forests

MDPI

*Article*

# Improvement of Rooting Performance in Stem Cuttings of Savin Juniper (*Juniperus sabina* L.) as a Function of IBA Pretreatment, Substrate, and Season

**Maliheh Abshahi [1], Francisco Antonio García-Morote [2,*], Hossein Zarei [3], Bahman Zahedi [1] and Abdolhossein Rezaei Nejad [1]**

[1] Department of Horticultural Sciences, Faculty of Agriculture, Lorestan University, P.O. Box 465, Khorramabad 68151-44316, Iran
[2] Department of Science and Agroforestry Technology and Genetics, Higher Technical School of Agricultural and Forest Engineering, University of Castilla-La Mancha, 02071 Albacete, Spain
[3] Department of Horticulture, College of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan 49138-15739, Iran
* Correspondence: fcoantonio.garcia@uclm.es

**Abstract:** *Juniperus sabina* is an interesting species for forest restoration and ornamental purposes. The seeds of this plant have several types of dormancies; therefore, seed propagation is difficult and time consuming. The production of cuttings can be an alternative way to produce plants more quickly. The main objective of this experiment was to determine the best propagation conditions (indole butyric acid dose, substrate, and season) for this species using stem cuttings. Rooting performance of the cuttings was evaluated based on the rooting percentage (%), root biomass, and specific root length (SRL). In addition, we examined the internal composition (auxin and peroxidase content) in treated stem cuttings. Cuttings were pretreated with five doses of indole butyric acid (IBA; 0 (control), 1000, 2000, 4000, and 8000 ppm) and were rooted in four substrates (perlite, perlite-cocopeat, pumice, and mixed substrate) during the four seasons (winter, spring, summer, and autumn). The best treatments, with more than 60% rooting, were applied in spring, and IBA at 1000 ppm in perlite–cocopeat substrate obtained 62% rooting. The highest rooting percentage correlated with the highest root biomass production and the lowest SRL. IBA pretreatment decreased the concentration of peroxidase in spring (coinciding with maximum rooting), representing an indicator of rooting performance. Based on these results, we recommend a new protocol for *Juniperus sabina* production: (i) prepare cuttings in spring, (ii) treat cutting bases with 1000 ppm IBA, and (iii) plant cuttings in a substrate of perlite–cocopeat (1:1).

**Keywords:** auxin concentration; juniper production; peroxidase concentration; root development; vegetative propagation



**Citation:** Abshahi, M.; García-Morote, F.A.; Zarei, H.; Zahedi, B.; Nejad, A.R. Improvement of Rooting Performance in Stem Cuttings of Savin Juniper (*Juniperus sabina* L.) as a Function of IBA Pretreatment, Substrate, and Season. *Forests* **2022**, *13*, 1705. https://doi.org/10.3390/f13101705

Academic Editors: Carol Loopstra, Ricardo Javier Ordás and José Manuel Álvarez Díaz

Received: 30 July 2022
Accepted: 13 October 2022
Published: 16 October 2022



## 1. Introduction

The genus *Juniperus* comprises about 50 species of coniferous trees and shrubs widely distributed throughout the temperate and subtropical regions of the Northern Hemisphere. Savin juniper, or Savin (*Juniperus sabina* L.; Cupressaceae), is native to the mountains of Central and Southern Europe and Western and Central Asia, from Spain to Eastern Siberia, typically growing at altitudes of 700–3300 m [1,2]. The species has its origin in the remote times of the Tertiary, when it grew in colder climates. It is well adapted to continental climates with very cold winters and hot, dry summers, which are typical of semi-arid climates [3].

This species can be utilized for forest restoration on poor sites with low potential productivity, such as arid and semi-arid areas. This plant is native to mainly steep slopes, and its increased strength in these conditions is due to its deep roots) which can penetrate up to one meter), making the plant last on slopes over 30° [4]. In addition, Savin is one of

the most beautiful juniper species and is suitable for ornamental use [5]. Thus, information about *Juniperus sabina* plant production could be useful for forest managers and plant producers in some areas of Europe and Asia.

Seed propagation of *Juniperus* is difficult and time consuming [2]. The sexual regeneration of *Juniperus sabina* is low due to poor seed quality [6], and reproductive propagation is difficult due to the prolonged period between pollen production and fertility (fertilization), seed loss, and the long duration of seed dormancy [7]. The success of asexual propagation using rooting cuttings of juniper branches is often reported as less than 30% [8]. To enhance vegetative propagation in juniper, using a tissue culture system based on micropropagation and in vitro culture can be an option to produce plants [8]. However, in conifers, the rapid loss of the callus multiplication capacity under in vitro culture is a serious problem [9]. In this sense, the production of cuttings can be an alternative way to produce plants of this conifer more quickly in a greenhouse. However, problems with the asexual propagation of *Juniperus* species by rooting vegetative cuttings has not been well studied, and the rooting efficiency of juniper branch cuttings is often less than 30% [9]. For this reason, we think that it is necessary to investigate new techniques or protocols for the vegetative reproduction of this species.

To propagate plants via cuttings, the growth regulator indole butyric acid (IBA) has been widely used as pretreatment [10]. In general, treated cuttings have shown higher rooting percentage and number of roots and longer root length than untreated cuttings, which indicates a strong influence of IBA on rooting capacity [11]. Thus, to produce junipers by stem cuttings, IBA was used in previous studies on *Juniperus* genus [12,13]. A certain amount of indole acetic acid (IAA) may also be necessary for root formation [11]. IAA is the most important auxin, and seems to be an important internal factor for the regulation of adventitious root formation, and it is a stimulant hormone [14]. During the propagation of plants, the amount of IAA (as an internal hormone) can vary based on the time the cuttings are prepared for rooting [15,16]. Measuring the amount of internal IAA in treated cuttings can show the effect of the treatment. The peroxidase enzyme level is another indicator because a high level of peroxidase decreases rooting percentage [17]. In fact, peroxidases are often introduced as biochemical markers [18]. Moreover, in plants with hydric stress, increasing the peroxidase, will decrease the percentage of rooting [17].

Previous studies also reported differences in rooting of cuttings affected by substrate [19]. Analyzing the rooting of *Juniperus horizontalis*, Hong-wei et al. [20] found that the best substrate was 1:3 ($v/v$) vermicelli and 2:3 ($v/v$) perlite, with 36% rooting. In the study of Sabina et al. [21] on *Juniperus sabina*, the results showed that a substrate containing 60% foliar soil (fragmented leaves of the trees) and 40% fragmented oats provided a suitable growth rate. The size and age of cuttings can also be important factors to maximize rooting and cutting growth. Maintaining stock plants at 15 cm height sometimes reduced the production of stem cuttings, but often increased the percentage of cuttings that formed roots by 30–53% [22]. Choosing the right time to achieve the best rooting is also important. For example, the best rooting of *Juniperus virginiana* was from cuttings collected in winter [13].

Finally, to evaluate the level of rooting of cuttings, it is necessary to measure root morphology and development. Roots take up minerals and water from the soil, and larger amounts of adventitious roots could improve the root system symmetry, stability, survival, and growth rate [23]. Thus, root biomass should be a good indicator of the growth strategy of root development and the capacity to endure water stress in juniper trees [24]. Another indicator is the specific root length (SRL; calculated as root length per unit of root biomass), which depends on root diameter and tissue density [25]. Roots with high SRL have a high surface-to-volume ratio for the same C investment. This maximizes the root–soil interface, and a high SRL can be achieved by having roots with a small diameter to maximize water uptake [26]. For this, both root production and structure can be considered fundamental parameters to assess the level of rooting in plant material.

Therefore, the present study was intended to investigate an efficient method (dose of IBA and type of substrate) for vegetative propagation of Savin juniper using stem

cuttings, and the effects on morphological (root development) and phytochemical (auxin and peroxidase) characteristics. We also hypothesized that several parameters of roots (root biomass and specific root length) and the levels of auxins and peroxidase could be indicators of rooting performance in cuttings of *Juniperus sabina*. Thus, the main objective of this research was to analyze the effects of five concentrations of IBA as pretreatment and four substrate types (perlite, perlite–cocopeat, pumice, and mixed substrate) on the rooting performance and levels of auxin and peroxidase in cuttings. The experiment was conducted in the four seasons of the year to determine the effects of harvesting time on the rooting capacity of cuttings.

## 2. Materials and Methods

### *2.1. Cutting Preparation, Pretreatment with Indole Butyric Acid (IBA), and Substrate Composition*

Cuttings of *Juniperus sabina* were sampled from its natural habitat in the Chaharbagh mountains of Gorgan (northern Iran; Figures 1 and 2), one of the main Mediterranean populations at higher altitude (2700 m.a.s.l.).


**Figure 1.** Worldwide distribution of different populations of *Juniperus sabina*, in gray [27]; sampling area of collected stem cuttings is in red circle (Northern Iran).

The climate in the study area is cold and semi-arid, type BSk [28]. Based on 30-year averages, the mean annual temperature at the site is 9.2 °C and the mean annual precipitation is 429 mm. Temperature extremes (in summer and winter) range from 23 °C to −5 °C (data from Gorgan climatic station, 46°06′ N, 28°00′ W, 2600 m.a.s.l.). Soils are sandy loam. In this area, the mean age of Savin juniper plants is 50 years. The size of crowns is approximately 2 × 2 m (length × width; Figure 2). The ring diameter of shrubs is on average 20.0 cm, and the height is 1.5 m (these are a type of old and horizontal shrub; Figure 2). Generally, 20 male shrubs were used for this experiment, growing in the same area with a similar environment (climate and soil). The experiment was conducted at Gorgan University of Agricultural Sciences and Natural Resources in winter 2016 and spring, summer, and autumn 2017. Stem cuttings were only collected from the upper crown in male trees.

