Rooting of cuttings of *Feijoa sellowiana* with the use of antioxidants

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Abstract – *Feijoa sellowiana* O. Berg is a native fruit with great potential for cultivation, but Brazil has few orchards on a commercial scale. One of the difficulties in expanding production is linked to obtaining clonal plantlets due to the lack of efficient protocols for vegetative propagation. Thus, this study aimed to evaluate the efficiency of the antioxidant polyvinylpyrrolidone (PVP) in cuttings of *F. sellowiana*. Different concentrations of PVP (0, 2,000, and 4,000mg L^{-1}), applied systemically, were tested in immersion and associated with a solution of indolebutyric acid (IBA). The application of 4,000mg L^{-1} of PVP in systemic form resulted in the highest percentages of survival and shooting (47.1% and 25%, respectively) and maximized callus formation (50%) at 90 days of cultivation and rooting (47.5%) at 120 days. The use of PVP favors the vegetative propagation of *F. sellowiana* by cuttings and may be a relevant tool in the clonal propagation of the species.

Index terms: Vegetative propagation; Adventitious rooting; PVP.

Enraizamento de estacas de *Feijoa sellowiana* com uso de antioxidante

Resumo – *Feijoa sellowiana* O. Berg é uma frutífera nativa de grande potencial de cultivo, porém no Brasil existem poucos pomares em escala comercial. Uma das dificuldades à expansão da produção está vinculada a obtenção de mudas clonais pela escassez de protocolos eficientes para a propagação vegetativa. Assim, o presente estudo teve como objetivo avaliar a eficiência do antioxidante polivinilpirrolidona (PVP) na estaquia de *F. sellowiana*. Para isso foram testadas diferentes concentrações de PVP (0, 2000 e 4000mg L^{-1}) aplicados de forma sistêmica, em imersão e associado à solução de ácido indolbutírico (AIB). A aplicação de 4000mg L^{-1} de PVP na forma sistêmica resultou nas maiores porcentagens de sobrevivência e brotação (47,1 e 25,0%, respectivamente), bem como foi maximizou a formação de calos (50,0%) aos 90 dias e de raízes (47,5%) aos 120 dias de cultivo. O uso de antioxidante polivinilpirrolidona favorece a propagação vegetativa de *F. sellowiana* por estaquia, podendo apresentar-se como relevante ferramenta na propagação clonal da espécie.

Termos para indexação: Propagação vegetativa; Enraizamento adventício; PVP.

Introduction

Brazil is a country rich in plant biodiversity, with many native fruit species (NEGRI et al., 2016) with potential for commercial production and income generation (AMARANTE et al., 2017). Among these species, *Feijoa sellowiana* O. Berg, a synonym of *Acca sellowiana* and popularly known as pineapple guava (*goiabeira-serrana* in Portuguese), belongs to the Myrtaceae family and is native to the Southern Brazilian plateau, northeastern Uruguay, and parts of Argentina (MATTOS, 1954; MATTOS, 1986; MATTOS, 1990; KELLER & TRESSENS, 2007). Despite its natural occurrence and potential, the cultivation of *F. sellowiana* is restricted to small areas, but with prospects for expansion (AMARANTE et al., 2017). It is cultivated commercially in New Zealand and Colombia, countries that have significant production and export the fruit, including to Brazil (PARRA-CORONADO et al., 2015). In this sense, the possibility of use in several by-products, the nutraceutical and pharmacological properties of the fruit, its adaptation to the climatic conditions of the region, and, above all, the fact that it is native to the country evidences the need for investment in research aimed at advances related to autecology and cultural management of the species (OLIVEIRA et al., 2021; SANTOS et al., 2021).

One of the significant limitations that hinder the popularization of the species in Brazil is the need for efficient methodologies for plantlets production (AMARANTE et al., 2013), especially by vegetative propagation. Although Souza (2013) obtained promising results with the grafting technique, other researchers consider that the propagation of *F. sellowiana* presents difficulties when using the cutting, grafting, micropropagation, and stump layering methods (ROSS & GRASSO, 2010; PASA et al., 2018). This situation

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hinders the homogeneity of both the orchards (FRAZON et al., 2004; PASA et al., 2018) and, in the case of *F. sellowiana*, the expansion of productive areas.

