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Impact of PGPR Formulations Combined with Exogenous IBA Levels to Enhance Root Capacity in Poinsettia Cuttings

Fazilet Parlakova Karagöz

Department of Horticulture, Faculty of Agriculture, Atatürk University, TR-25240 Erzurum, Turkey; f.parlakova@atauni.edu.tr; Tel.: +90-0-442-231-1489

Abstract: The commercial production of poinsettias begins with the propagation of apical cuttings from stock plants. The aim of the study was to use bacterial mixtures prepared with PGPRs in the cultivation of stock mother poinsettia plants and determine an effective IBA dose to increase root development and root yield of cuttings prepared from these stock mother plants. Rooted cuttings of *Euphorbia pulcherrima* Willd.ex Klotzsch were used for planting stocks of mother plants. Eight different bacterial isolates were mixed in triple combinations to form 4 different bacterial formulations: (BI) formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79); (BII) formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C); (BIII) formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C); and (BIV) formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D). In the first year, rooted cuttings of stock mother plants were dipped in liquid microbial solution (bacterial formulations) for 15 min and then planted in plastic pots. In the second year, the same bacterial formulations were freshly prepared and applied to the 1-year-old stock mother plants as irrigation water for the second time. Amounts of 0 (control), 1000, 1500, and 2000 mg L^{-1} IBA doses were used for rooting cuttings taken from the 2-year-old stock mother plants. In the experimental group treated with the BI bacterial formulation, the number of rooted cuttings (NR) increased by 14.26% and 19.00%, compared with the control, in response to IBA 1500 mg L^{-1} and IBA 2000 mg L^{-1} treatment, respectively. Compared to the mean root length of the cuttings in the control treatment, the mean root length of the cuttings in the BIV treatment increased by 18.83%. The mean root length values decreased with the increase in IBA doses. The highest value of the number of mean shoots (NAS) was in cuttings treated with BI + 1500 treatment, which was 1.93 times higher than the control. The highest nitrogen content (5.73%) was determined in the bract leaf samples of the BIV application, and an 81.33% nitrogen increase was detected in the related application when compared to the control. In terms of P, Ca, and Fe contents, higher values were obtained from the BIV application when compared to the control application. The highest values of K content were determined in BIII and BII applications. This study provides positive effects on the feedback of stock mother plants with PGPR to provide sprout production by cuttings technique. It has been revealed that lower IBA dose applications can be recommended for rooting cuttings taken from mother plants treated with the BIV bacterial formulation.

Keywords: *Euphorbia pulcherrima* Willd.ex Klotzsch; bacterial formulation; stock mother plant; cutting rooting; indole 3-butyric acid; plant growth-promoting rhizobacteria

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1. Introduction

Ornamental plants have nowadays gained importance not only for their aesthetic and social values but also for their economic contribution. Production and trade of these products make an important contribution to the national economies of developed countries. Poinsettia (*Euphorbia pulcherrima* Wild.) (in the *Euphorbiaceae* family) is one of the most expensive and high-volume crops in the floriculture industry [1]. Although this plant originates from Central America, it is one of the most widely grown shrubs worldwide.

Poinsettia has brightly colored bracts ranging from red, scarlet, and red to yellow and white. The fact that the gorgeous bracts of the poinsettia stay fresh and intact for three to four months [2] contributes to its demand as an ornamental plant in the global market as well as in the internal market [3].

Many horticultural plants, especially ornamental plants, are propagated by vegetative means for commercial purposes. Commercial production of poinsettias begins with the propagation of apical cuttings from stock plants. One of the important problems encountered in the propagation of commercial poinsettia by cuttings is the high loss rate in cutting rooting [4]. The genetic, physiological, and biochemical characteristics of the stock mother plants from which the cuttings are derived are one of the main causes of the high loss rate in cutting rooting [3,5,6]. The level of rooting of cuttings is closely tied to the proper management of light and temperature in the environment where rooting will occur, as well as the water and fertilizer resources employed [7]. There are research results indicating that cuttings are better rooted as a result of fertilization and plant nutrition programs performed on the stock mother plants [8–10].