The harvesting of cuttings took place in the morning. After harvesting, stem cuttings 15 cm in length and 0.5–0.7 cm in diameter [29] were prepared for treatment and cultivation in a greenhouse (Figure 2). Substrates were prepared, and cuttings were placed in the greenhouse equipped with an automatic system to control humidity (micro-irrigation) and temperature at the root level. The average daily temperature during the experiment was 22 °C, and average relative humidity was 77%. The photoperiod was based on nature in each season.

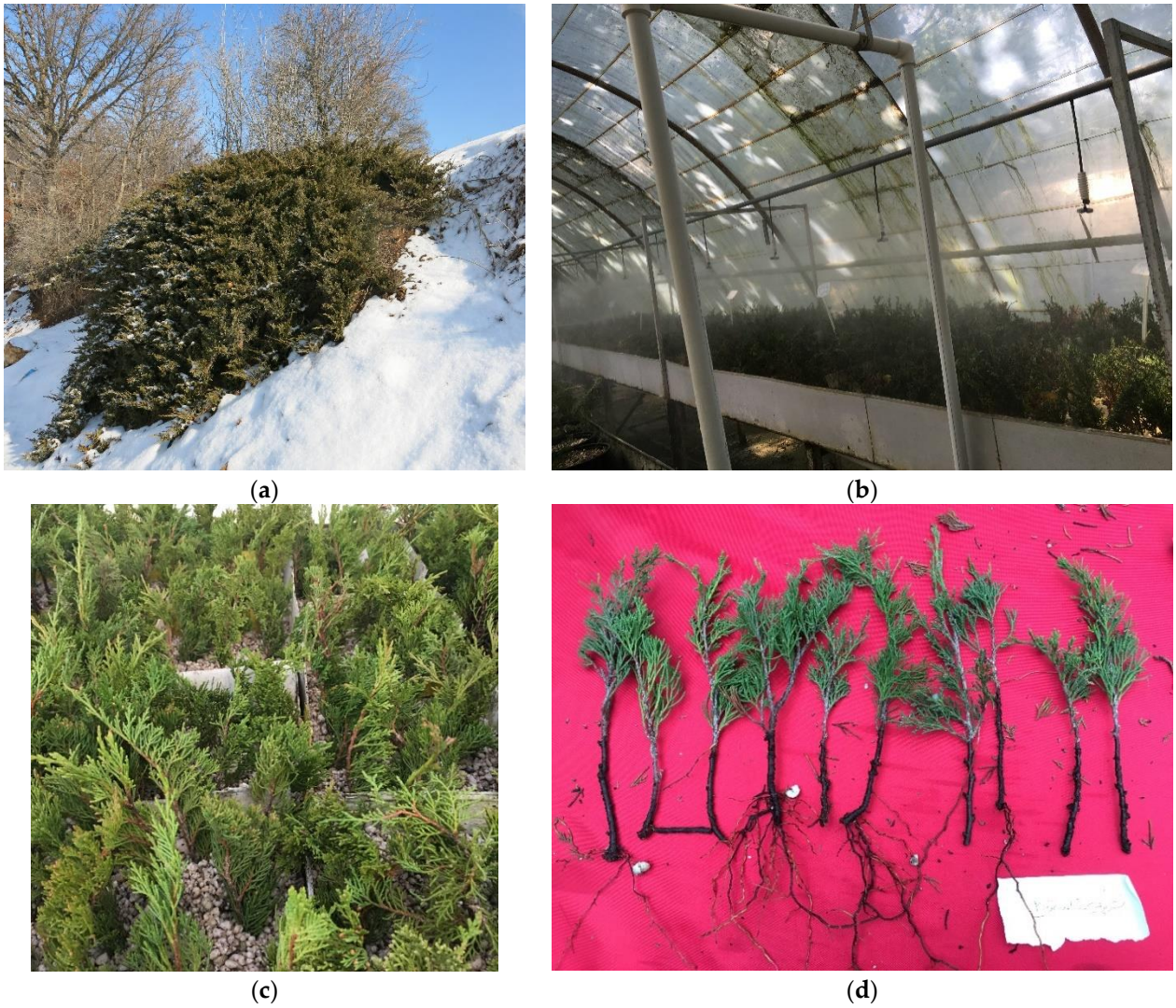

**Figure 2.** (**a**) Example of Savin juniper plants for collection of stem cuttings in study area; (**b**) greenhouse used to culture stem cuttings under different treatments; (**c**) cuttings growing on substrate; (**d**) example of rooted and unrooted stem cuttings of Savin juniper.

For the pretreatment of stem cuttings, 5 IBA concentrations (Merk, Darmstadt, Germany) were used: 0 (control), 1000, 2000, 4000, and 8000 parts per million (ppm). The base of each cutting was placed in IBA for 5 s and then inserted into the substrate. The 4 substrates were (i) perlite; (ii) mixed rooting substrate, a combination of sand (20%), perlite (20%), cocopeat (20%), vermicompost (20%), and potash (20%); (iii) perlite–cocopeat (1:1); and (iv) mineral pumice (all substrates were developed at the University of Gorgan, Golestan, Iran). For each treatment (combination of pretreatment and substrate), 3 replicates were prepared, with 9 cuttings per replicate. Thus, a total of 540 cuttings were planted in the greenhouse each season (Figure 2).

### *2.2. Rooting Performance: Rooting (%), Root Biomass, and Specific Root Length (SRL)*

To determine the rooting percentage, roots were counted in all rooted cuttings in each treatment (Figure 2). The root length (in mm) was obtained by separating the roots from the stem cuttings and then measuring them with a digital caliper (Mitutoyo, Kanagawa, Japan). To measure the root biomass (dry weight in grams), the roots of each cutting after separation were placed in an oven (Unico, E. Dayton, NJ, USA) at 70 °C, and after 24 h, they were weighed separately. To measure average root biomass, the dry weights of all roots

of cuttings in each treatment were combined, and the total was divided by the number of cuttings. To calculate the specific root length (SRL; cm $g^{-1}$), the length of roots (cm) was divided by the root biomass (g) [30].

### 2.3. Internal Chemical Compounds of Stem Cuttings: Auxin and Peroxidase Enzyme Concentration

Internal auxin (μg $g^{-1}$) was measured following the methodology of Sridhar et al. [31], with slight modification. First, 5 g of plant sample (bark or periderm of the end part of stem cuttings for each treatment) was ground into powder and filtered with 10 mL methanol (Merk, Darmstadt, Germany). After adding methanol several times, it was evaporated in a rotary evaporator (Unico, E. Dayton, NJ, USA) at 30 °C.

In the next step, the followed materials were added (all made by Tetrachem Company, Cardiff, UK): 10 mL of potassium phosphate, 10 mL of petroleum spirit (3 times), 10 mL of petroleum spirit, and 3 mL of phosphoric acid. Then 10 mL of this solution was extracted, and these materials were added: 10 mL of potassium phosphate solution, phosphoric acid (0.28 M), and 10 mL of ethyl ether. The ether was evaporated in the rotary evaporator. The residue was dissolved in 5 mL of methanol at low temperature, and 0.2 mL of tri-fluoro acetic acid and 3 mL water were added to each sample.

The amount of internal auxin was measured with a spectrophotometer (Unico, E. Dayton, NJ, USA) at 440 and 490 nm wavelengths. Samples of unrooted and rooted cuttings totaled 152 and 88, respectively.

To measure the level of peroxidase enzyme in stem cuttings (mg $g^{-1}$), 1 g of leaves from cuttings of each treatment was weighted. The following combination was prepared (all chemical materials made by Tetrachem Company, Cardiff, UK) [31]: 1.2 g of Tris, 2 g of ascorbic acid, 3.8 g of sodium borate, 2 g of ethylene diamine tetra acetate (EDTANa), and 50 g of polyethylene glycol 2000, mixed with distilled water to achieve a volume of 100 mg $L^{-1}$.

Each plant sample was mixed with 4 mL of the above solution and placed on a shaker, then kept in a refrigerator (LG, Seoul, South Korea) at 4 °C. In the next step, the samples were centrifuged (Unico, E. Dayton, NJ, USA), then filtered. In the final step, 0.1 mg $g^{-1}$ of each plant sample was combined with 2 mg of buffer acetate 0.3 M (pH = 5.0), 0.4 mL of 3% hydrogen peroxide, and 0.2 mL of benzidine dissolved in alcohol at 50 °C (0.01 M). Using the spectrophotometer at a wavelength of 530 nm, they were measured against the control. The total numbers of samples for unrooted and rooted cuttings were 122 and 67, respectively.

Auxin and peroxidase were measured in rooted and unrooted stem cuttings to detect differences in the internal compounds between cuttings that have the capacity for rooting and those that do not. To evaluate the treatments and compare the chemical compounds in cuttings at the beginning of sampling and the amount of increase or decrease between the time of planting and rooting (between the beginning and end of each season), samples were taken from freshly harvested cuttings in each season (the first of each season) and compared with the results at the end of the season.