In order to contribute to overcoming these limitations, cuttings are included, since this is one of the most common, accessible, and practical clonal multiplication techniques, consisting of the adventitious rooting of vegetative propagules known as cuttings, which are taken from mother plants with the desired characteristics. Adventitious rooting, in turn, is an essential process for successful propagation and depends on factors such as temperature, brightness, humidity, type of cutting, cutting time, plant hormones, and genotypes (PASA et al., 2018).

In general, species are classified according to their ability to form adventitious roots, from easy-to-root to recalcitrant species, that is, species that are difficult to root. *F. sellowiana* is considered recalcitrant, which, according to Timm et al. (2014), is related to the high amount of phenolic compounds produced by the species, promoting the rapid oxidation of cuttings. Phenolic compounds are released in lesions or cuts as a rapid defense against infections, serving as substrates for enzymes such as peroxidases, polyphenol oxidases (JANEIRO, 2011), and oxidases. Oxidative enzymes degrade and inhibit indoleacetic acid (AIA), an auxin associated with cell hypertrophy and plant growth (FERREIRA et al., 2017).

Thus, the use of antioxidants in asexual propagation is an alternative to circumvent this limitation, since these substances inactivate free radicals and reduce or prevent intoxication of cuttings by the release of phenolic compounds (ARAÚJO, 1985). However, although phenolic compounds are harmful to cuttings, these compounds are also essential for plants, as they regulate the oxidation of AIA, which are phytohormones responsible for root induction (HARTMANN et al., 2018; BEZERRA et al., 2014; STEFANEL et al., 2021). Therefore, the adjustment between these compounds in the plant is necessary to promote an antioxidant effect. Among the substances with this effect, we highlight citric acid, ascorbic acid, activated charcoal, polyvinylpyrrolidone (PVP), dithiothreitol, among others (GOULART et al., 2010).

Thus, considering the commercial potential of *F. sellowiana*, the difficulty in adventitious rooting, and the need to find a fine adjustment in the use of antioxidant compounds that efficiently enable plantlets production, this study aimed to evaluate the antioxidant PVP in the vegetative propagation of *F. sellowiana* by cuttings.

Material and methods

The plant materials used in this study were epicormic shoots formed at the base of 10 *Feijoa sellowiana* plants selected considering shoot production. The donor plants were from the agricultural experimental area of the Federal University of Santa Catarina, Campus Curitibanos. Access to native genetic material followed the requirements of Law No. 13,123/2015, with registration at the Genetic Heritage Management Council No. A3B7DCD.

Three ways of applying the antioxidant PVP, at three concentrations (0, 2,000, and 4,000mg L^{-1}), were tested: systemic application; treatment in a phytohormone solution of indolebutyric acid (IBA); and immersed cuttings. In systemic application, immediately after collection, the bases of part of the epicormic shoots were immersed in PVP solution at different concentrations (0, 2,000, and 4,000mg L^{-1}) (Figure 1A) for 3,600 seconds. The principle of this treatment is that the antioxidant solution placed in contact with the base of the shoot is absorbed and translocated with the sap to all parts of the plant via the vascular system (xylem). The control treatment (0mg L^{-1}) consisted of keeping the bases of the shoots in distilled water. After 3,600 seconds, the shoots were sectioned into 2 to 5cm long cuttings, keeping two pairs of leaves with 50% of their leaf area. All cuttings were treated for 10 seconds in a hydroalcoholic solution of IBA at a concentration of 2,000mg L^{-1} (Figure 1). For the other two forms of application (phytohormone solution and immersed cuttings), the epicormic shoots were collected and immediately sectioned into 2 to 5cm long cuttings, keeping two pairs of leaves with 50% of the leaf area. Therefore, these two treatments differ from the first because PVP is applied after the cuttings are made, while in the systemic treatment, it is applied to the entire epicormic shoot and the cuttings are made only after 3,600 seconds of treatment in an antioxidant solution.

For the application in a phytohormone solution, the cuttings were treated for 10 seconds in a hydroalcoholic solution of IBA at a concentration of 2,000mg L^{-1} containing, in association, the different concentrations of PVP (0, 2,000, and 4,000mg L^{-1}) (Figure 1B). For the treatment with immersed cuttings, immediately after making the cuttings, they were immersed in containers with a solution with different concentrations of PVP (0, 2,000, and 4,000mg L^{-1}) for 3,600 seconds (Figure 1C). After this period, the bases of the cuttings were treated for 10 seconds in a hydroalcoholic solution with 2,000mg L^{-1} of IBA.

