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Iron Chelate Improves Rooting in Indole-3-Butyric Acid-Treated Rosemary (*Rosmarinus officinalis*) Stem Cuttings

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Abstract: Adventitious root formation in stem cuttings is affected by exogenous and endogenous factors. The study assessed the effect of Fe(III)-EDDHA (ethylenediamine-*N*,*N*-bis 2-hydroxyphenyl acetic acid) on the rooting of 4 indol-3-butyric acid (IBA)-treated hardwood cuttings of the aromatic and medicinal species *Rosmarinus officinalis*. Cuttings treated with 0, 1000, 2000 or 3000 mg L^{-1} IBA were placed in pots filled with sand:perlite mixture and irrigated daily with nutrient solution pH 5.8, containing 0, 5, 10 or 20 µM Fe(III)-EDDHA. Ten days later, the number of new emerging roots were recorded. After 20 days, leaf photosynthetic pigments and morphological traits, including root number, fresh (FW) and dry weight (DW), shoot FW and DW, mean length of the longest roots, number of new shoots and new growth in old shoots, were measured. Finally, plants were transplanted to pots filled with a sand:soil mixture and survival was measured after 10 days. Results indicate that Fe application promotes root emergence and improves root and shoot biomass, leaf photosynthetic pigment concentrations and survival percentage. This indicates that using low concentrations of Fe(III)-EDDHA (5–20 µM) in the growth medium could be a good management strategy to facilitate the production of vigorous *R. officinalis* plants from hardwood cuttings.

Keywords: rooting; hardwood cuttings; iron chelates

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1. Introduction

Adventitious root formation in plant cuttings is influenced by a large set of exogenous and endogenous factors [1]. Root initiation involves de-differentiation of specific cells leading to the formation of the root meristems [1]. Endogenous factors that could act as rooting co-factors and auxin transport modulators are transferred from the stock plants to the propagules [2]. These include auxin and carbohydrates [3], mineral nutrients [4] and other metabolites, including phenolic compounds [5].

Among the exogenous rooting factors, the auxin IBA (indole-3-butyric acid) is widely used to stimulate rooting processes in cuttings, because of its high ability to promote root initiation. This effect of IBA is thought to be due to its conversion in the plant tissue to indole-3-acetic acid (IAA), which is needed for the rooting process. Endogenous IAA can be readily oxidized in plants by peroxidase, but IBA is quite stable and is only slowly transported from the site of application at the base of the cuttings, resulting in a localized IAA production [6]. Exogenous IBA application has been shown to have positive rooting effects in many woody plant species, including *Citrus medica* [7], *R. damascena* [8], *Hibiscus rosa-sinensis* [9], *Olea europaea* [10,11], *Zizyphus jujuba* [12], *Tilia rubra* [13], *Eucaliptus* spp. [14,15], *Sterculia foetida* [16], *Castanea* spp. [17] and *Populus* [18]. For instance, in *Cinnamomum bodinieri*, exogenous IBA was shown to modify the auxin signaling pathway and carbohydrate metabolism, improve the formation of lateral root initiation site and root cell elongation, and enhance d-glucose synthesis as well as sucrose and starch utilization [19].

Other exogenous factors involved in rooting are mineral nutrients, which are involved in many metabolic processes associated with differentiation and root meristem formation, which is essential for root initiation [1]. Transcriptome analysis of adventitious root formation in *Petunia* × *hybrida* revealed an increase, starting from the initiation phase, in the expression of 18 genes involved in the uptake and assimilation of N, P, K, S, Fe and Zn [4]. For instance, within this period a high transcript abundance was observed for a plasma membrane H-ATPase, which may energize nutrient uptake [4]. The mineral nutrient composition of the cuttings, especially regarding micronutrients such as Fe, Zn, Mn and B, plays a key role in controlling root morphogenesis. Iron and Mn are cofactors and structural components of peroxidase and can therefore directly affect IAA catabolism [20]. Iron is an essential micronutrient for plants, which plays vital roles in many metabolic processes in plants, including photosynthesis, respiration and N_2 fixation [21]. Additionally, auxin is involved in the root responses to Fe deficiency [21–23]. A boosting effect of mineral nutrients (including Fe) on propagation of plant cuttings has also been reported in different studies [24,25]. In hardwood cuttings from Fe-deficient peach trees, the application of Fe compounds significantly reduced chlorotic symptoms and improved rooting [26].

Rosemary (*Rosmarinus officinalis*) is a xerophytic, aromatic, evergreen shrub widely used for food and as an ornamental species in gardens. Because of its hardiness under environmental stress, it is also used to protect against soil erosion and is planted in fire-damaged areas. Rosemary is a medicinal species that contains polyphenols, resulting in a number of pharmacological effects, including antioxidant, antitumor, antidiabetic and antibacterial ones [27–29]. Rosemary plants can be propagated by seed and stem cuttings, but propagation from seeds is rarely used, because of the long times needed for blooming and germination and the low germination rates (10–20%) [30,31]. Rooting of *R. officinalis* cuttings is facilitated by using hormone treatments [32,33], and different approaches are being used to further improve the rooting ability, with the aim to reduce costs and allow for mass production. For instance, it has been recently shown that using blue light induces an upregulation of auxin signaling and leads to better root formation [30].