### 2.4. Statistical Analysis

A factorial arrangement of treatments [32] was applied to analyze the effects of the 3 main factors on the 5 dependent variables. The first factor was pretreatment or a concentration of IBA (5 levels: 0, 1000, 2000, 4000, and 8000 ppm), the second was substrate (4 types: perlite, perlite–cocopeat, pumice, and mixed substrate), and the third was season (winter, spring, summer, and autumn). This represents a 5 × 4 × 4 factorial design. The dependent variables were the indicators of rooting performance (% of rooting, root biomass, and SRL) and internal compounds of unrooted and rooted cuttings (auxin and peroxidase enzyme).

In addition, for the dependent variables concerning internal chemical compounds, another level was added as pretreatment, fresh samples (stem cuttings not planted and prepared at the beginning of each season), in order to compare the effects of treatments between untreated and treated cuttings at the end of each season.

SAS® statistical software (SAS Institute Inc., Cary, NC, USA) was used to detect significant factors and to compare mean values between factors and treatments. Means were compared using the PROC GLM procedure. We utilized multifactor analysis of variance (3-way ANOVA) at a probability level of 5% ($p < 0.05$). The analysis within seasons was performed by 2-way ANOVA (excluding season as a main factor in the complete model). We performed independent ANOVAs (not mixed-design or repeated-measures) because the measurements were independent (different stem cuttings were used for each treatment and season). Three-level interactions were not performed because the degrees of freedom were cero in all cases.

Fisher's least significant difference (LSD) test ($p < 0.05$) was used to determine significant differences between treatments [33]. To apply this statistical method, it is desirable for the data to be normally distributed. This is not the case for proportions, which have values that range between 0 and 1. In addition, errors must be independent and normally distributed with constant variance. To ensure that these assumptions were met, logarithmic transformation was used [21]: for % of rooting, the analyzed variable was [ln(r + 0.5)], where r is the percentage of rooting (divided by 100). As this transformation requires numerical data above zero, a small number (0.5) was added to this variable before transformation. The other dependent variables were normally distributed.

## 3. Results

### *3.1. Rooting Performance*

Table 1 shows that IBA pretreatment and substrate composition significantly affected rooting percentage and SRL, whereas season of collection significantly affected all the dependent variables ($p < 0.0001$ in all cases). There were also several significant interactions, but only the pretreatment × season interaction significantly affected the three dependent variables (Table 1). These results confirmed a strong seasonality (harvesting time) of the rooting capacity of stem cuttings.

**Table 1.** Results of multifactor ANOVA of effect of main factors on rooting performance of cuttings across four seasons. Shown are *p*-values for three principal effects (pretreatment with IBA, substrate, and season, and their two-way interactions) and effects within each season (pretreatment, substrate, and their interaction). Effects were considered significant when $p < 0.05$. A total of 540 stem cuttings were planted each season.

| | | Growing Season | | | | Annual Values |
|---|---|---|---|---|---|---|
| **Variables** | **Effects** | **Winter** | **Spring** | **Summer** | **Autumn** | |
| Rooting (log-transformed units) | Pretreatment | 0.10 | <0.001 | 0.0005 | 0.11 | <0.0001 |
| | Substrate | 0.91 | 0.03 | 0.22 | 0.08 | <0.0001 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | 0.40 | 0.24 | * | * | <0.0001 |
| | Pretreatment × Season | - | - | - | - | <0.0001 |
| | Substrate × Season | - | - | - | - | <0.0001 |
| Root biomass (g) | Pretreatment | <0.0001 | 0.63 | <0.0001 | 0.04 | 0.39 |
| | Substrate | 0.30 | 0.01 | 0.11 | 0.29 | 0.08 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | 0.64 | 0.04 | * | * | 0.76 |
| | Pretreatment × Season | - | - | - | - | 0.01 |
| | Substrate × Season | - | - | - | - | 0.87 |
| SRL (cm $g^{-1}$) | Pretreatment | <0.0001 | 0.04 | <0.0001 | 0.01 | <0.0001 |
| | Substrate | 0.08 | 0.29 | 0.02 | 0.95 | <0.0001 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | 0.32 | 0.86 | * | * | 0.93 |
| | Pretreatment × Season | - | - | - | - | <0.0001 |
| | Substrate × Season | - | - | - | - | <0.0001 |

* Summer and autumn: there were not enough living cuttings for analysis because many cuttings dried.

In this sense, the best root-growing season for cuttings of *Juniperus sabina* was spring (Figure 3). In spring, 502 cuttings rooted, and 38 cuttings did not root. In this season, the rooting of cuttings was 51.6 ± 9.3% (mean ± LSD interval; Figure 3a) with 4000 ppm IBA, but no significant difference was seen at 1000 and 2000 ppm ($p < 0.05$). Therefore, the best IBA level was 1000 to 4000 ppm. In the other seasons, rooting was very weak. In winter, cuttings in four treatments rooted (99 cuttings), and 441 cuttings did not root; in summer, cuttings in three treatments rooted (89 cuttings), and 451 cuttings did not root; and in autumn, cuttings in three treatments rooted (102 cuttings), and 438 did not root.

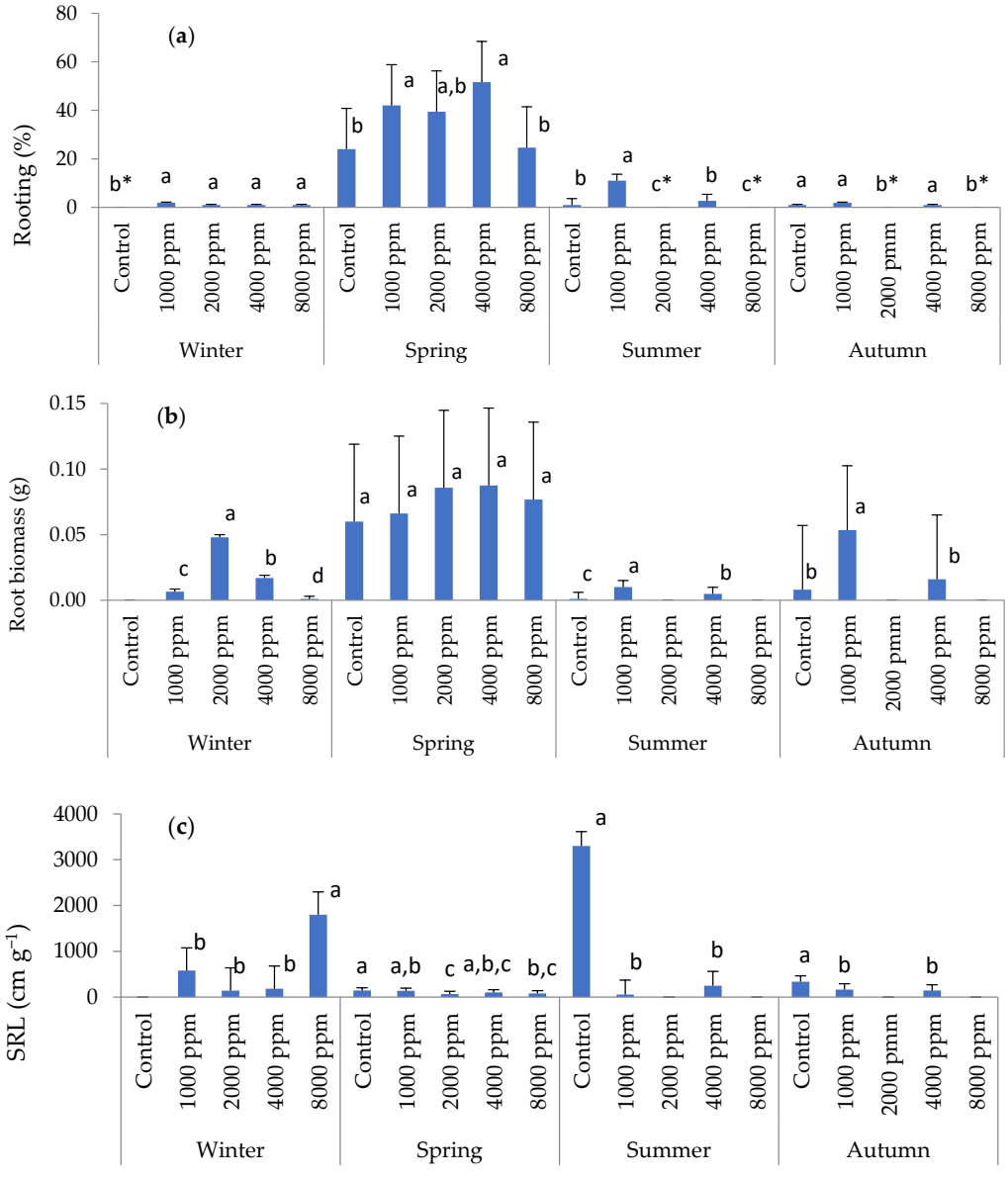


**Figure 3.** Mean values of rooting performance in rooted cuttings within seasons and with five indole butyric acid (IBA) pretreatments: (**a**) % of rooting, (**b**) root biomass, and (**c**) SRL. Mean values with the same letter do not differ at 0.05 level according to LSD test. Sample data = 540 cuttings in each season. Treatments in which all cuttings dried are not represented in the figure. Error bars: LSD intervals. * Rooting percentage was zero.