After the treatments, the cuttings were cultivated in Styrofoam trays with 128 alveoli and medium grain vermiculite, and kept in a humid chamber, with intermittent nebulization irrigation. The relative humidity was maintained at around 85% and the temperature at 25±2°C.

The experimental design used was completely randomized in a 3×3 factorial scheme (forms of application and concentrations of PVP), with ten repetitions of four cuttings each. At 30, 60, 90, and 120 days, the cuttings were evaluated for percentages of survival, sprouting, callus formation, and rooting of the cuttings. The data were tested for normality and homogeneity of variances and ANOVA was used to interpret the results. In cases of significant differences, the means were compared using Tukey's test at 5% error probability using the R Core Team (2020) software.

Results and discussion

We observed neither a significant interaction between the factors nor a significant influence of the

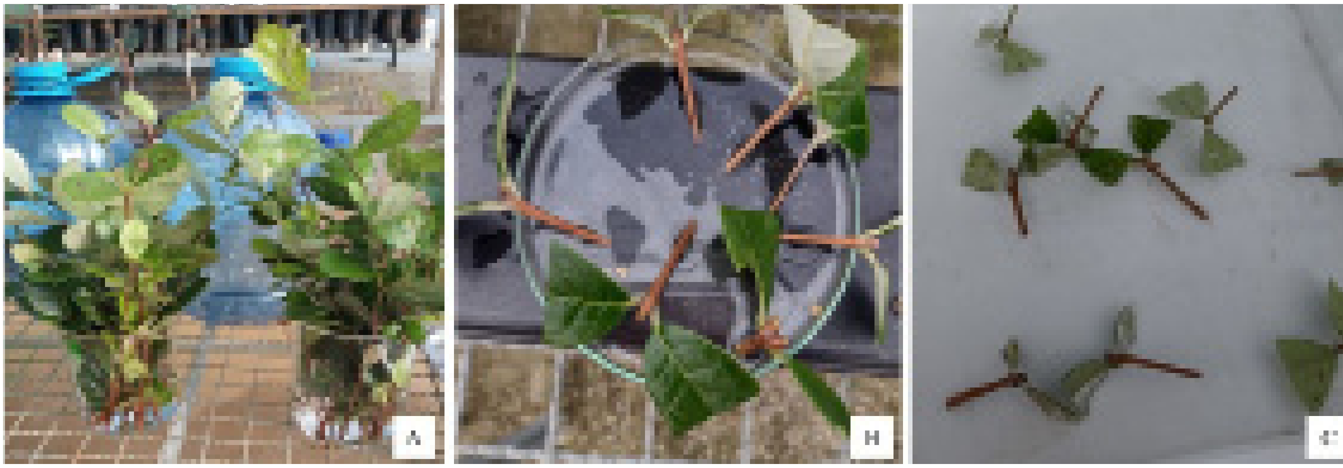

Figure 1. Epicormic shoots of *F. sellowiana* in PVP antioxidant solution for systemic treatment (A); cuttings with their bases immersed in IBA and PVP antioxidant solution (B); and cuttings immersed in an antioxidant solution (C)
Photos: Jacqueline Claudino da Silva
*Figura 1. Brotos epicórmicos de **F. sellowiana** em solução de antioxidante PVP para tratamento sistêmico (A); estacas com as bases imersas em solução de AIB e antioxidante PVP (B); e estacas imersas em solução de antioxidante (C)*
Fotos: Jacqueline Claudino da Silva

isolated factors on the percentage of survival and sprouting of cuttings of *F. sellowiana* at 30, 60, 90, and 120 days of cultivation in a humid chamber. Regardless of the treatment, mean survival rates remained high at 30 and 60 days of cultivation (95.0% and 86.1%, respectively), reducing to 64.2% at 90 days and 39.4% at 120 days (Figures 2A and 2B). Sprouting increased from 11.6% at 30 days to 35% at 60 days of cultivation and decreased to 21.9% at 120 days (Figures 2C and 2D). These results show that the cuttings reach their maximum sprouting at 60 days; after this period, if no roots are formed, the cuttings tend to die (Figure 2).

