the parameters of leaf abscission (LA), mortality (M), and rooting (R) percentages and average number of roots (NR) in 'Fepagro C 13' and 'Trifoliata' cuttings with presence/absence of leaves, treated or untreated with auxin (4000 mg L^{-1} of IBA), after 92 days in a greenhouse

Source of variation	DF	LA	DF	M	R	NR
Rootstock (RS)	1	11.63*	1	0.08^{ns}	62.96*	19.11*
Leaves (L)	-	-	1	82.75*	46.16*	22.46*
Auxin (A)	1	0.78^{ns}	1	0.96^{ns}	20.06*	13.89*
RS × L	1	0.18^{ns}	1	0.04^{ns}	5.66*	0.02^{ns}
RS × A	-	-	1	0.31^{ns}	0.04^{ns}	0.93^{ns}
A × L	-	-	1	0.33^{ns}	0.04^{ns}	0.36^{ns}
RS × A × L	-	-	1	0.21^{ns}	13.96*	2.59^{ns}
Block	3	0.37^{ns}	3	2.21^{ns}	2.31^{ns}	0.21^{ns}
Error	9		21			
CV%		28.51		34.67	31.92	28.13

*significant at 5% probability by the F test; ns = not significant ($p \geq 0.05$).

The absence of leaves on the cuttings significantly influenced cutting mortality, but there was no effect of rootstock or auxin application (Table 1). Cuttings with no leaves showed a quadratic behavior for mortality during the greenhouse period, with the highest rate of increase observed between 46 and 61 days (14%), reaching 30% at 92 days. On the other hand, in the cuttings on which the leaves were kept, mortality was only 2% (Figure 1B), demonstrating that the rooting environment conditions were adequate for the process. Therefore, the maintenance of leaves is essential for the survival of cuttings during the greenhouse period.

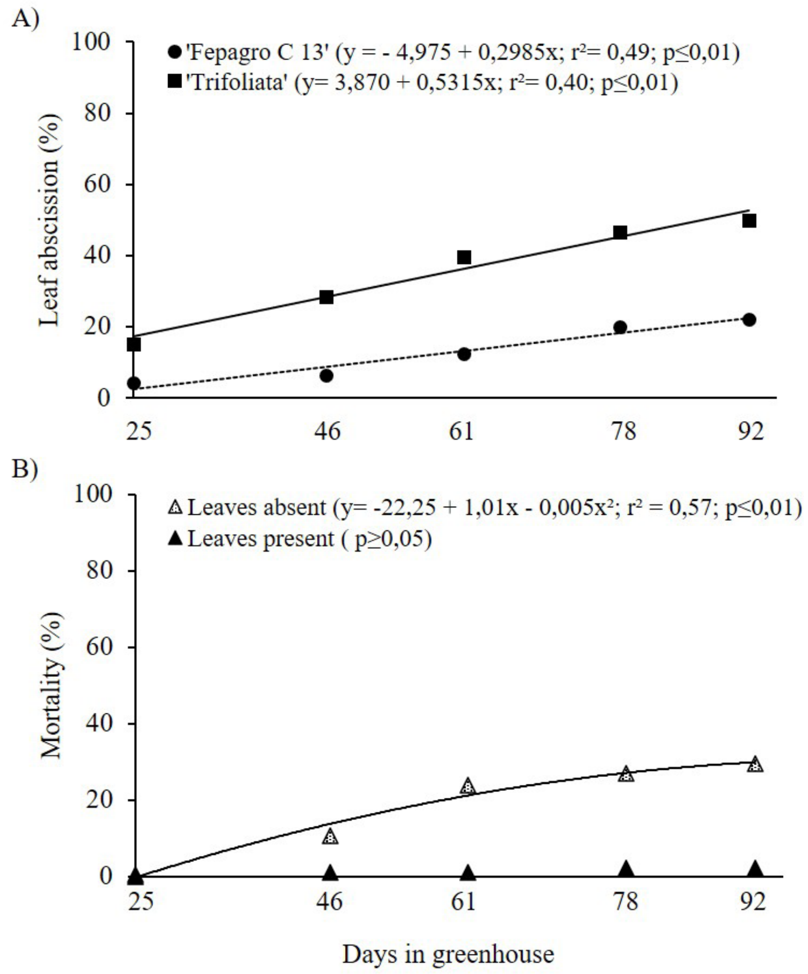

Figure 1. Percentages of leaf abscission (A) and mortality (B) in cuttings of 'Fepagro C 13' and 'Trifoliata' rootstocks with and without leaves, over 92 days in a greenhouse.