The highest biomass of roots also occurred in spring (0.088 ± 0.08 g at 4000 ppm IBA; mean ± LSD interval; Figure 3b), and there were no significant differences between treatment levels in this season. The lowest root biomass occurred with the control treatment in summer, and with 8000 ppm IBA in winter (Figure 3b). In addition, the highest SRL

occurred with the control treatment in summer (3300 ± 314 cm $g^{-1}$), whereas the lowest occurred with the treatments in spring (67.1 ± 20.3 cm $g^{-1}$ at 2000 ppm IBA (mean ± LSD interval); Figure 3c). Thus, the combination of no pretreatment of cuttings in the drier season increased SRL as an adaptation to capture water and nutrients under stress.

In several replicates, all of the cuttings dried, thus there were not sufficient cuttings to analyze chemical compounds. In our study, after harvesting the cuttings, a rooting percentage of zero was obtained with pretreatments of control in winter, and 2000 and 8000 ppm in summer and autumn, respectively (Figure 3a). This was due to the cuttings drying during maintenance in the substrates during those seasons.

The best substrate for % of rooting as a function of IBA level was perlite–cocopeat, with a maximum of 62.0 ± 15.6 % (mean ± LSD interval) at 1000 ppm IBA (Figure 4a). In the pumice substrate, the root biomass was significant with 2000 and 8000 ppm IBA (Figure 4b).

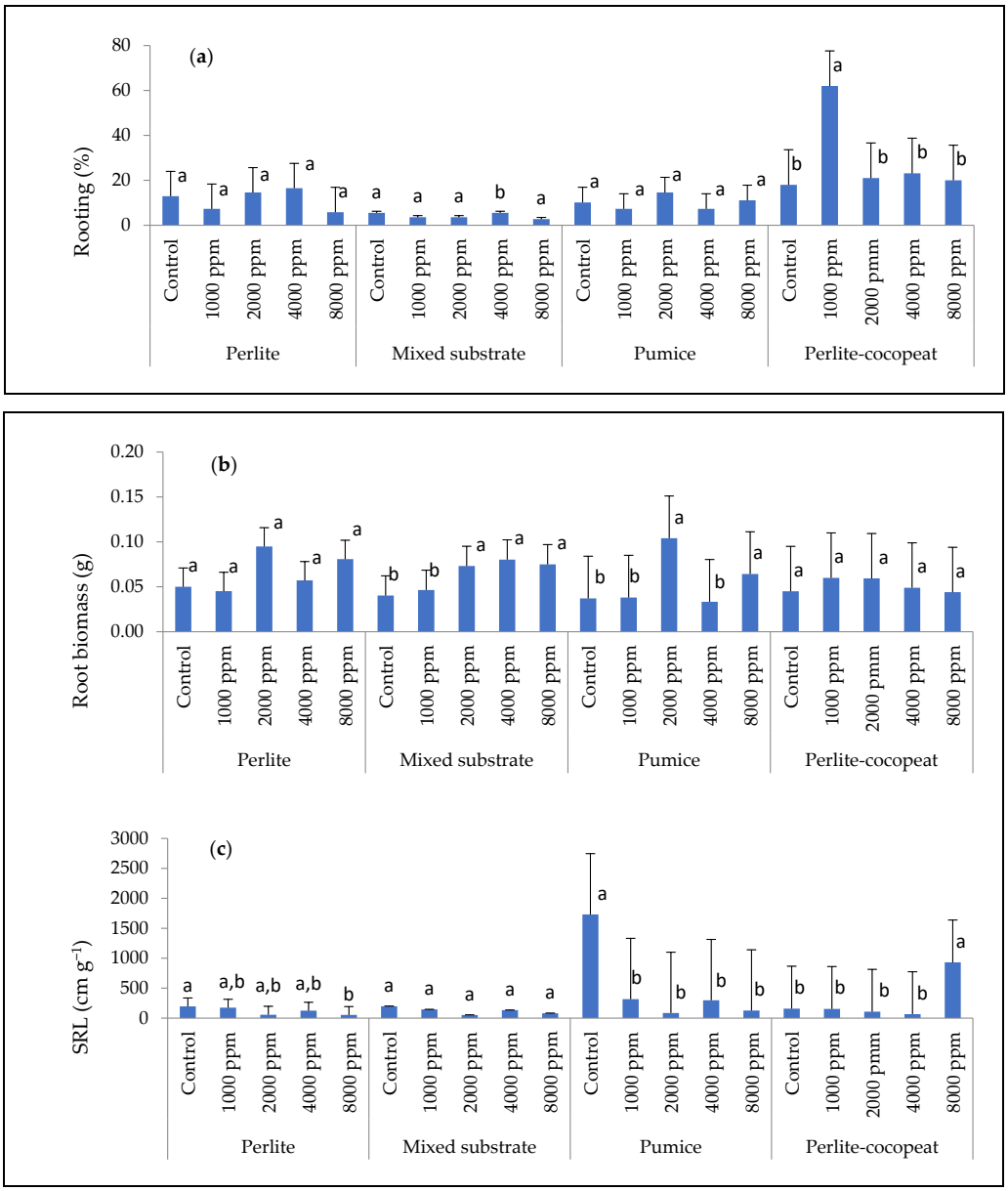


**Figure 4.** Mean values of rooting performance in rooted cuttings within substrates and with five IBA pretreatments: (**a**) % of rooting, (**b**) root biomass, and (**c**) SRL. Mean values with the same letter do not differ at 0.05 level according to LSD test. Sample data = 540 stem cuttings in each substrate. Error bars: LSD intervals.

There were no significant differences between the levels of root biomass in perlite and perlite–cocopeat substrates. In the mixed substrate, root biomass was significantly higher with 2000 to 8000 ppm IBA than control (Figure 4b). On the contrary, the highest SRL occurred with the control treatment in pumice and with 8000 ppm IBA in perlite–cocopeat (Figure 4c). Thus, it is notable that when rooting reached low levels, these values correlated with high SRL values.

Finally, the best rooting percentage also occurred in spring and in the perlite–cocopeat substrate (65.9 ± 9.3% (mean ± LSD interval); Figure 5a). This result was similar to that obtained in the previous analysis, and the combination of 1000 ppm, perlite–cocopeat, and spring resulted in a higher rooting value (62.3 ± 1.2% (mean ± standard error; third-order interaction value)). With this combination of factors, a rooting percentage of zero was obtained with mixed substrate in winter, perlite and mixed substrate in summer, and mixed substrate and perlite–cocopeat in autumn (Figure 5a).

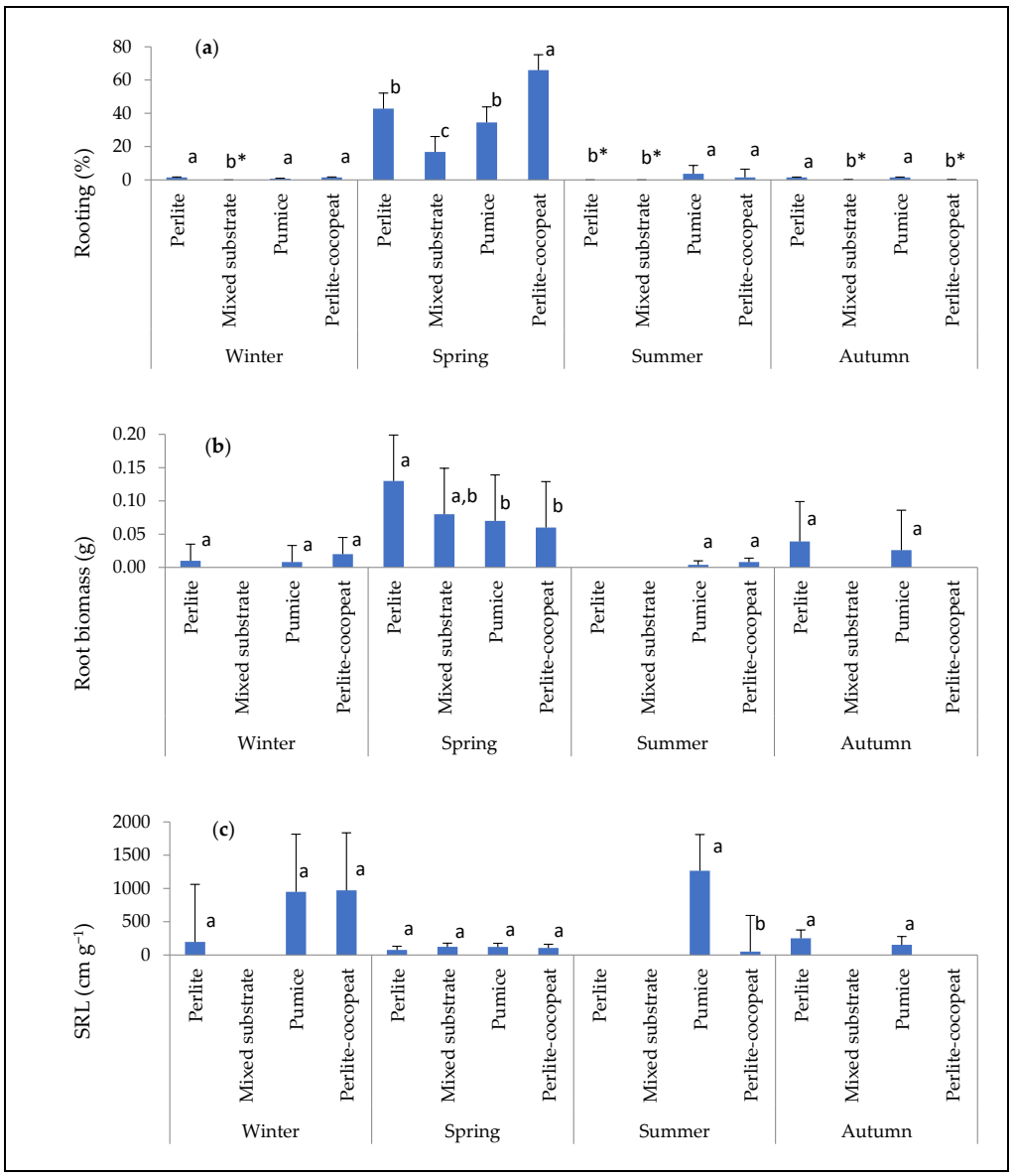


**Figure 5.** Mean values of rooting performance of rooted cuttings within seasons and with four substrates analyzed: (**a**) % of rooting, (**b**) root biomass, and (**c**) SRL. Mean values with the same letter do not differ at 0.05 level according to LSD test. Sample data = 540 cuttings in each season. Error bars: LSD intervals. *Rooting percentage was zero.