At 30 days, the base of the cuttings showed no callus formation. At 60 and 90 days, we observed formed calluses and a significant interaction between the factors tested for this variable. The highest callogenesis responses occurred in systemic treatments with 2,000 and 4,000mg L^{-1} of PVP and at 60 and 90 days in the treatment in IBA solution with 2,000mg L^{-1} of PVP (Table 1). For Bressaneli (2017), the presence of calluses at the bases of mini cuttings of *F. sellowiana* did not indicate the formation of adventitious roots, but they are indicative of cell differentiation. According to Silva et al. (2012), the presence of calluses in olive tree cuttings indicates cell differentiation, suggesting the formation of early adventitious roots, and *F. sellowiana* may have this same behavior (Table 2).

Regarding the rooting percentage, the tested treatments showed no significant interaction. At 30 days, we observed root formation in plants subjected to the systemic treatment, but without differing from the other forms of application (Figure 3A), and the application of 4,000mg L^{-1} of PVP, which showed the same behavior compared to the other concentrations (Figure 3B). At 60 and 90 days, the influence of isolated culture factors was evident (Figure 3). The systemic treatment resulted in the highest rooting percentage, but without differing from the treatment with immersed cuttings (Figure 3A). For the application of 4,000mg L^{-1} of PVP, the result was significantly higher than the other concentrations for the same cultivation period (Figure 3B).

Table 1. Percentage of callus formation in cuttings of *F. sellowiana* at 60 and 90 days in a humid chamber after treatment with different forms of application and concentrations of polyvinylpyrrolidone (PVP)
Tabela 1. Porcentagem de formação de calos em estacas de *F. sellowiana* aos 60 e 90 dias em câmara úmida após tratamento com diferentes formas de aplicação e concentrações de polivinilpirrolidona (PVP)

PVP concentration (mg L^{-1})	PVP treatments / % callus formation		
	Systemic at 60 days	IBA solution	Immersion
0	17.5 aA	20.0 abA	20.0 aA
2,000	37.52 aA	40.0 aA	22.5 aA
4,000	37.5 aA	7.5 bB	30.0 aAB
Mean	30.8	22.5	24.2
CV (%)		85.5	
		At 90 days	
0	17.5 bA	27.5 aA	27.5 aA
2000	40.0 abA	40.0 aA	30.0 aA
4000	50.0 aA	12.5 aB	35.0 aAB
Mean	35.8	26.7	30.8
CV (%)		79.5	

* Values followed by the same lower case letter in the vertical and capital letter in the horizontal do not differ by Tukey's test at 5% probability of error.