The aim of this work was to assess the hypothesis that Fe supplementation in the form of Fe(III)-chelate can promote rooting in stem cuttings of the medicinal and aromatic plant *R. officinalis* treated with IBA. Four concentrations each of Fe and IBA were used and shoot and root biomass as well as rooting parameters were studied.

2. Materials and Methods

2.1. Greenhouse and Propagation Conditions

This study was carried out in March 2018, in an experimental greenhouse of the Lorestan University, Khorramabad, Lorestan Province, in the western part of Iran (33°45′ N 48°26′ E). The greenhouse was north–south oriented, the mean temperature and relative humidity were maintained at 22/28 °C (night/day) and 55–75%, respectively, and the light intensity was approximately 500 ± 100 µmol quanta m^{-2} s^{-1} (photosynthetically active photon flux density).

2.2. Plant Material and Growth Conditions

Cuttings were taken from vigorous and healthy bushes of *R. officinalis*, 10 years old, growing in the Faculty of Agriculture, Lorestan University (originated from cuttings obtained at the National Botanical Garden of Iran, Tehran). Cuttings consisted of hardwood branches (including the apical meristem), excised approximately 1 cm below a leaf node, at least 15 cm in length and with 7–8 nodes. In each cutting, leaves in the lower 5 cm were removed, and the basal 1 cm was dipped for 5 s [34] in a solution containing different IBA (CAS number 133-32-4) concentrations (0, 1000, 2000 or 3000 mg IBA L^{-1} –0, 4.9, 9.8 or 14.8 mM, respectively; these concentrations were thereafter called 0, 1000, 2000 and 3000 IBA). Cuttings were then placed in pots (28.0 cm height and 25.5 cm in diameter; ten cuttings per pot) filled with a sand:perlite (1:1, w:w) mixture, and irrigated daily with a nutrient solution containing (in mM) 0.1 KH_2PO_4, 0.1 $MgSO_4$, 0.25 $CaCl_2$ and 2 NH_4NO_3, and (in µM) 50 H_3BO_3 and 5 $MnSO_4$; the solution also

contained 1 mM MES (2-(*N*-morpholino)ethanesulfonic acid), and the pH was 5.8. The nutrient solution was supplemented with 0, 5, 10 or 20 μM Fe(III)-EDDHA (ethylenediamine-*N*,*N*-bis 2-hydroxyphenyl acetic acid; Sequestrene 138 Fe, 6% chelated Fe, Syngenta, Basel, Switzerland). These Fe concentrations are thereafter called 0, 5, 10 and 20 Fe. Pictures of the plants are shown in Figure S1 in the Supplementary File. Pots were covered with a clear polyethylene sheet to keep the medium moist. Pots were irrigated daily with the same nutrient solutions (1 L per pot), with the excess being drained. After 10 days new roots had emerged, and after 20 days new shoot tissue and leaves had developed. At that date, three plants per treatment were transferred to pots filled with a sand:soil mixture (1:2, w:w) and grown for 10 more days. Four replications (pots) were used for all treatments.

2.3. Plant Morphological Traits

Ten days after the root induction treatment, three cuttings were taken from each pot (a total of 12 cuttings per treatment) and used to count the number of newly developed roots. Twenty days after rooting induction, four plants from each pot were used to determine morphological traits, including root number, fresh (FW) and dry weight (DW), shoot FW and DW, mean length of the longest roots, number of new shoots and new growth in old shoots (a total of 16 cuttings were considered per treatment). The three remaining plants from each pot were transferred to the sand:soil mixture, and plant survival was recorded after 10 days.

2.4. Pigment Analysis

Chlorophyll a (Chl a), Chl b, total Chl (Chl a + b) and carotenoid (Car) concentrations were determined 20 days after the rooting induction treatment, in leaves of the same plants considered for analysis of morphological parameters. Leaf tissue (0.1 g FW) was collected from young, fully expanded leaves pooled from four plants in each pot, ground in liquid N_2 with mortar and pestle, homogenized with 10 mL 100% acetone, centrifuged for 15 min at 4000 rpm, and the supernatant collected. The absorbance of the extracts was measured as 470, 662 and 645 nm using a spectrophotometer (Mapada UV-1800, Shanghai, P.R. China), and the leaf pigment concentrations were calculated as follows: Chl a = $11.24 \times A_{662} - 2.04 \times A_{645}$; Chl b = $20.13 \times A_{645} - 4.19 \times A_{662}$; Chl a + b = Chl a + Chl b; Car = $(1000 \times A_{470} - 1.90$ Chl a $- 63.14$ Chl b$)/214$ [35]. Leaf pigments were expressed as (mg g FW^{-1}).