In summer, rooting only occurred in pumice and perlite–cocopeat, with an average of <5%. In autumn, cuttings rooted only in pumice and perlite (<3%), whereas in winter, rooting occurred with substrates of perlite, pumice, and perlite–cocopeat, with an average of <2% (Figure 5a). The greatest root biomass occurred in the spring, and the lowest in summer and winter (Figure 5b). The highest SRL occurred in pumice in summer (again in the warmer season) and perlite–cocopeat and pumice in winter (Figure 5c). On the contrary, in spring, SRL was low in all substrates (Figure 5c). So, in spring, when rooting percentage was the highest, SRL was lowest in all substrates. Additionally, the highest SRL calculated in winter and summer also correlated with a low rooting percentage.

### *3.2. Auxin and Peroxidase Concentration in Cuttings*

Pretreatment with IBA significantly affected the levels of peroxidase enzyme in both rooted ($p < 0.00$) and unrooted ($p = 0.009$) stem cuttings (Table 2).

**Table 2.** Results of multifactor ANOVA of effects of main factors on auxin and peroxidase composition of stem cuttings across seasons. *p*-values for principal effects (IBA pretreatment, substrate, and season, and their two-way interactions) and for effects within each season (pretreatment, substrate, and their interaction) are given. Effects were significant at $p < 0.05$.

| | | Growing Season | | | | Annual Values |
|---|---|---|---|---|---|---|
| **Chemical Compound** | **Effects** | **Winter** | **Spring** | **Summer** | **Autumn** | |
| Auxin (μg $g^{-1}$) (Rooted cuttings) | Pretreatment | 0.02 | 0.33 | 1.00 | 0.22 | 0.95 |
| | Substrate | 0.69 | 0.05 | 0.99 | 0.94 | 0.97 |
| | Season | - | - | - | - | 0.39 |
| | Pretreatment × Substrate | 0.37 | 0.53 | * | 0.98 | 0.99 |
| | Pretreatment × Season | - | - | - | - | 0.69 |
| | Substrate × Season | - | - | - | - | 0.99 |
| Auxin (μg $g^{-1}$) (Unrooted cuttings) | Pretreatment | 0.01 | 0.57 | 0.94 | 0.11 | 0.70 |
| | Substrate | 0.83 | 0.07 | 0.99 | 0.36 | 0.66 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | <0.0001 | 0.38 | * | 0.89 | 0.92 |
| | Pretreatment × Season | - | - | - | - | 0.01 |
| | Substrate × Season | - | - | - | - | 0.99 |
| Peroxidase (mg $g^{-1}$) (Rooted cuttings) | Pretreatment | <0.0001 | <0.0001 | 0.73 | <0.0001 | <0.0001 |
| | Substrate | 0.67 | 0.43 | 0.71 | 0.99 | 0.91 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | 0.62 | 0.86 | * | 0.80 | 0.81 |
| | Pretreatment × Season | - | - | - | - | <0.0001 |
| | Substrate × Season | - | - | - | - | 0.99 |
| Peroxidase (mg $g^{-1}$) (Unrooted cuttings) | Pretreatment | 0.0001 | <0.0001 | 0.13 | 0.0003 | 0.009 |
| | Substrate | 0.47 | 0.14 | 0.99 | 0.77 | 0.86 |
| | Season | - | - | - | - | <0.0001 |
| | Pretreatment × Substrate | 0.96 | 0.0005 | * | 0.43 | 0.68 |
| | Pretreatment × Season | - | - | - | - | <0.0001 |
| | Substrate × Season | - | - | - | - | 0.77 |

* There were not enough living cuttings for analysis because many cuttings dried.

It can be seen in Table 2 that season significantly affected the auxin level in unrooted stem cuttings ($p < 0.000$) and peroxidase enzyme in both rooted and unrooted stem cuttings ($p < 0.00$), whereas substrate was not significant ($p > 0.05$). Regarding interactions, only IBA pretreatment × season was significant for the variables auxin (in unrooted cuttings) and peroxidase (rooted and unrooted cuttings) ($p < 0.05$; Table 2). The results within seasons (two-way ANOVA model) also showed several significant effects (Table 2). IBA pretreatment was significant in winter for all dependent variables ($p < 0.05$), and in spring and autumn for peroxidase enzyme in both rooted and unrooted cuttings ($p < 0.05$). For

rooted cuttings, there was no significant difference in auxin with IBA pretreatment in spring, summer, and autumn.

Figure 6 shows that the highest amount of auxin (1067 ± 337 µg $g^{-1}$ (mean ± LSD interval) occurred in unrooted cuttings at 8000 ppm in winter. The amount of internal auxin in this pretreatment was slightly increased compared with fresh samples in unrooted cuttings. However, the amount of internal auxin in the unrooted cuttings did not change significantly during the rest of the seasons (spring, summer and autumn).

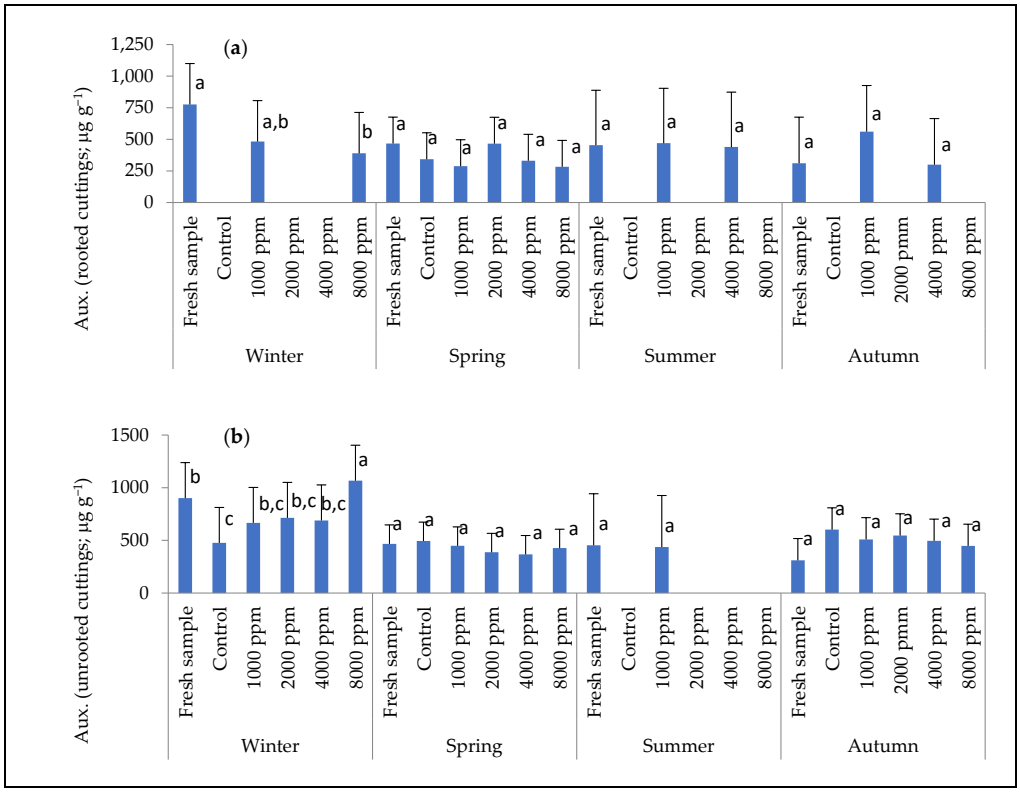


**Figure 6.** Mean auxin levels within seasons with different IBA treatments for (**a**) rooted and (**b**) unrooted cuttings. Mean values with the same letter were not significantly different at the 0.05 level (LSD test). Error bars: LSD intervals.

Due to the cuttings drying during maintenance in the substrates (mainly in winter and summer), there were not enough samples to measure chemical components in several treatments. Therefore, in those treatments, the response variables were not measured and there were no numerical values (Figure 6).