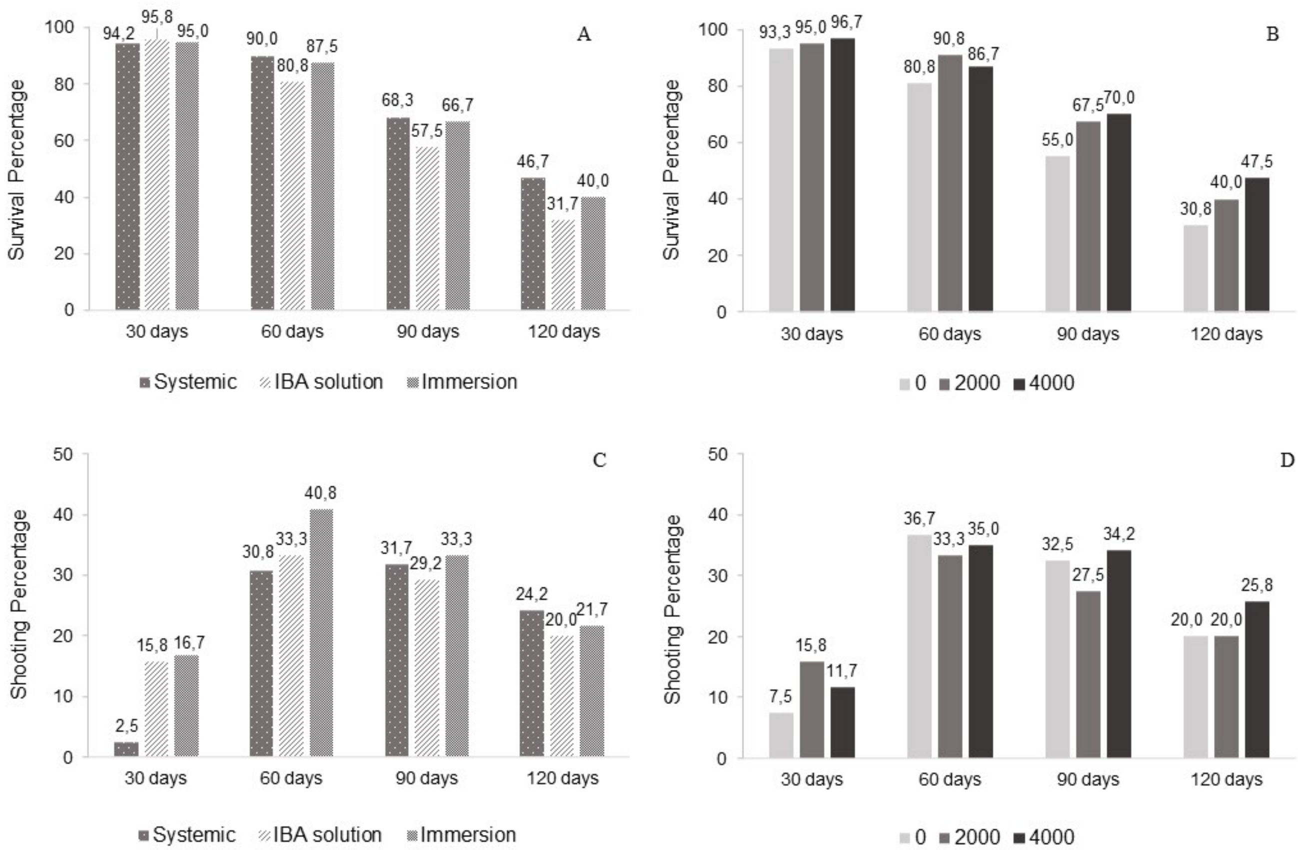

Figure 2. Survival and sprouting percentage of cuttings of *F. sellowiana* in different forms of application (A and C, respectively) and with different concentrations of PVP (B and D, respectively) at 30, 60, 90, and 120 days of cultivation in a humid chamber
*Figura 2. Porcentagem de sobrevivência e de brotação de estacas de **F. sellowiana** em diferentes formas de aplicação (A e C, respectivamente) e com diferentes concentrações de PVP (B e D, respectivamente) aos 30, 60, 90 e 120 dias de cultivo em câmara úmida*

Although Hartmann et al. (2018) explain that early-sprouting cuttings do not emit adventitious roots due to competition for carbohydrates for root and shoot formation and hormonal imbalance in the cuttings, this apparently did not occur in *F. sellowiana*, since the rooting percentage showed an increasing trend after 60 days.

At 120 days, we observed a significant interaction between the factors tested, and the systemic treatment with 4,000mg L^{-1} of PVP showed the highest rooting (Table 2). *F. sellowiana* is a species of the Myrtaceae family that has, at the cutting point of the propagules, high rates of oxidation of phenolic compounds present in large quantities in the tissues, a factor responsible for the reduced rooting capacity of these species (FACHINELLO et al., 1995). Thus, the use of antioxidant substances, such as PVP, can be beneficial in the adventitious rooting process, since PVP adsorbs phenols released by plant tissues through hydrogen bonds, preventing oxidation and polymerization and adsorbing products related to phenolic oxidation (GOULART et al., 2010).

The results obtained in this study are in line with Goulart et al. (2010), who observed the best results with the application of 4,000 mg L^{-1} of PVP in the rooting of mini cuttings in clones of *Eucalyptus grandis* × *E. urophylla* (Myrtaceae). In their study, the PVP application method used was associated in IBA solution and they did not test other methods (GOULART et al., 2010).

On the other hand, the study by Martins et al. (2022) found high concentrations of phenolic compounds in the cambial region of rooted cuttings, suggesting a positive influence of these compounds on adventitious rooting. Similarly, Wu & Toit (2011) recommend the exogenous application of phenolic compounds at the bases of cuttings of *Protea cynaroides* L. to stimulate root formation. This would explain the ineffectiveness of applying antioxidants for the rooting of cuttings of species such as *Coffea arabica* L. (Rubiaceae) (BERGO & MENDES, 2000) and *Vaccinium myrtillus* (Ericaceae) (HOFFMANN et al., 1995).