The rootstock, the presence of leaves, and the application of auxin influenced the rooting of the cuttings, which exhibited a significant triple interaction (Table 1). For both rootstock varieties, cuttings from the control treatment (without leaves and without the use of auxin) showed incipient root formation, less than 2% (Figure 2). Leafless 'Fepagro C 13' cuttings treated with auxin showed 30.5% rooting, with a significant linear behavior. Cuttings of the same variety with leaves, without auxin, showed 58% rooting, with a linear rooting behavior throughout the period. Cuttings with leaves that were treated with auxin exhibited a similar rooting percentage of 62.5%, with a quadratic rooting behavior. At 25 days after staking, 6% of the cuttings in this treatment already showed root formation, whereas in the others it occurred later. The period of greatest increase in rooting occurred after 61 days in the greenhouse.

In the 'Trifoliata' rootstock, both the cuttings with leaves untreated with auxin and the leafless ones treated with auxin showed incipient rooting (< 1.5%). Only the cuttings with leaves treated with auxin showed rooting

(30%). Compared with ‘Fepagro C 13’, the ‘Trifoliata’ cuttings show slower rooting, and the beginning of root formation was observed only in the evaluation carried out after 46 days in the greenhouse, with a greater increase seen after 78 days.

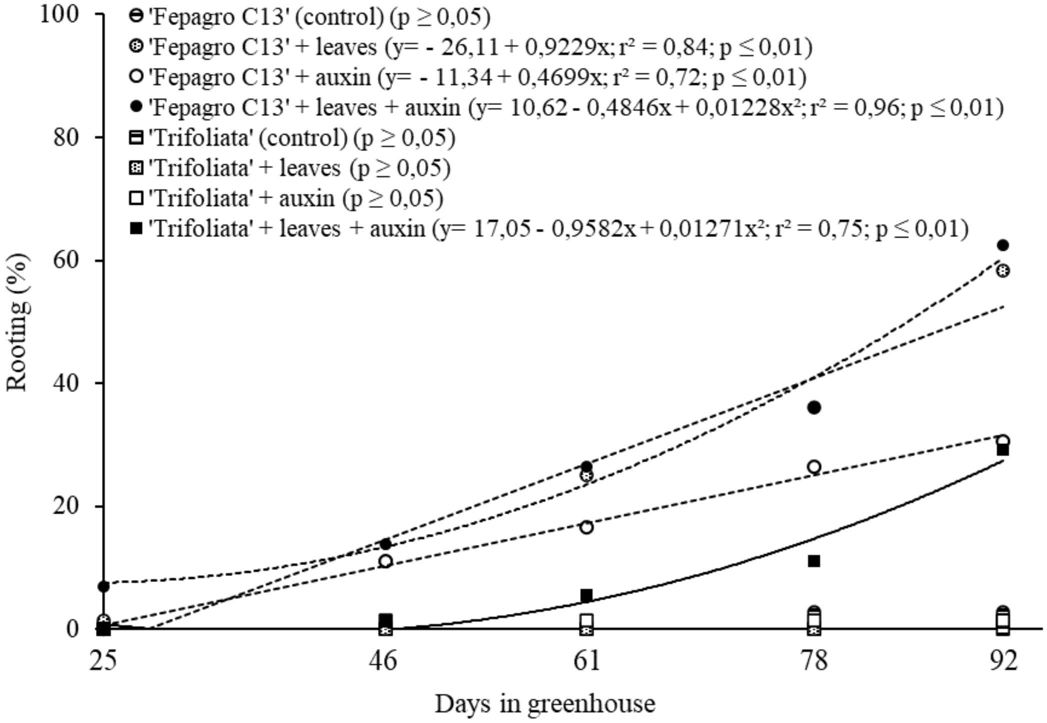

Figure 2. Rooting percentage in semi-hardwood cuttings of ‘Fepagro C 13’ and ‘Trifoliata’ rootstocks with and without leaves, treated or untreated with exogenous auxin (4000 mg L^{-1} of IBA), over 92 days in a greenhouse.

The average number of roots varied with the rootstock, the presence of leaves, and the use of auxin, but there was no interaction between the factors (Table 1). Between the rootstocks, cuttings of ‘Fepagro C 13’ had a higher number of roots than those of ‘Trifoliata’ (Figure 3). The presence of leaves and the application of auxin resulted in the formation of a greater number of roots than absence of leaves and lack of auxin application (Figure 3).