2.5. Statistical Analysis

The experiment was carried out with a completely randomized design with four replications per IBA × Fe treatment (16 treatments in total, four IBA and four Fe concentrations; 64 pots in total). All data were subjected to analysis of variance (SAS 9.1.3, SAS Institute Inc., Cary, NC, USA), and normality and homogeneity tested. Post hoc multiple comparison of means corresponding to the different treatments was carried out (at $p \leq 0.05$) using a LSD test; comparisons were carried out for the Fe treatments in a given IBA treatment, and also for the IBA treatments in a given Fe treatment (significance letters are shown in all Figures in lower case and capitals, respectively). Values shown are means of four replications—pots—each averaging values from three and four plants per pot in the cases of root number and the rest of parameters, respectively.

3. Results

Both factors that were used, IBA and Fe doses, had statistically significant effects on all parameters analyzed, with the only exception of Car content for Fe, and the interaction IBA × Fe was also significant (Table S1 in the Supplementary File).

3.1. Application of Fe Enhance the Rooting Performance in IBA-Treated Cuttings

Ten days after the IBA/Fe treatments, the percentage of rooting was between 0 and 100% (Figure 1). At that time, the control 0 IBA/0 Fe cuttings did not show any root-

ing signs, whereas cuttings under the 2000–3000 IBA/5 Fe, 1000–3000 IBA/10 Fe and 1000–3000 IBA/20 Fe treatments showed 100% rooting. The 1000–3000 IBA/0 Fe, 0–1000 IBA/5 Fe, 0 IBA/10 Fe and 0 IBA/20 Fe treatments led to intermediate percentages of rooting. In the absence of Fe, treatments with 1000–3000 IBA increased this parameter.

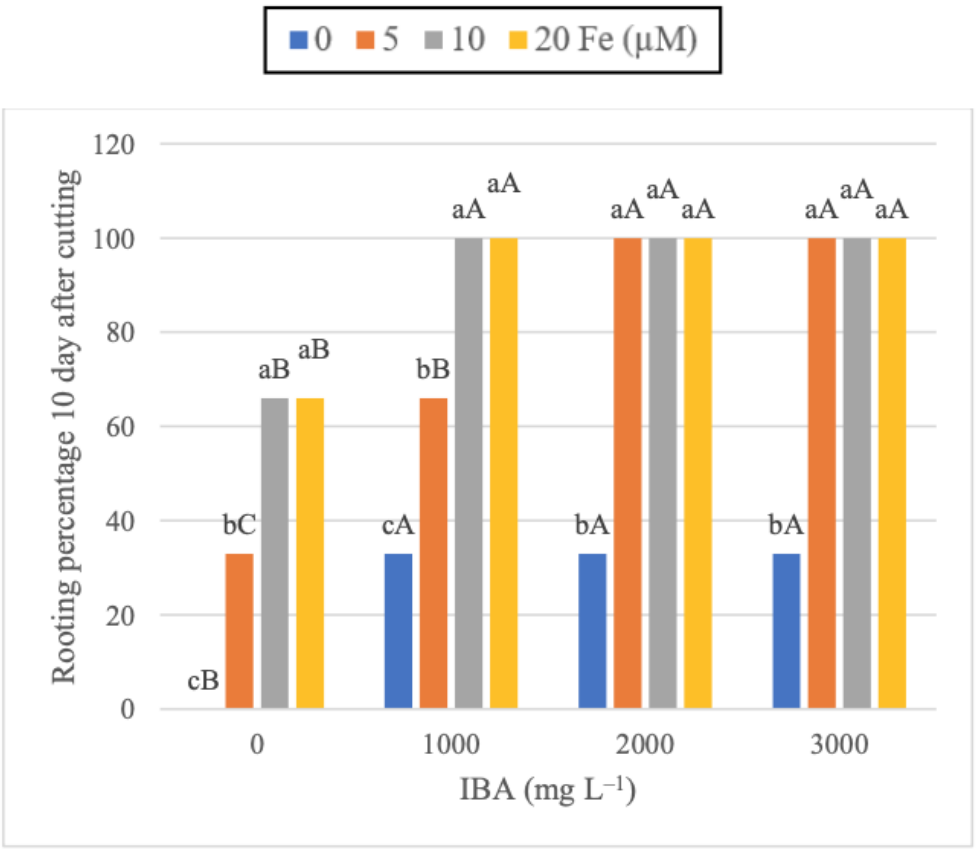

Figure 1. Percentages of rooting 10 days after the rooting induction treatments in *Rosmarinus officinalis* stem cuttings. Cuttings were treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment and then grown with different concentrations of Fe (0, 5, 10 and 20 µM). Values shown are means ± SE (n = 4 pots). Letters above the columns indicate significant differences at $p \leq 0.05$ for the Fe treatments in a given IBA treatment (in lower case) and for the IBA treatments in a given Fe treatment (in capitals).

3.2. Application of Fe Increase Biomass in IBA-Treated Root Cuttings

Twenty days after the start of the experiment, the root FW was between 0.19 and 0.66 g per plant, depending on the treatment (Figure 2A). The highest values were observed in some treatments including IBA and Fe (3000 IBA/5 Fe, 2000 IBA/10 Fe and 1000 IBA/20 Fe), and the lowest in the 0 IBA/0 Fe and the 0 IBA/5 Fe treatments, whereas other treatments led to intermediate values. The 10–20 Fe treatments increased root FW in cuttings not treated with IBA. On the other hand, in the absence of Fe treatments with 1000–3000 IBA increased this parameter.