The highest concentration of peroxidase enzyme occurred in winter for both rooted and unrooted stem cuttings (Figure 7), with a maximum value of 0.108 ± 0.03 mg $g^{-1}$ (mean ± LSD interval) for 8000 ppm concentration in unrooted cuttings (Figure 7b). In winter, the lowest peroxidase enzyme level occurred in fresh samples (0.04 mg $g^{-1}$) for both rooted and unrooted cuttings (Figure 7a,b). In spring, the maximum peroxidase enzyme level also occurred in the fresh samples (Figure 7a,b), at the beginning of this season.

In conclusion, we observed interesting relationships between rooting performance, IBA pretreatment, and peroxidase content throughout the seasons. For example, in winter, when rooting % was lower (see Section 3.1), the amount of peroxidase enzyme in rooted cuttings was higher, and in the following season (spring), when rooting was higher, the amount of peroxidase enzyme significantly decreased. In spring, the highest peroxidase enzyme level occurred in fresh cuttings in the early season. On the contrary, in autumn, the lowest concentration occurred in fresh cuttings (early autumn). Therefore, IBA pretreatment seemed to decrease the concentration of peroxidase in spring (coinciding with maximum rooting) and increase the concentration in winter and autumn.

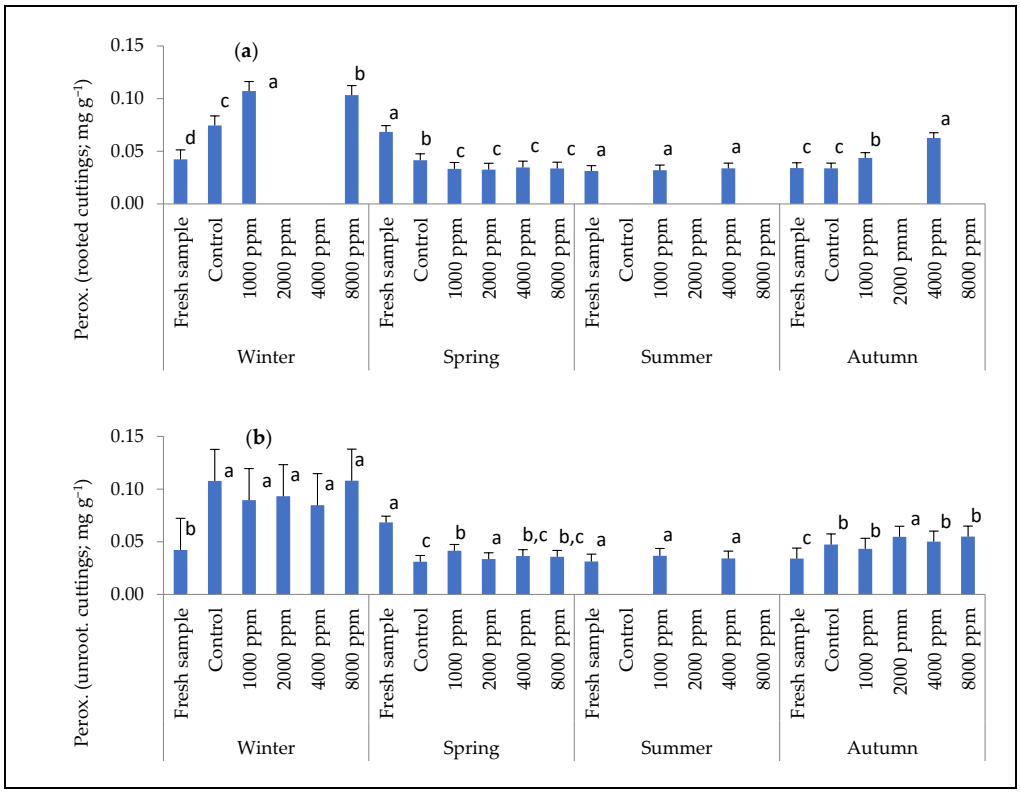


**Figure 7.** Mean peroxidase within seasons with different IBA treatments for (**a**) rooted and (**b**) unrooted cuttings. Mean values with the same letter were not significantly different at the 0.05 level (LSD test). Error bars: LSD intervals.

## 4. Discussion

Our results show, as a novelty, an elevated rooting percentage, greater than 60%, in the best protocol (1000 ppm IBA concentration, perlite–cocopeat substrate, and spring as the harvesting time). In previous studies, the success of rooting cuttings of juniper branches was often reported as less than 30% [8]. For example, only 24% of branch cuttings of *Juniperus procera* obtained from 1.5-to-2-year-old stock plants rooted 32 weeks after treatment, and such low rooting success makes the large-scale propagation of this tree impossible [9].

In our research, pretreatment with IBA increased rooting in stem cuttings, in agreement with previous studies [11,34–36]. This is because the compound induces adventitious roots [23,37] and shortens rooting time [38]. Another function of IBA comes from the direct effect of auxin, because it slowly releases a source of IAA [15,39,40]. Thus, IBA has been used for rooting of *Juniperus* species with different types of treatment, but showing diverse results. For example, the results were best with 8000 ppm of IBA in *Juniperus osteosperma* [41], 5000 ppm IBA for *Juniperus virginiana* [13], 1000 to 9000 ppm in *Juniperus scopulorum* [42], and 6000 ppm in *Juniperus excelsa* [43]. In their research on *Juniperus virginiana*, Henry et al. [13] noted that in preliminary studies, IBA concentrations up to 2000 ppm did not stimulate rooting beyond that obtained with 5000 ppm. The capacity of cuttings can vary among species [44], as shown by our results. In our study, the best result was obtained from intermediate levels of IBA (1000–4000 ppm), and we suggest that IBA at 8000 ppm can damage the cuttings and reduce rooting. Therefore, we do not recommend applying this dose as a pretreatment for cuttings of *Juniperus sabina*.

In general, our results are more in agreement with those of Chowdhuri et al. [45], who proposed an IBA concentration of 1000 to 3000 ppm for cuttings of *Juniperus chinensis*, Rifaki et al. [12], who proposed an IBA concentration of 4000 ppm for cuttings of *Juniperus excelsa*, and Esmaeil et al. [7], who proposed 3000 to 6000 ppm for the same. On the contrary,

our result was not in agreement with Stuepp et al. [46] and Fragoso et al. [47], because the application of IBA was not efficient at propagating cuttings of *Juniperus chinensis*. Nevertheless, the novelty of our results is that the selected concentration of IBA (1000 ppm) was lower.

The substrate is another relevant factor in the production of seedlings from cuttings in a greenhouse [20]. The bottom heat and mist system used in our experiment was also necessary to favor rooting, in agreement with previous research [21,41,48]. In our study, rooting was more than 60% in the substrate of perlite–cocopeat. In the study by Hong-wei et al. [20] on *Juniperus procumbens*, the best substrate was 1.3 (*v*/*v*) vermiculite and 2.3 (*v*/*v*) perlite, with only 36% rooting. The results of our study are also better than those obtained by Cuevas-Cruz et al. [49] in *Pinus*, with 43.5% rooting (in a mixture of peat–perlite–vermiculite substrate); Khoushnevis et al. [35], with 28% rooting in *Juniperus oblonga* (fine and harsh bed); Stuepp et al. [46], with 16% rooting in *Juniperus chinensis* (fine-grained vermiculite and carbonized rice hull, 1:1); and Ayan et al. [50], with 24% rooting in *Juniperus foetidissima*, 31.5% rooting in *Juniperus excelsa*, 38.42% rooting in *Juniperus sabina*, and 31.83% rooting in *Juniperus oxycedrus* (perlite).

In addition to IBA pretreatment, by creating favorable conditions for growth (porosity, cation exchange capacity, salinity, and proper pH), the perlite–cocopeat substrate provides better absorption of water and nutrients for cuttings, resulting in increased maintenance and rooting in comparison with other substrates [51]. This could be due to the differences in substrates in terms of creating suitable conditions for plants, including moisture, soil aeration, and other physical and chemical properties. Due to the high CEC, cocopeat facilitates the storage and distribution of nutrients and improves water management. Perlite also improves aeration in the substrate. In this study, perlite–cocopeat in a 50/50 ratio had moderate water reserve, adequate substrate aeration, and low salinity, and thus could increase the maintenance and rooting of cuttings [52].

Our results reveal that root variables can explain the differences in rooting performance between treatments (and throughout the seasons) because root structure is an adequate indicator of the hydraulic architecture in seedlings [53]. In our research, the worst treatments for rooting percent had the highest values of specific root length (SRL), which occurred in the control treatment in summer, and the lowest occurred in spring. In winter, SLR, especially with 8000 ppm IBA, was high. So, in *Juniperus sabina*, the best time to prepare and plant cuttings for rooting performance is spring. This harvesting time differs from the studies of Guerrero-Campo et al. [54], who found the best rooting of several species of cuttings in different seasons, and Chowdhuri [45], who showed that the best time for rooting *Juniperus chinensis* was summer. On the contrary, our result is in agreement with Fragoso et al. [47] and Tektas et al. [43], who both suggested that the best season for rooting of *Juniperus* is spring.

Roots with a high SRL have a high surface-to-volume ratio for the same C investment, and this can be a strategy to maximize the root–substrate interface, and hence root absorption under water stress, due to the small amount of root biomass available. The persistence and growth of cuttings in seasons with high SRL are compromised, because survival strongly depends on the capacity to take up soil water and nutrients. To counteract their lower root production and maximize seedling survival, cuttings with higher SRL have root traits that confer higher efficiency for the acquisition of soil resources, in agreement with [25]. Thus, the lower SRL values obtained in spring confirm the higher potential of cuttings to take up water and nutrients in this season, and thus the rooting % is higher in spring.