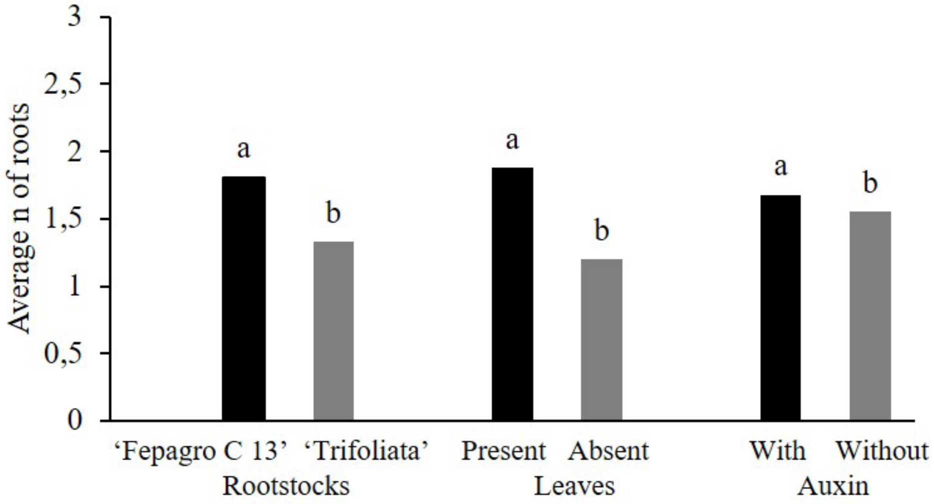

Figure 3. Average number of roots in cuttings of 'Fepagro C 13' and 'Trifoliata' rootstocks with presence/absence of leaves and treated or untreated with auxin (4000 mg L^{-1} of IBA), after 92 days in a greenhouse.

These results show that maintaining the leaves is essential for the survival and rooting of the cuttings of the evaluated rootstocks, in addition to allowing the formation of more roots. However, the behavior regarding auxin application differed between the rootstocks. These differences are expected, since the rooting ability varies between citrus genotypes (Oliveira et al., 2014). For 'Fepagro C 13', the treatment with auxin did not significantly influence rooting percentage, but anticipated the rooting process in relation to the other treatments and induced the formation of a greater number of roots. On the other hand, in 'Trifoliata' cuttings, the presence of leaves in isolation did not provide root formation, requiring association with the use of auxin. Even so, the rooting percentage observed in the latter was low (30%), which, in this case, may be related to the high leaf abscission observed throughout the greenhouse period (50%), especially in the initial phase after staking. Leaves and buds are sites of endogenous auxin synthesis and a source of carbohydrates and other photoassimilates for the base of the cutting (Hartmann et al., 2017). Thus, the early leaf fall may have contributed to the lower rooting of the cuttings, preventing the carbohydrates necessary for the rooting process from being translocated from the leaves to the cuttings in sufficient time (Denaxa et al., 2012).

The obtained results may be linked to the occurrence of a period of lower water availability for the parent plants, which took place prior to the collection of propagating material. According to data from the meteorological station installed at the site, the accumulated precipitation in the 20 days before the collection was only 56 mm, of which 25 mm occurred on the day immediately before collection. In this way, the water status of the parent plants may have been affected, thereby influencing the behavior of the cuttings in the greenhouse, including their leaf abscission and rooting potential. This was especially true for rootstock 'Trifoliata', a deciduous species highly sensitive to water deficit.

Chlorophyll fluorescence

Table 2 shows the results of analysis of variance for the different chlorophyll a fluorescence parameters evaluated. Initial fluorescence (Fo) and maximum fluorescence (Fm) varied with rootstock and evaluation date, with a significant interaction between the factors. On the other hand, for photochemical efficiency (Fv/Fm) and Fv/Fo ratio, there was an effect of rootstock and evaluation date and a significant interaction both between rootstock and evaluation date and between auxin application and evaluation date.

Initial fluorescence represents fluorescence when quinone A (QA), the primary electron acceptor of PSII, is fully oxidized and the reaction center is open, preceding the activation of photochemical reactions. The increase in Fo reflects the destruction of the PSII reaction center or the decrease in the ability to transfer the excitation energy from the antenna to the reaction center (Baker & Rosenqvst, 2004). In field plants, 'Fepagro C 13' showed higher Fo than 'Trifoliata' (Figure 4A), a difference that may be linked to genetic and/or environmental variations. At the establishment of the experiment (day 1), there was an increase in Fo in both rootstocks, which showed similar values. This was probably due to the stress caused by the collection of propagation material, followed by the displacement and implementation of the experiment. Nevertheless, throughout the greenhouse period, 'Fepagro C 13' cuttings recovered their initial condition and even reduced Fo below the initial condition of plants in the field, reaching 182 electrons quantum^{-1}. On the other hand, 'Trifoliata' cuttings showed impairment. In the evaluations after the experimental onset, at 50 and 83 days, there was an increase in Fo, which reached 224 electrons quantum^{-1}, a value statistically superior to the condition of the plants in the field and that observed in 'Fepagro C 13' cuttings. This result is possibly related to the rooting behavior of the two rootstocks. As observed, 'Fepagro C 13' cuttings showed greater and faster rooting than 'Trifoliata'. After root formation, the leaves of the 'Fepagro C 13' cuttings were under less stress and, therefore, the photosynthetic apparatus was less affected. In contrast, 'Trifoliata' cuttings with low rooting remained under stressful conditions until the end of the greenhouse period, having its photosynthetic apparatus more compromised.