The root DW was between 0.013 and 0.077 g per plant depending on the treatments (Figure 2B). The highest values were observed in some treatments including IBA and Fe (1000–3000 IBA/5 Fe, 1000–2000 IBA/10 Fe and 1000–3000 IBA/20 Fe), and the lowest in the 0 IBA/0 Fe control, whereas other treatments led to intermediate values. All treatments with Fe increased root DW in cuttings not treated with IBA. In the absence of Fe, treatments with 1000–3000 IBA increased this parameter.

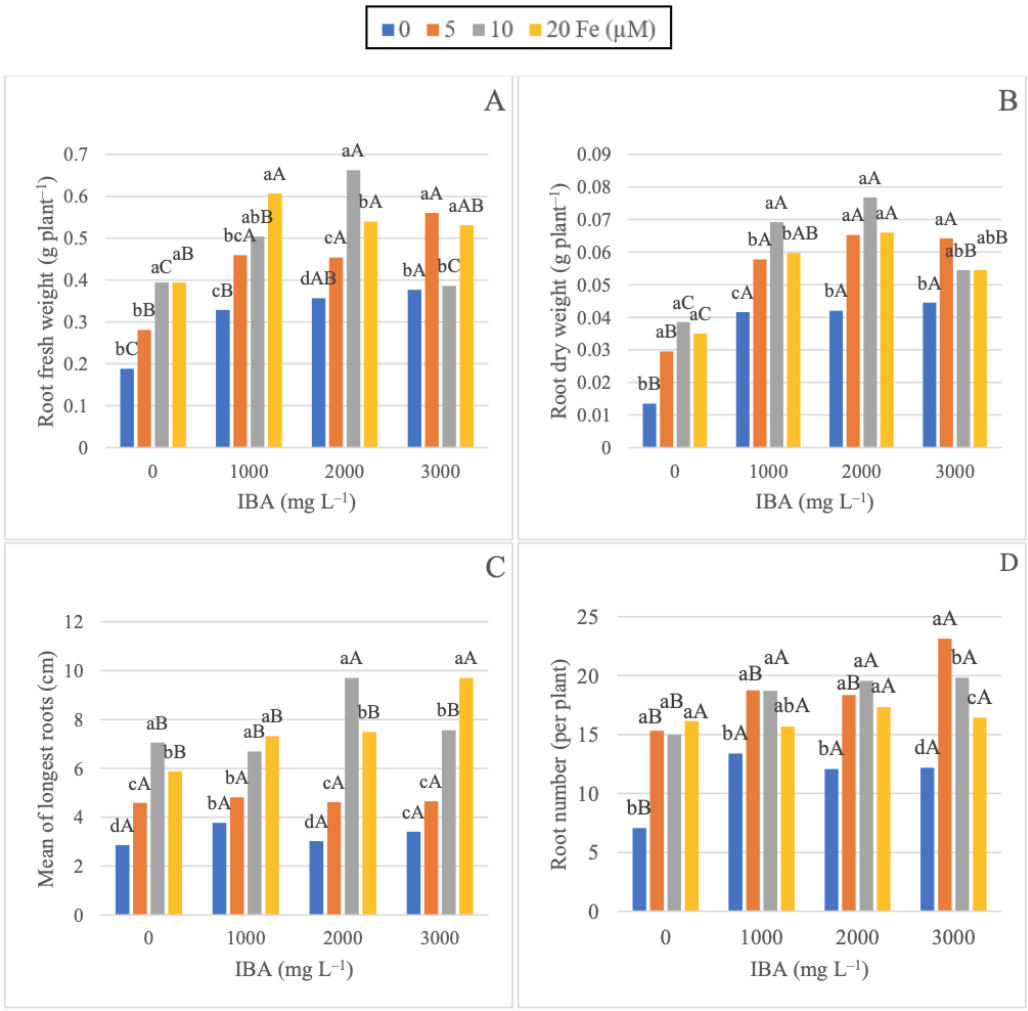

Figure 2. Root parameters 20 days after the rooting induction treatments in *Rosmarinus officinalis* stem cuttings. Cuttings were treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment and then grown with different concentrations of Fe (0, 5, 10 and 20 µM). Root fresh weight ((**A**), in g $plant^{-1}$), root dry weight ((**B**), in g $plant^{-1}$), mean of longest roots ((**C**), in cm) and root number per plant (**D**). Values shown are means ± SE (n = 4 pots). Letters above the columns indicate significant differences at $p \leq 0.05$ for the Fe treatments in a given IBA treatment (in lower case) and for the IBA treatments in a given Fe treatment (in capitals).