Interestingly, Coutinho et al. (1992), when testing the use of PVP associated with IBA, observed no rooting stimulatory effect in cuttings of *F. sellowiana*, the same species evaluated in this study. Thus, in this case, the ineffectiveness of PVP in rooting is more related to how the antioxidant is applied. The antioxidant may have been influenced only during the period of immersion of the base of the cuttings, but after placement in the substrate,

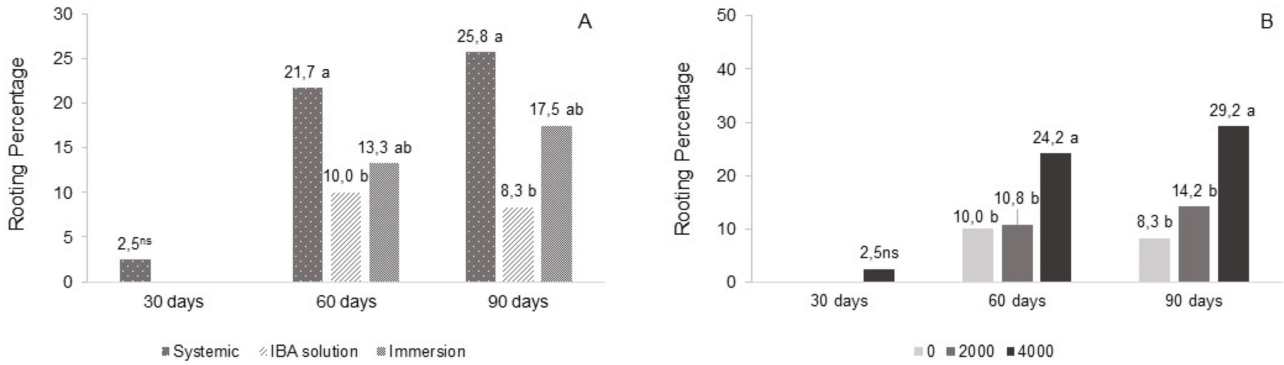

Figure 3. Rooting percentage of cuttings of *F. sellowiana* in different forms of application (A); and with different concentrations of PVP (B); at 30, 60, and 90 days of cultivation in a humid chamber
Values followed by the same letter in bars of the same color were not different from each other by Tukey's test at 5% probability of error
*Figura 3. Porcentagem de enraizamento em estacas de **F. sellowiana** em diferentes formas de aplicação (A); e com diferentes concentrações de PVP (B); aos 30, 60 e 90 dias de cultivo em câmara úmida*
Valores seguidos de mesma letra nas barras de mesma cor não diferentes entre si pelo teste de Tukey a 5% de probabilidade de erro

oxidation may have occurred again (HOFFMANN et al., 1995).

The cutting of epicormic shoots treated systemically with 4,000mg L^{-1} of PVP resulted in root formation in 47.5% of the cuttings, which is a promising result for cloning the species, since propagation by cuttings, to date, has not been feasible due to the recalcitrance to adventitious rooting obtained in studies performed with the species, with values ranging from 0% to 32% (DUARTE et al., 1992; FIGUEIREDO et al., 1995; BRESSANELI, 2017). Thus, further studies are recommended, addressing especially the invigoration/rejuvenation of propagules, the evaluation of other antioxidant substances, and the concentrations of substrate and IBA that favor adventitious rooting and make the technique viable for cloning the species.

Table 2. Rooting percentage in cuttings of *F. sellowiana* subjected to different forms of application and concentrations of polyvinylpyrrolidone (PVP) after 120 days in a humid chamber
Tabela 2. Porcentagem de enraizamento em estacas de *F. sellowiana* submetida a diferentes formas de aplicação e concentrações de polivinilpirrolidona (PVP) aos 120 dias em câmara úmida

PVP concentration (mg L^{-1})	PVP treatments / % rooting		
	Systemic	IBA solution	Immersion
0	12.5 bA	10.0 aA	10.0 aA
2,000	20.0 bA	12.5 aA	17.5 aA
4,000	47.5 aA	12.5 aB	27.5 aB
Mean	26.7	11.7	18.33
CV (%)		92.8	

* Values followed by the same lower case letter in the vertical and capital letter in the horizontal do not differ by Tukey's test at 5% probability of error.

Conclusion

The use of the antioxidant polyvinylpyrrolidone favors the vegetative propagation of *F. sellowiana* by cuttings.

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