Maximum fluorescence behaved similarly to Fo (Figure 4B). This variable (Fm) represents the maximum intensity of fluorescence, when QA is completely reduced and the reaction centers reach maximum capacity for photochemical reactions (Baker & Rosenqvst, 2004). Field plants of 'Fepagro C 13' showed higher Fm than 'Trifoliata'. At establishment, there was an increase in Fm in both rootstocks, with 'Fepagro C 13' showing higher values. During the greenhouse period, Fm decreased significantly in both rootstocks. At 83 days, both reached values lower than on other evaluation dates and the initial condition of the plants in the field, with similar behaviors. The reduction in Fm shows that, over the period, there was a deficiency in the photoreduction of QA and in the flow of electrons between the photosystems (Tatagiba et al., 2014).

Table 2

Analysis of variance for the parameters of initial fluorescence (Fo), maximum fluorescence (Fm), photochemical efficiency (Fv/Fm), and Fv/Fo ratio in adult plants of 'Fepagro C 13' and 'Trifoliata' (period zero) in the field and on cuttings at the implementation of the experiment (day 1) and 50 and 83 days in a greenhouse, as a function of auxin application (4000 mg L^{-1} of IBA)

Source of variation	DF	Fo	Fm	Fv/Fm	Fv/Fo
Rootstock (RS)	1	10.30*	31.64*	44.63*	48.04*
Leaves (L)	1	2.37 ^{ns}	0.09 ^{ns}	4.31*	3.38 ^{ns}
Auxin (A)	3	13.43*	96.75*	45.25*	47.47*
RS × L	1	1.21 ^{ns}	2.99 ^{ns}	0.01 ^{ns}	0.32 ^{ns}
RS × A	3	14.04*	10.41*	7.45*	5.79*
A × L	3	1.21 ^{ns}	2.74 ^{ns}	5.04*	4.09*
RS × A × L	3	0.57 ^{ns}	0.36 ^{ns}	0.3 ^{ns}	0.67 ^{ns}
Block	3	2.27 ^{ns}	2.56 ^{ns}	0.81 ^{ns}	0.64 ^{ns}
Error	45				
CV%		7.42	4.23	1.77	7.73

*significant at the 5% probability level by the F test; ns = not significant (p≥0.05).

The Fv/Fm ratio is an estimate of the maximum quantum efficiency of photochemical activity of PSII, when all PSII reaction centers are open (Baker & Rosenqvst, 2004). It is considered a reliable indicator when plants are subject to environmental and biotic stresses, and a drop in this ratio reflects the presence of photoinhibitory damage (Campostrini, 2001). Although differences were seen in the other fluorescence variables analyzed in the plants in the field (Fo and Fm), in terms of photochemical efficiency (Fv/Fm), the rootstocks showed equivalent values, close to 0.82 electrons quantum^{-1} (Figure 4C). Despite the stress detected at the establishment of the experiment, the photochemical efficiency of the leaves present on the cuttings was not compromised, as both rootstocks maintained the Fv/Fm ratio. However, there was a reduction in efficiency during the greenhouse period. At 83 days, 'Trifoliata' cuttings exhibited an Fv/Fm of 0.74 electrons quantum^{-1}, which was significantly lower than those measured on the other evaluation dates and that of 'Fepagro C 13' (0.79 electrons quantum^{-1}). Auxin application also influenced the photochemical efficiency of the leaves. At 83 days, the treated cuttings showed higher efficiency (0.78 electrons quantum^{-1}) than their untreated counterparts (0.75 electrons quantum^{-1}), which is believed to be linked to the overall higher rooting percentage observed in the treated cuttings. Thus, the results indicate that throughout the rooting process there is an impact on the photosynthetic apparatus of the leaves, even after the rooting of the cuttings. However, rooted cuttings show less impairment of leaf photochemical efficiency.