3.3. Application of Fe Increase Root Length and Number in IBA-Treated Root Cuttings

The mean length of the longest roots was in the range 2.9–9.7 cm, and it was markedly affected by the IBA/Fe regimes (Figure 2C). The highest value was observed in the 2000 IBA/10 Fe and 3000 IBA/20 Fe, and the lowest in the 0 Fe treatments. All treatments with Fe increased this parameter in cuttings not treated with IBA, whereas in the absence of Fe treatments with IBA did not have any effect.

The number of roots per plant was in the range 7–23, and it was markedly affected by the IBA/Fe regimes (Figure 2D). The highest value was observed in the 3000 IBA/5 Fe treatment, and the lowest in the 0 IBA/0 Fe. All treatments with Fe increased root number in cuttings not treated with IBA. In the absence of Fe, treatments with 1000–3000 IBA increased root number.

3.4. Application of Fe-Chelate Causes Positive Changes in Shoot Parameters by IBA

Shoot FW was between 2.1 and 4.7 g per plant depending to the treatment (Figure 3A). The highest values were observed in the 1000–3000 IBA/5 Fe, 1000–2000 IBA/10 Fe and 1000 IBA/20 Fe treatments, and the lowest in the 0 IBA/0 Fe control one. All treatments

with Fe increased shoot FW in cuttings not treated with IBA. Treatments with 1000–3000 IBA increased this parameter in the absence of Fe.

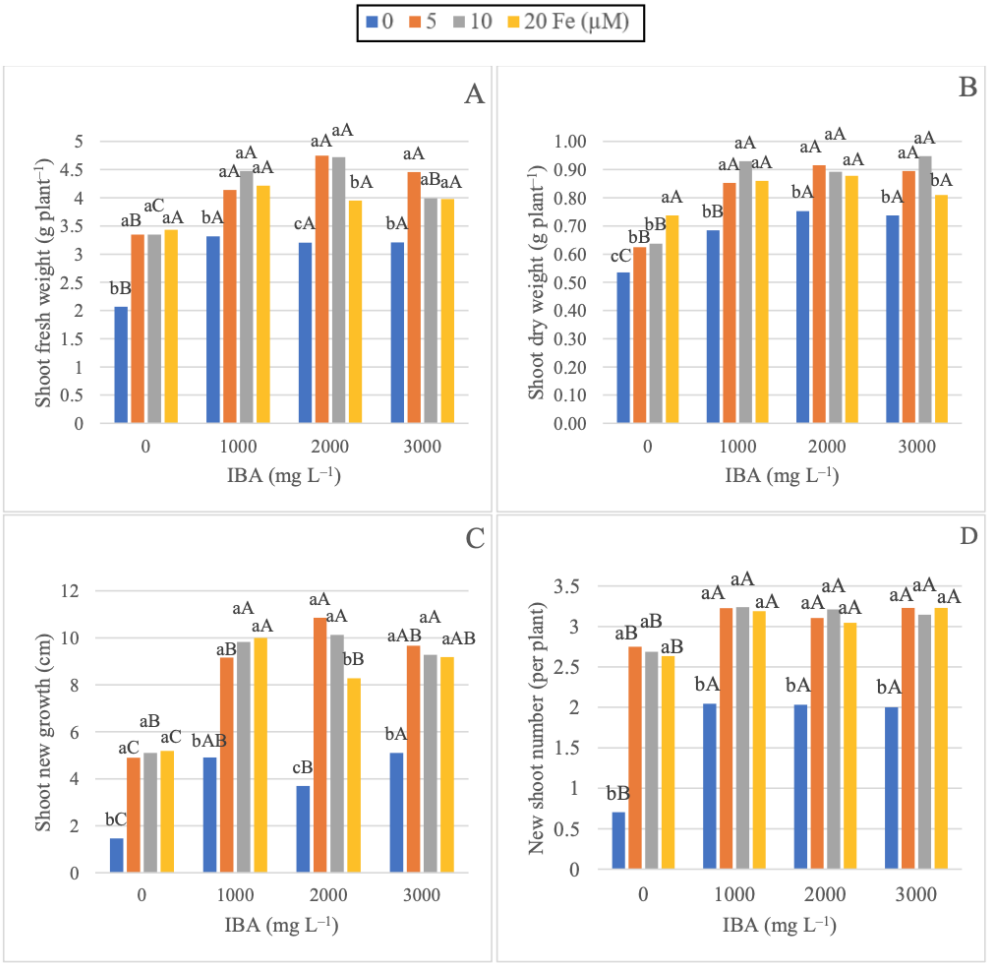

Figure 3. Shoot parameters 20 days after the rooting induction treatments in *Rosmarinus officinalis* stem cuttings. Cuttings were treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment and then grown with different concentrations of Fe (0, 5, 10 and 20 μM). Shoot fresh weight ((**A**), in g $plant^{-1}$), shoot dry weight ((**B**), in g $plant^{-1}$), shoot new growth ((**C**), in cm), and new shoot number per plant (**D**). Values shown are means ± SE (n = 4 pots). Letters above the columns indicate significant differences at $p \leq 0.05$ for the Fe treatments in a given IBA treatment (in lower case) and for the IBA treatments in a given Fe treatment (in capitals).