Regarding internal chemical compounds of stem cuttings, Aux/IAA plays various roles in plant growth, such as root development [16,55]. Although Blakesley et al. [56] indicated that auxin content varies by season, in our study, the auxin in rooted stem cuttings was not different between seasons, in agreement with Aliahmad Koruri et al. [18]. Moreover, the results of our study are not opposed to those of Wendling et al. and Blakesley

et al. [22,56] linking rooting to IAA concentration. Those studies reported that a decrease in internal auxin in cuttings was accompanied by a decline in the power of rooting.

Several studies on different species have also shown the important role of peroxidase in controlling growth and rooting [57,58]. In our study, the amount of this enzyme varied in different seasons, and when the peroxidase levels were higher (in winter), rooting % was lower. Increasing this enzyme could reduce the rooting of cuttings, which agrees with studies on other forest species, such as *Phoenix dactylifera* and *Populus tremuloides* [58,59]. In fact, major isoperoxidases are considered to be IAA oxidase; therefore, they are able to change the amount of internal auxin. The root appearance phase (the first visible signs of rooting) is accompanied by a gradual decline in peroxidase activity [58].

Increased rooting after the use of hormonal treatments is related to peroxidase activity inhibitors such as polyphenols [58]. In this regard, peroxidase is the most sensitive plant enzyme to environmental stress [60]. On the other hand, many reports have suggested that increasing IAA accumulation by reducing the amount of IAA oxidase/peroxidase can lead to increased rooting [36,61]. Cuttings can show a decrease in the amount of peroxidase after collection, which is associated with an increase in the concentration of IAA. However, the level of IAA can be reduced by increasing peroxidase activity [56], as shown in our results.

## 5. Conclusions

Based on the results of this experiment, the following protocol is recommended for the vegetative propagation of *Juniperus sabina*: (1) prepare cuttings in spring, (2) treat cutting bases with 1000 ppm IBA, and (3) planting them in a substrate of perlite–cocopeat (1:1). This should yield more than 60% rooting in a few months (approximately 3 months; more efficient than traditional seed production methods). Thus, it is an easy and rapid method for nurseries to propagate *Juniperus sabina*, which is a very interesting shrub for forest restoration and ornamental purposes, worldwide. In the future, our results will be confirmed with new experiments to determine whether spring is the best season to obtain stem cuttings in other juniper species (trees and shrubs).

**Author Contributions:** Conceptualization, M.A., B.Z., H.Z. and A.R.N.; formal analysis, M.A., F.A.G.-M. and A.R.N.; investigation, M.A. and F.A.G.-M.; methodology, M.A., B.Z. and H.Z.; project administration, B.Z.; resources, B.Z.; supervision, F.A.G.-M., B.Z., H.Z. and A.R.N.; validation, A.R.N.; writing—original draft, M.A.; writing—review and editing, F.A.G.-M. and B.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Data is available upon request to the first author.

**Acknowledgments:** This study was part of a PhD thesis supported by the University of Lorestan (Iran) and a grant for scientific stay by Maliheh Abshahi at the University of Castilla-La Mancha (Spain) supported by the Ministry of Science, Research and Technology, Iran. We are grateful to three anonymous reviewers for their comments, which allowed us to improve the initial manuscript.

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

## References

1. Farjon, A. *A Monograph of Cupressaceae and Sciadopitys*; Royal Botanic Gardens, Kew: Richmond, Surrey, UK, 2005; p. 648.
2. Adams, R.P. *The Junipers of the World: The Genus Juniperus*, 4th ed.; Trafford Publishing: Victoria, BC, Canada, 2014; p. 422.
3. García Morote, F.A.; Andrés Abellán, M.; Rubio, E.; Pérez Anta, I.; García Saucedo, F.; López Serrano, F.R. Stem $CO_2$ Efflux as an Indicator of Forests' Productivity in Relict Juniper Woodlands (*Juniperus thurifera* L.) of Southern Spain. *Forests* **2021**, *12*, 1340. [CrossRef]
4. Comino, E.; Marengo, P. Root tensile strength of three shrub species: Rosa canina, Cotoneaster dammeri and *Juniperus horizontalis* Soil reinforcement estimation by laboratory tests. *Catena* **2010**, *82*, 227–235. [CrossRef]
5. Piotto, B.; Di Noi, A. *Seed Propagation of Mediterranean Trees and Shrubs*; Agency for the Protection of the Environment and for Technical Services: Roma, Italy, 2003; p. 108.
6. Lu, D.; Huang, H.; Wang, A.; Zhang, G. Genetic Evaluation of *Juniperus sabina* L. (Cupressaceae) in Arid and Semi-Arid Regions of China Based on SSR Markers. *Forests* **2022**, *13*, 231. [CrossRef]

7. Esmaeilnia, M.; Jalali, S.G.; Tabari, M.; Hosseini, S.M. Influence of plant growth regulator IBA on vegetative propagation of Juniperus excelsa. *Iran. J. For. Poplar Res.* **2006**, *14*, 221–227.
8. Momeni, M.; Ganji-Moghadam, E.; Kazemzadeh-Beneh, H.; Asgharzadeh, A. Direct organogenesis from shoot tip explants of *Juniperus polycarpos* L.: Optimizing basal media and plant growth regulators on proliferation and root formation. *Plant Cell Biotechnol. Mol. Biol.* **2018**, *19*, 40–50.
9. Hazubska-Przybył, T. Propagation of Juniper Species by Plant Tissue Culture: A Mini-Review. *Forests* **2019**, *10*, 1028. [CrossRef]
10. Amri, E.; Lyaruu, H.; Nyomora, A.; Kanyeka, Z. Vegetative propagation of African Blackwood (*Dalbergia melanoxylon* Guill. & Perr.): Effects of age of donor plant, IBA treatment and cutting position on rooting ability of stem cuttings. *New For.* **2010**, *39*, 183–194. [CrossRef]
11. Nordstrom, A.C.; Jacobs, F.A.; Eliasson, L. Effect of exogenous Indole-3-Acetic-acid and Indole-3-Butyric acid on internal levels of the respective auxins and their conjugation with aspartic-acid during adventitious root-formation in Pea cuttings. *Plant Physiol.* **1991**, *96*, 856–861. [CrossRef]
12. Rifaki, N.; Economou, A.; Hatzilazarou, S. Factors affecting vegetative propagation of Juniperus excelsa bieb. by stem cuttings. *Propag. Ornam. Plants* **2002**, *2*, 9–13.
13. Henry, P.H.; Blazich, F.A.; Hinesley, L.E. Vegetative propagation of Eastern redcedar by stem cuttings. *Hortscience* **1992**, *27*, 1272–1274. [CrossRef]
14. Pacurar, D.; Perrone, I.; Bellini, C. Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiol. Plant.* **2014**, *151*, 83–96. [CrossRef] [PubMed]
15. Ludwig-Muller, J. Indole-3-butyric acid in plant growth and development. *Plant Growth Regul.* **2000**, *32*, 219–230. [CrossRef]
16. Daveis, P.J. *Plant Hormones: Biosynthesis, Signal Transduction, Action!* Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; p. 802.
17. Gao, J.; Xiao, Q.; Ding, L.; Chen, M.; Yin, L.; Li, J.; Zhou, S.; He, G. Differential responses of lipid peroxidation and antioxidants in Alternanthera philoxeroides and Oryza sativa subjected to drought stress. *Plant Growth Regul.* **2008**, *56*, 89–95. [CrossRef]
18. Aliahmad Koruri, S.; Khoushnevis, M.; Matinizadeh, M. *Comprehensive Studies of Juniper Species in Iran*; Publication of Pooneh, Forest & Ranges and Watershed Organization of Iran: Tehran, Iran, 2011; p. 554.
19. Kentelky, E. The Analysis of Rooting and Growth Peculiarities of *Juniperus* Species Propagated by Cuttings. *Horticulture* **2011**, *68*, 380–385.
20. Hong-wei, Y.; Yong-sheng, G.; Hai-jun, S.; Yan-he, R. Experiment on Cutting Propagation of Sabina procumbens. *J. Inn. Mong. For. Sci. Technol.* **2011**, *4*, 6.
21. Sabina, P.; Cornelia, H. Researches concerning the production of planting material using vegetative propagation on *Juniperus horizontalis* mnch. *J. Hortic. For. Biotechnol.* **2009**, *13*, 462–464.
22. Wendling, I.; Warburton, P.; Trueman, S. Maturation in *Corymbia torelliana* x *C. citriodora* Stock Plants: Effects of Pruning Height on Shoot Production, Adventitious Rooting Capacity, Stem Anatomy, and Auxin and Abscisic Acid Concentrations. *Forests* **2015**, *6*, 3763–3778. [CrossRef]
23. Bryant, P.; Trueman, S. Stem Anatomy and Adventitious Root Formation in Cuttings of Angophora, Corymbia and Eucalyptus. *Forests* **2015**, *6*, 1227–1238. [CrossRef]
24. Garcia Morote, F.A.; Lopez Serrano, F.R.; Andres, M.; Rubio, E.; Gonzalez Jimenez, J.L.; de las Heras, J. Allometries, biomass stocks and biomass allocation in the thermophilic Spanish juniper woodlands of Southern Spain. *For. Ecol. Manag.* **2012**, *270*, 85–93. [CrossRef]
25. Paula, S.; Pausas, J. Root traits explain different foraging strategies between resprouting life histories. *Oecologia* **2011**, *165*, 321–331. [CrossRef]
26. Hernandez, E.; Vilagrosa, A.; Pausas, J.; Bellot, J. Morphological traits and water use strategies in seedlings of Mediterranean coexisting species. *Plant Ecol.* **2010**, *207*, 233–244. [CrossRef]
27. Adams, R.P.; Schwarzbach, A.E. Chloroplast capture by a new variety, *Juniperus sabina* var. *balkanensis* R. P. Adams and A. N. Tashev, from the Balkan peninsula: A putative stabilized relictual hybrid between *J. sabina* and ancestral *J. thurifera*. *Phytologia* **2016**, *98*, 100–111.
28. Kottek, M.; Grieser, J.; Beck, C.; Rudolf, B.; Rubel, F. World Map of the Köppen-Geiger climate classification. *Meteorol. Z.* **2006**, *15*, 259–263. [CrossRef]
29. Bohlenius, H.; Fransson, T.; Holmstrom, E.; Salk, C. Influence of Cutting Type and Fertilization in Production of Containerized Poplar Plants. *Forests* **2017**, *8*, 164. [CrossRef]
30. Judd, L.A.; Jackson, B.E.; Fonteno, W.C. Advancements in Root Growth Measurement Technologies and Observation Capabilities for Container-Grown Plants. *Plants* **2015**, *4*, 369–392. [CrossRef]
31. Sridhar, R.; Mohanty, S.; Anjaneyulu, A. Physiology of rice tungro virus-disease-increased cytokinin activity in tungro-infected rice cultivars. *Physiol. Plant.* **1978**, *43*, 363–366. [CrossRef]
32. Hoshmand, H.R. *Design of Experiments for Agriculture and the Natural Sciences*, 2nd ed.; Champan & Hall/CRC: New York, NY, USA, 2006; p. 437.
33. Neter, J.; Kutner, M.; Wasserman, W.; Nachtsheim, C. *Applied Linear Statistical Models*, 4th ed.; McGraw-Hill-Irwin: Chicago, IL, USA, 1996; p. 720.