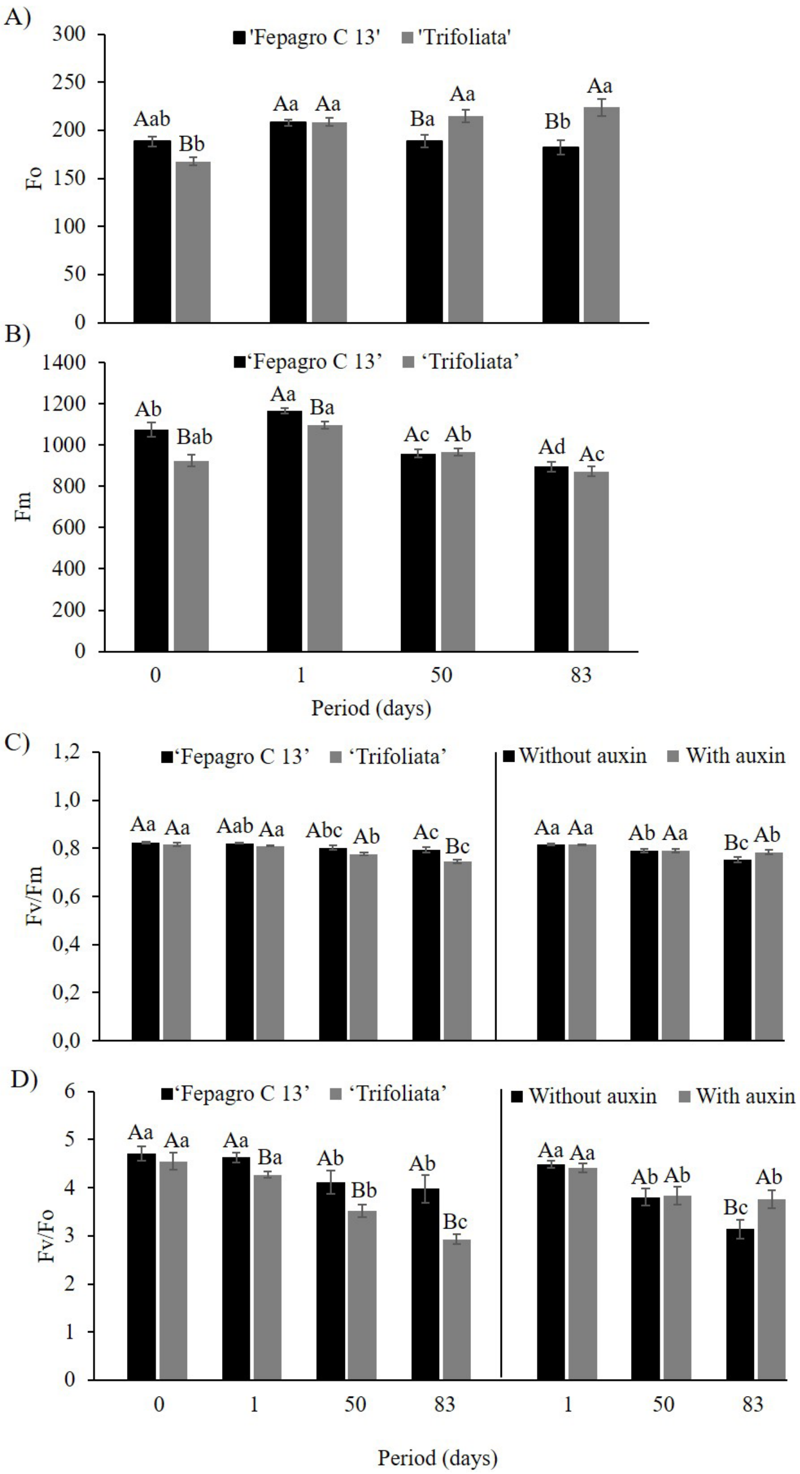

Figure 4. A) Minimum fluorescence (Fo); B) maximum fluorescence (Fm); C) photochemical efficiency (Fv/Fm); and D) Fv/Fo ratio in adult 'Fepagro C 13' and 'Trifoliata' plants in the field (period zero) and in cuttings at the implementation of the experiment (day 1) and at 50 and 83 days in the greenhouse, as a function of the application of auxin (4000 mg L^{-1} of IBA). Uppercase letters compare rootstocks or auxin application, and lowercase letters compare evaluation data.

According to Suassuna et al. (2010), a plant with an intact photosynthetic apparatus has an Fv/Fm ratio between 0.75 and 0.85 electrons quantum^{-1}. In citrus rootstock hybrids under irrigation with saline water, Sousa et al. (2014) considered normal values as between 0.79 and 0.82 electrons quantum^{-1}. Therefore, even with the variations observed during the study, the observed Fv/Fm values are within the normal range.

The observed differences in photochemical efficiency were amplified by the Fv/Fo ratio (Figure 4D). During the greenhouse period, the Fv/Fo ratio decreased significantly, confirming the reduction of photochemical efficiency. This indicator is commonly used in studies with different species and types of stress, such as water deficit, as it acts as an indicator of maximum efficiency in the photochemical process in PSII and/or potential photosynthetic activity, with values between 4 and 6 electrons quantum^{-1} (Silva et al., 2015). Thus, among the Fv/Fo values obtained in the study, cuttings of 'Trifoliata' rootstock and of both rootstocks not treated with auxin exhibited Fv/Fo values below normal: 2.12 and 3.14, respectively, reinforcing the occurrence of photoinhibitory damage in the leaves of the respective treatments.