Total shoot DW was between 0.54 and 0.95 g per plant, and values were markedly affected by the Fe regime (Figure 3B). The highest values were observed in the 1000–3000 IBA/5–10 Fe and 1000–2000 IBA/20 Fe treatments and the lowest in the 0 IBA/0 Fe control. Treatments with Fe increased shoot DW in cuttings not treated with IBA, and in the absence of Fe treatments with 1000–3000 IBA increased this parameter.

Shoot new growth was between 1.5 and 10.9 cm depending on the treatment (Figure 3C). The highest values were observed in the 1000–3000 IBA/5–10 Fe and 1000 and 3000 IBA/20 Fe treatments, and the lowest in the 0 IBA/0 Fe control one. Treatments with Fe increased shoot new growth in cuttings not treated with IBA, whereas in the absence of Fe treatments with 1000–3000 IBA increased this parameter.

The number of new shoots per plant was in the range 0.7–3.2, and it was affected by the IBA/Fe regimes (Figure 3D). The highest values were observed in the 1000–3000 IBA/5–20 Fe and the lowest in the 0 IBA/0 Fe control one. All treatments with Fe increased the number of

new shoots in cuttings not treated with IBA, and treatment with 1000–3000 IBA increased this parameter in the absence of Fe.

3.5. Fe-Chelate Increase Leaf Photosynthetic Pigment Concentration of Cuttings

The concentrations of Chl a, Chl b, total Chl and Car were in the ranges 4.0–9.7, 1.1–6.0, 5.2–15.2 and 0.7–2.5 mg g FW^{-1}, respectively, and values were markedly affected by the IBA/Fe regimes (Figure 4). The highest total Chl was observed in the 2000–3000 IBA/10 Fe and 1000–3000 IBA/20 Fe treatments, the highest Car value was in the 1000 IBA/20 Fe treatment, and the minimum value for all pigments were observed in the 0 Fe treatments. In cuttings not treated with IBA, all treatments with Fe increased the level of Chls, and treatments with 10–20 Fe increased total Car. On the other hand, in the absence of Fe treatments with IBA did not cause any change in the leaf concentration of photosynthetic pigments.