34. Davies, F.T.; Geneve, R.L.; Kester, D.E.; Hartmann, H.T. Techniques of Propagation by Cuttings. In *Plant-Propagation-Principles and Practices*, 8th ed.; Hartmann, K., Davies, G., Eds.; Pearson Education Limited: Harlow, Essex, UK, 2014; p. 927.
35. Khoushnevis, M.; Teimouri, M.; Matinizadeh, M.; Shirvany, A. Effects of hormones, light and media treatments on rooting of Juniperus oblonga cuttings. *Iran. J. For.* **2012**, *4*, 135–142.
36. Moncousin, C.; Gaspar, T. Peroxidase as a marker for rooting improvement of Cynara scolymus L. cultured in vitro. *Biochem. Physiol. Pflanz.* **1983**, *178*, 263–271. [CrossRef]
37. Kocer, Z.A.; Gozen, A.G.; Onde, S.; Kaya, Z. Indirect organogenesis from bud explants of *Juniperus communis L.*: Effects of genotype, gender, sampling time and growth regulator combinations. *Dendrobiology* **2011**, *66*, 33–40.
38. Negash, L. Successful vegetative propagation techniques for the threatened African pencil cedar (*Juniperus procera* Hoechst. ex Endl.). *For. Ecol. Manag.* **2002**, *161*, 53–64. [CrossRef]
39. Epstein, E.; Ludwig-Muller, J. Indole-3-butyric acid in plants- ocurrence, synthesis, metabolism and transport. *Physiol. Plant.* **1993**, *88*, 382–389. [CrossRef]
40. Poupart, J.; Waddell, C. The rib1 mutant is resistant to indole-3-butyric acid, an endogenous auxin in arabidopsis. *Plant Physiol.* **2000**, *124*, 1739–1751. [CrossRef] [PubMed]
41. Cope, K.; Rupp, L. Vegetative propagation of Juniperus osteosperma (Utah Juniper) by cuttings. *Nativ. Plants J.* **2013**, *14*, 76–84. [CrossRef]
42. Bielenin, M. Rooting and gas exchange of conifer cuttings treated with indole butyric acid. *J. Fruit Ornam. Plant Res.* **2003**, *11*, 99–105.
43. Tektas, I.; Türkoğlu, N.; Cavusoğlu, S. Effects of auxin doses on rooting of *Juniperus* L. *Prog. Nutr.* **2017**, *19*, 130–136.
44. Ma, W.; Zhang, S.; Wang, J.; Sun, X.; Zhao, H.; Ning, Y. Endogenous hormones, nutritive material and phenolic acid variation in cuttings of japanese larch during rooting. *Acta Bot. Boreali-Occident. Sin.* **2013**, *1*, 19.
45. Chowdhuri, T.K. Performance evaluation of different growth regulators on propagation of Chinese juniper (*Juniperus chinensis* Var. *pyramidalis)* in subtropical zone. *J. Pharmacogn. Phytochem.* **2017**, *6*, 2190–2193.
46. Stuepp, C.A.; Ruffellato-Ribas, K.C.; Macanhão, G.; Fragoso, R.; Rickli, H.C. Rooting of *Juniperus chinensis* var. *kaizuka* for different concentrations of IBA and heights collection. *Rev. Agrar.* **2014**, *7*, 496–503.
47. Fragoso, R.; Zuffellato-Ribas, K.; Macanhão, G.; Stuepp, C.A.; Koehler, H. Vegetative propagation of *Juniperus chinensis*. *Comun. Sci.* **2015**, *6*, 307–316. [CrossRef]
48. Chong, C. Influence of Bottom Heat and Mulch on Rooting of Evergreen Cuttings. *Comb. Proc. Int. Plant Propagators Soc.* **2003**, *53*, 496–500.
49. Cuevas-Cruz, J.; Jimenez-Casas, M.; Jasso-Mata, J.; Perez-Rodriguez, P.; Lopez-Upton, J.; Villegas-Monter, A. Asexual propagation of Pinus leiophylla Schiede ex Schltdl. et Cham. *Rev. Chapingo Ser. Cienc. For. Ambiente* **2015**, *21*, 81–95. [CrossRef]
50. Ayan, S.; Küçük, M.; Ulu, F.; Gerçek, V.; Sahin, A.; Sivacioğlu, A. Vegetative propagation possibilities of some natural Juniper (Juniperus L.) species. *J. For. Fac. Gazi Uni. Kast.* **2004**, *4*, 1–12.
51. Manios, V.I.; Papadimitriou, M.D.; Kefakis, M.D. Hydroponic culture of tomato and gerbera at different substrates. *Acta Hortic.* **1995**, *408*, 11–15. [CrossRef]
52. Maloupa, E.; Samartzidis, P.; Couloumbis, P.; Komnin, A. Yield quality and photosynthetic activity of greenhouse-grown "Madelom" roses on perlite-zeolite substrate mixtures. *Acta Hortic.* **1999**, *481*, 97–99. [CrossRef]
53. Pratt, R.; North, G.; Jacobsen, A.; Ewers, F.; Davis, S. Xylem root and shoot hydraulics is linked to life history type in chaparral seedlings. *Funct. Ecol.* **2010**, *24*, 70–81. [CrossRef]
54. Guerrero-Campo, J.; Palacio, S.; Perez-Rontome, C.; Montserrat-Marti, G. Effect of root system morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in north-east Spain. *Ann. Bot.* **2006**, *98*, 439–447. [CrossRef]
55. Luo, J.; Zhou, J.; Zhang, J. Aux/IAA Gene Family in Plants: Molecular Structure, Regulation, and Function. *Int. J. Mol. Sci.* **2018**, *19*, 259. [CrossRef]
56. Blakesley, D.; Weston, G.; Hall, J. The role of endogenous auxin in root initiation. 1. Evidence from studies on auxin application, and analysis of endogenous levels. *Plant Growth Regul.* **1991**, *10*, 341–353. [CrossRef]
57. Hausman, J. Changes in Peroxidase-activity, auxin and ethylene production during root-formation by Poplar shoots raised in-vitro. *Plant Growth Regul.* **1993**, *13*, 263–268. [CrossRef]
58. Rival, A.; Bernard, F.; Mathieu, Y. Changes in peroxidase activity during in vitro rooting of oil palm (*Elaeis guineensis* Jacq.). *Sci. Hortic.* **1997**, *71*, 103–112. [CrossRef]
59. Yan, S.; Yang, R.; Wang, F.; Sun, L.; Song, X. Effect of Auxins and Associated Metabolic Changes on Cuttings of Hybrid Aspen. *Forests* **2017**, *8*, 117. [CrossRef]
60. Torabian, Y.; Korori, S.; Adeli, A.; Falahchai, M. Quantitative and qualitative study of peroxidase and amylase enzymes of seeds and shoots of Juniper trees (*Juniperus excelsa*) depending on the quality of the bases in the habitat of Razavi Khorasan. *J. Biol. Sci.* **2009**, *3*, 19–27.
61. Moncousin, C. Rooting of in vitro cuttings. In *High-Tech and Micropropagation I*; Springer: Berlin/Heidelberg, Germany, 1991; pp. 231–261.