Anatomical evaluation

The sections at the base of the cuttings of both rootstock varieties revealed no differences in terms of stem anatomy, as shown in Figure 5. Despite the difference in rooting capacity seen in this study, this variation does not seem to be related to the anatomical characteristics of the cuttings. No anatomical barriers to rooting were identified, e.g. the presence of a continuous ring of sclerenchyma between the phloem and the cortex, outside the point of origin of the adventitious roots, common in difficult-to-root woody species (Hartmann et al., 2017).

In 'Trifoliata' cuttings, although the rooting evaluations indicated root formation only after 46 days in the greenhouse, at 25 days it was possible to identify the presence of meristematic cells and root primordia developing at the base of the cuttings. The observations show that the root primordia have their origin in the vascular cambium (Figure 6B), a characteristic considered common to easy-to-root species, since the root formation process is direct, that is, it is not linked to callus formation at the base of cuttings (Hartmann et al., 2017). At 78 days, formed roots with vascular connection were visible (Figure 6D).

In 'Fepagro C 13' cuttings, the root primordium also appears to arise from the vascular cambium, but with likely participation of phloem cells, potentially meristematic parenchyma cells (Figures 7A and 7B). However, it was not possible to identify the exact root origin, warranting further studies for this variety. As previously discussed, compared with what was observed in 'Trifoliata', in the 'Fepagro C 13' rootstock the rhizogenesis process occurs more quickly, with observed roots developed after 25 days in the greenhouse in auxin-treated cuttings (Figure 7C). This aspect affected the observation of meristematic cells and root primordia in development, requiring a shorter interval for sample collection.

Despite the observed differences regarding the rooting potential of cuttings of the evaluated rootstock varieties, these are not attributed to the stem anatomy, but to the genetic potential and physiological conditions for root formation.