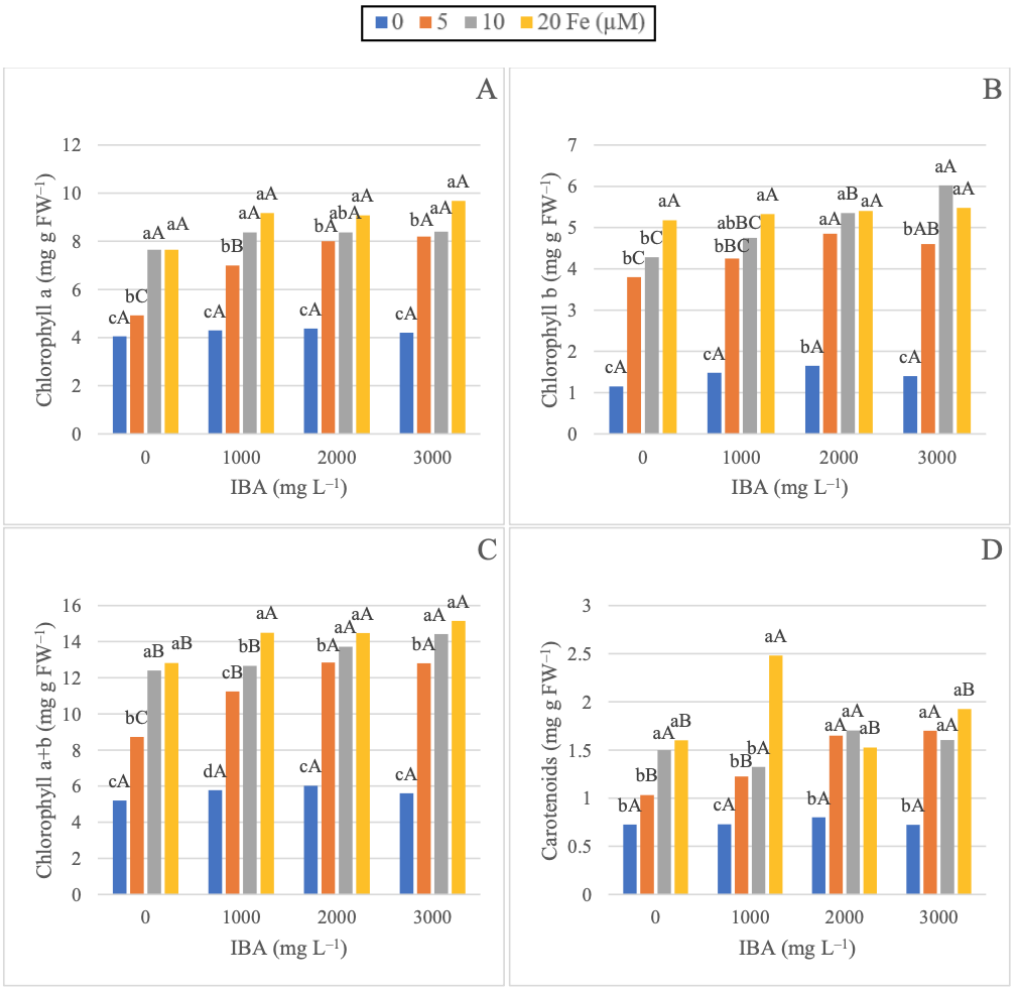

Figure 4. Leaf photosynthetic pigment concentrations 20 days after the rooting induction treatments in *Rosmarinus officinalis* stem cuttings. Cuttings were treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment and then grown with different concentrations of Fe (0, 5, 10 and 20 µM). Chlorophyll a (**A**), chlorophyll b (**B**), total chlorophyll (**C**), and carotenoid (**D**) concentrations. Values shown are means ± SE (n = 4 pots). Letters above the columns indicate significant differences at $p \leq 0.05$ for the Fe treatments in a given IBA treatment (in lower case) and for the IBA treatments in a given Fe treatment (in capitals).

3.6. Cutting Survival Percentage Increased by Applying IBA and Fe-Chelate Simultaneously

Ten days after transfer to the sand:soil substrate, cutting survival was between 35.3 and 89.3%, and values were markedly affected by the IBA/Fe regimes (Figure 5). The highest

values were observed in the 1000–3000 IBA/5–20 Fe and the lowest in the 0 IBA/0 Fe control one. All treatments with Fe increased survival significantly in cuttings not treated with IBA. In the absence of Fe, treatments including 1000–3000 IBA increased survival.

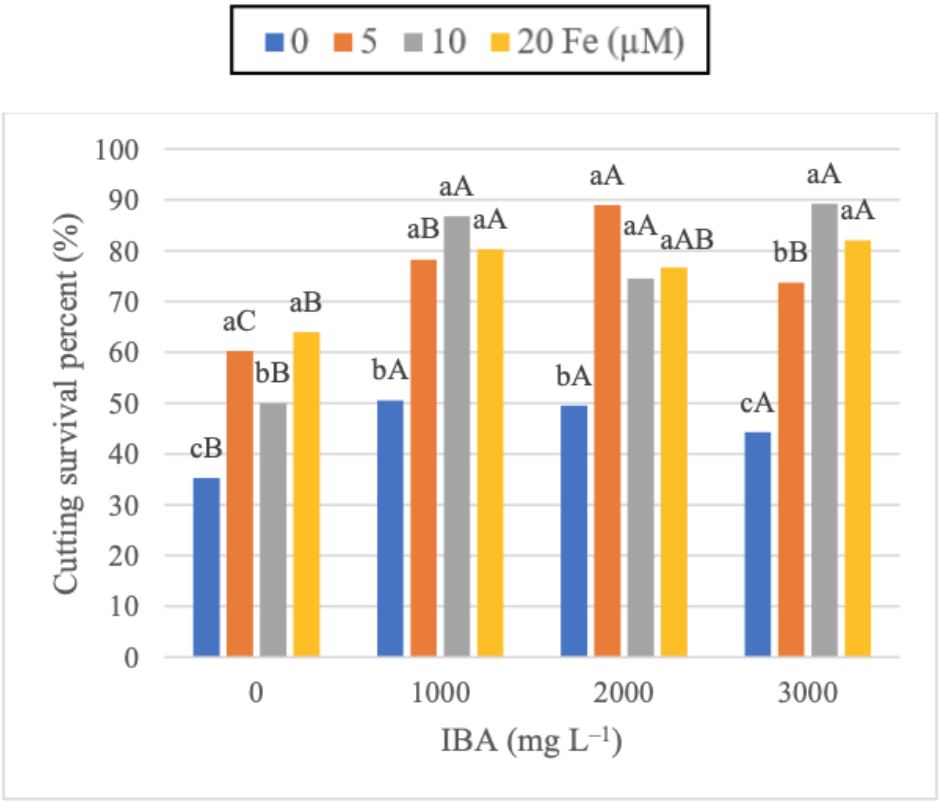

Figure 5. Survival (in %) of *Rosmarinus officinalis* stem cuttings 10 days after transfer to a sand:soil mixture. Cuttings were treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment, then grown for 20 days with different concentrations of Fe (0, 5, 10 and 20 μM) and finally transplanted to the sand:soil mixture. Values shown are means ± SE (n = 4 pots). Letters above the columns indicate significant differences at $p \leq 0.05$ for the Fe treatments in a given IBA treatment (in lower case) and for the IBA treatments in a given Fe treatment (in capitals).

4. Discussion

Results confirm that the application of IBA improves rooting in *R. officinalis* cuttings, in line with previous results obtained in this plant species [30–33], as well as in many other woody plants [7–18]. Data shown here indicate that when Fe supplementation is not used, an IBA concentration of 1000 mg L^{-1} appears to be adequate for *R. officinalis*, since higher IBA concentrations (2000–3000 mg L^{-1}) do not provide any supplementary advantage. Each plant species needs an appropriate concentration of IBA to promote cell proliferation and expansion [19,36], and excessive IBA concentrations may impair development [11,15,37]. For instance, *O. europaea* needs a 3500 mg L^{-1} IBA concentration [11], and 2000 mg L^{-1} IBA induced a higher percentage of adventitious rooting in *Eucalyptus benthamii* [15].