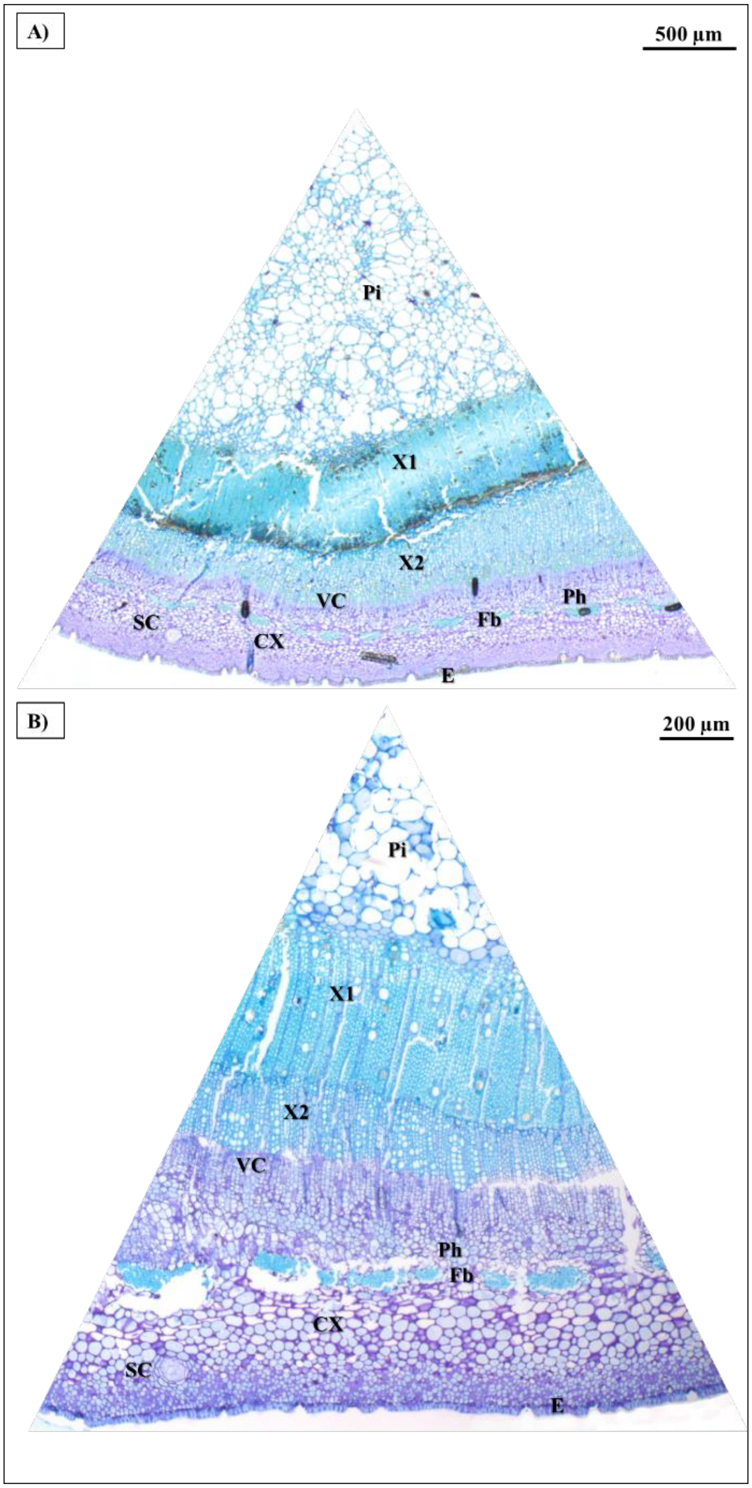

Figure 5. Cross-sectional anatomy of the stem, obtained in the basal zone of the cutting of two rootstocks in the study, A) 'Trifoliata' and B) 'Fepagro C 13'. Legend: pith (Pi), primary xylem (X1), secondary xylem (X2), vascular cambium (VC), fibers (Fb), phloem (Ph), cortex (CX), secretory cells (SC), epidermis (E).

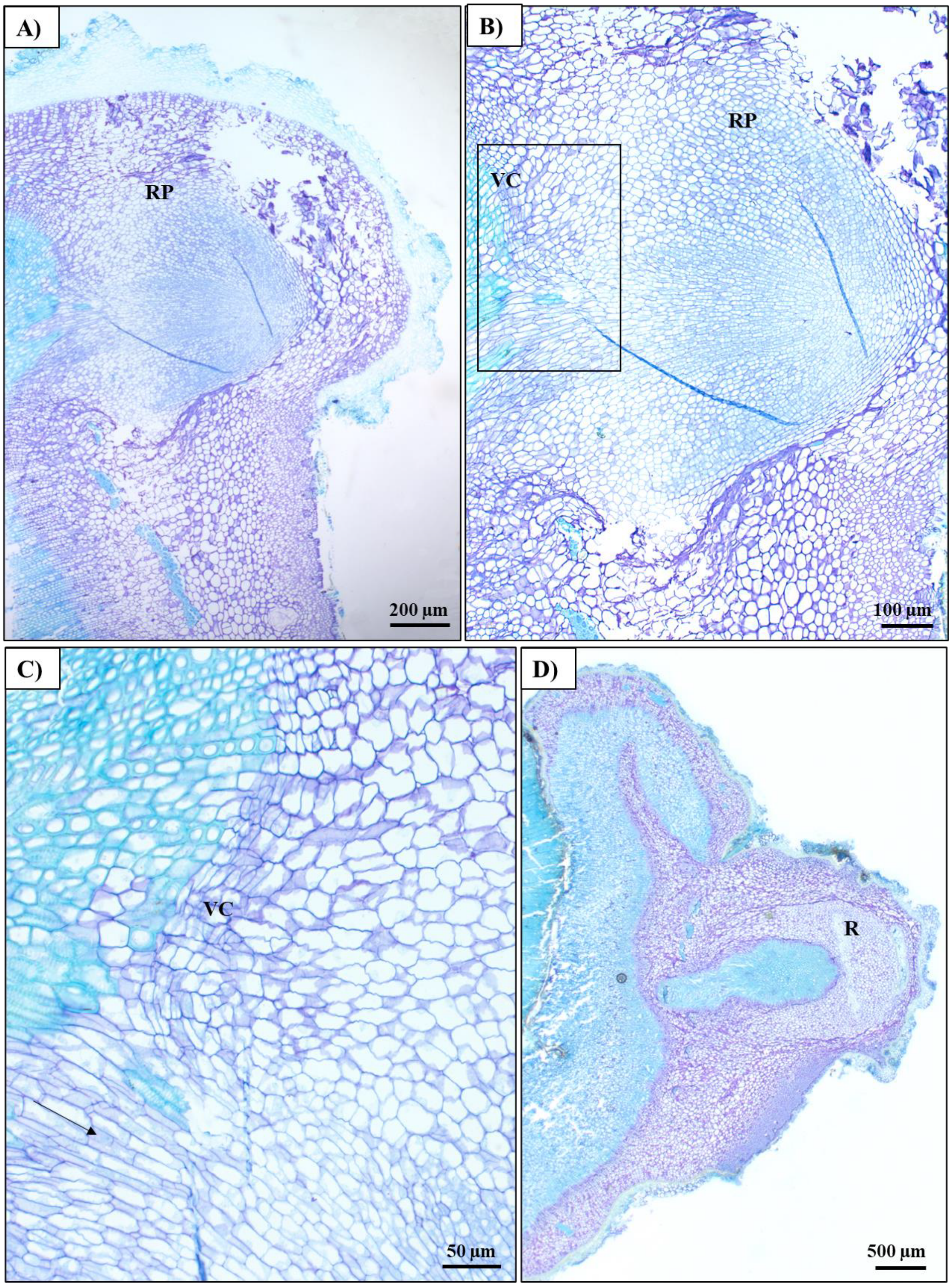

Figure 6. Cross-sections of the basal zone of 'Trifoliata' cuttings. A) Development of the root primordium (RP); B) Root primordium with emphasis on cell multiplication and no vascular cambium (VC); C) Detail of vascular cambium cells in cell division, originating the root primordium; D) Morphogenetic fields of the formed root (R).

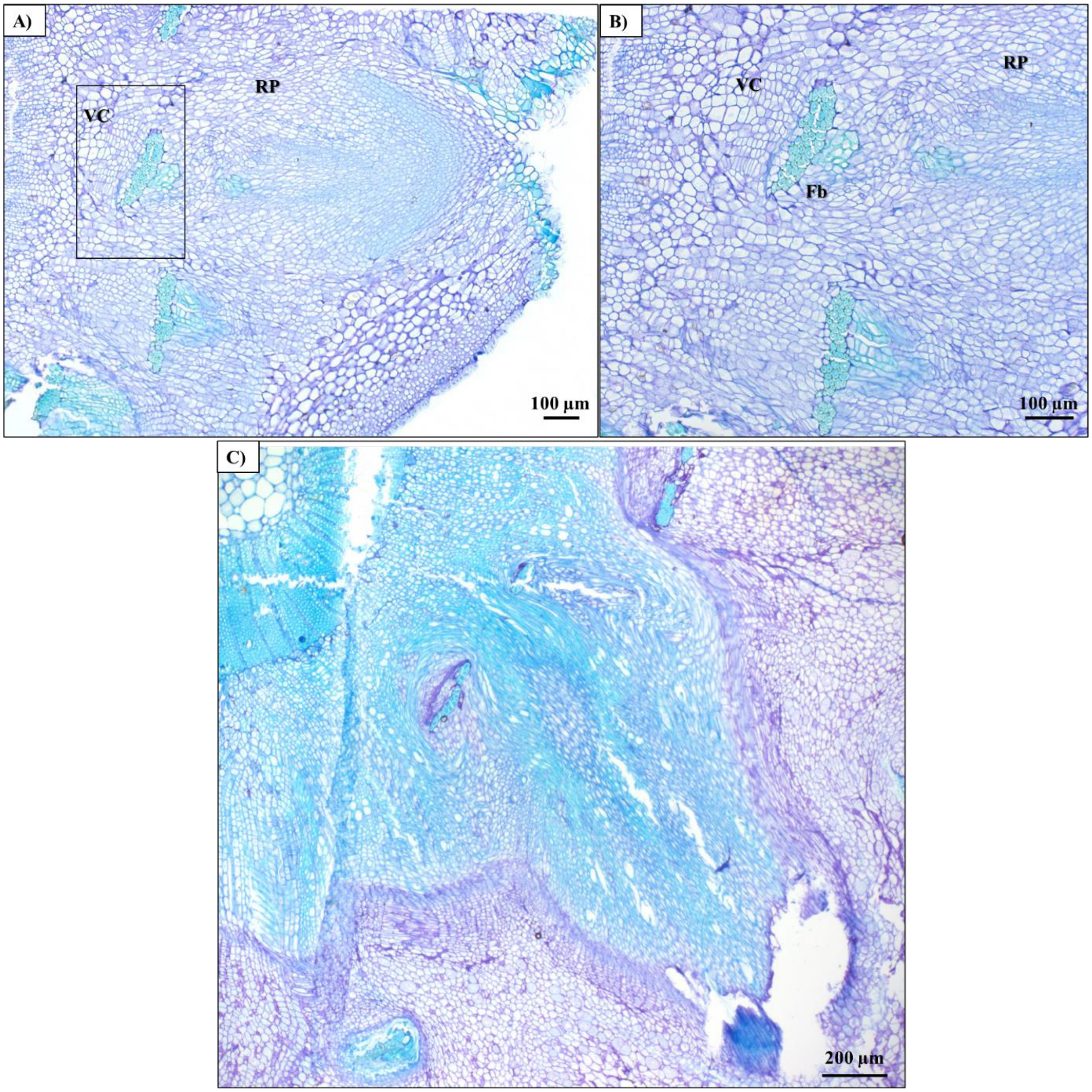

Figure 7. Cross-sections of the basal zone of ‘Fepagro C 13’ citrus cuttings. A) Development of the root primordium (RP) with emphasis on the cell multiplication site; B) Detail of vascular cambium (VC) cells in cell division, with possible participation of phloem cells in root formation; C) Morphogenetic fields of the formed root.

Conclusions

The presence of leaves in ‘Trifoliata’ and ‘Fepagro C 13’ cuttings is essential for adventitious rooting.

The use of indolebutyric acid increases the photochemical efficiency of the leaves on the cuttings.

In ‘Trifoliata’ cuttings, adventitious roots originate from the vascular cambium.

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