Even in the absence of IBA, Fe(III)-EDDHA supplementation improves to some extent rooting (at 10 days), root biomass and number, shoot biomass, new growth and number of new shoots, and leaf photosynthetic pigment levels (at 20 days), as well as cutting survival (10 days after transplant to sand:soil mixture). The low root biomass in cuttings grown with 0 μM Fe may be related to the auxin increases known to occur in Fe-deficient plants, which usually exhibit in roots morphological changes such as inhibition of elongation and swollen root tips [21,22,38]. The reason behind the positive effects of Fe(III)-EDDHA in the absence of IBA are not known at the current stage, although Fe is a co-factor of peroxidase, which is known to mediate the catabolism of auxin in the rooting process [38]. Evidence for

a role of mineral nutrients (including Fe) in the basal part of the cutting during rooting has been shown in *Petunia* × *hybrida* [4] and *Euphorbia pulcherrima* [39], as well as in the woody species *Eucalyptus globulus* [14], *Prunus persica* [26] and *Pinus taeda* [40]. In *Petunia* × *hybrida* leaf cuttings, it has been shown that adventitious root formation depends on the local provision of Fe, since shoot-to-root translocation of Fe from the aerial part of the cuttings is ineffective [4]. Stimulation of adventitious root development by Fe requires auxin and involves auxin polar transport, but both the fact that spatial distribution and activity of the auxin-reporter GFP-GUS are not affected by Fe supply and the additive effect of Fe and 1-naphthaleneacetic acid suggest that Fe and auxin may have parallel mechanisms of stimulation of adventitious root formation [4].

The Fe-mediated improvements in all parameters studied are generally more marked in the presence than in the absence of IBA. These results confirm the hypothesis that supplementing IBA-treated *R. officinalis* cuttings with 5–20 μM Fe(III)-EDDHA improves rooting (at 10 days), and root biomass and number, shoot biomass and number of new shoots and the leaf levels of photosynthetic pigments (at 20 days), as well as cutting survival (10 days after transplant to sand:soil mixture). Generally speaking, the treatments including 10 μM Fe and 2000 mg L^{-1} IBA appear to give adequate values for most parameters measured.

Application of Fe increased leaf photosynthetic pigment levels, in line with previous studies with other plant species [41,42], including woody ones such as *Pyrus communis* [43] and *P. persica* [44]. The increase with Fe was more marked for Chl b than for Chl a and Car, also in agreement with previous studies [43]. Iron plays roles in chlorophyll [45] and carotenoid biosynthesis [46] and is also part of many components in the chloroplast thylakoid membrane, which can be assembled only when all of them are present [45,47]. For instance, *Calendula officinalis* grown under low Fe showed decreases in Chl and Car concentrations under low Fe in the growth media [48,49]. An increase in photosynthetic pigment levels leads to higher photosynthetic rates, and therefore increases the resources for the formation and development of the root system. This would better facilitate water and nutrient uptake, therefore favouring plant survival [42,50].

In the present study, shoot new growth, number of new shoots, and shoot FW and DW were positively correlated with the root number (R^2 values of 0.70, 0.76, 0.72 and 0.64, respectively, data not shown). This is in line with the finding that in IBA-treated *Hibiscus rosa-sinensis* rootstock, propagated using stenting, there was a positive correlation between shoot and root number [51]. This may be caused by a higher cytokinin generation in cuttings with a higher root number. Cytokinins are mainly synthesized in roots and transported to the shoot in the xylem transpiration stream, and they affect many aspects of plant development, including morphogenesis and shoot initiation [52,53].

5. Conclusions

Results indicate that the application of 5–20 μM Fe(III)-EDDHA and 1000–3000 mg L^{-1} IBA can improve rooting, root and shoot biomass, photosynthetic pigment levels and plant survival in cuttings of the aromatic and medicinal species *R. officinalis*. These results show that the application of Fe(III)-chelate during rooting can lead to the production of vigorous new plants in a shorter time. The application of this type of treatment for the propagation of other rare and valuable aromatic and medicinal plant species via cuttings would deserve further studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12020210/s1, Figure S1: Pictures of the cuttings a few days after placing them in pots with the sand:perlite mixture; Table S1: Analysis of variance (ANOVA) of morphological and biochemical traits in *R. officinalis* treated with different concentrations of IBA (0, 1000, 2000 and 3000 mg L^{-1}) at the start of the experiment and then grown with different concentrations of Fe (0, 5, 10 and 20 μM).

Author Contributions: Design of the experiment and methodology, Z.I. and A.R.N.; data analysis, Z.I., A.R.N. and J.A.; writing—original draft preparation, Z.I., A.R.N. and J.A.; writing—review and editing,

Z.I., A.R.N. and J.A.; supervision and project administration, A.R.N. and J.A.; and funding acquisition, A.R.N. and J.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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