The initial carbohydrate content of the cutting should be sufficient to provide energy reserves during the rooting period [11,12]. According to Ahkami et al. [13], early carbohydrate accumulation at the rooting zone is an important metabolic event for adventitious root formation in *Petunia hybrid.* Similarly, higher soluble sugar and starch at the rooting site were associated with a higher rooting response in *Tectona grandis* cuttings [14].

The other factors affecting the rooting performance of plants in propagation by cuttings are the type of cuttings, the rooting medium, and the type and concentration of hormones used [3,15,16]. Indole 3-butyric acid (IBA), one of the rooting-promoting hormones, plays a key role in both root and shoot development. Among the different auxin types, IBA draws attention due to its higher chemical stability and slow transport in the plant system [17,18]. Nordström et al. [19] and Štefančič et al. [20] found that IBA levels remained higher than those of IAA and that IBA had higher stability in rooting solution than IAA. The effects of the type and concentration of auxin hormone on the cuttings of poinsettia have been studied in previous studies [3,4,21–24].

In recent years, it has been reported that plant growth-promoting rhizobacteria (PGPR) can be achieved [25,26]. Plant nutrient balance can be achieved with the use of nitrogen fixers in a mixture with phosphate solvents as a biological fertilizer, and soil pathogens can be better controlled [27]. There are few studies on the use of PGPR in the rooting of poinsettia: The effects of treatment with IAA-producing rhizobacteria [28] and the use of PGPR (plant growth-promoting rhizobacteria) for the treatment of root diseases [29] on cuttings were examined. However, after the use of PGPRs as a biofertilizer in stock mother plant nutrition, no research was found in which the rooting performance of cuttings taken from these plants was examined. The effects of PGPRs, which are known to have positive effects on plant growth and development [30], and the use of stock mother plant nutrition on cutting rooting are important research topics. Therefore, the objectives of this research were to: (1) evaluate the rooting status of apical cuttings taken from stock mother plants grown using different bacterial formulations under greenhouse conditions; and (2) investigate the effects of different doses of IBA applied on the rooting success of poinsettia cuttings and the relationship between bacterial formulation and feeding. The study's findings are anticipated to be very instructive and useful for nurseries, particularly those that cultivate poinsettias from rooted cuttings.

2. Materials and Methods

2.1. Description of the Study Area

The study was conducted in a greenhouse in Erzurum, Turkey. The cultivation of stock mother plants and the rooting of cuttings were carried out in the same greenhouse. The greenhouse had heating and ventilation set points of 27 and 22 °C throughout the day and night, respectively, and the average relative humidity was 70–75% (Figure 1). Curtains (55% reduction in photosynthetic photon flux, PPF) were used to shade the plants.

Figure 1. Stock mother plants (**a**); preparation of apical cuttings (**b**); soaking the bottom of the cuttings in perlite powder (**c**); soaking the bottom of the cuttings in IBA solutions (**d**); planting of cuttings in perlite medium (**e**,**f**).

2.2. Experimental Materials and Propagation Procedure

Stock mother plants: Poinsettia rooted cuttings (*Euphorbia pulcherrima* Willd.ex Klotzsch cv. Christmas Feelings) used for planting stock mother plants were obtained from a private company. The cultivation medium was prepared by mixing peat and pumice (diameter: 3–7 mm) as the volume in a ratio of 2:1 (Figure 1a). The main components of the peat (Peat Klasmann TS1 (Peat Moss)) used are decomposed white sphagnum peat and frozen black sphagnum peat, EC; 40 mS m^{-1} (+/−25%), pH value (H_2O); 5.5–6.5 and nutrient content (NPK fertilizer 14-10-18) 1.5 kg m^{-3} and sterile. The pumice used has the characteristics of 45–90% porosity, water absorption of 30–70 wt%, pH value of 7.0–7.3, and a water-soluble matter amount (wt%) $\leq$ 0.15.

Bacterial strains used in the study: All of the bacterial strains used in this study were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University (Table 1). The bacterial strains had been isolated from the rhizosphere of cultivated plants or wild plants growing in the Eastern Anatolia Region of Turkey by Kotan et al. [31]. Based on the results of previous studies and some biochemical test results [32–35], bacterial strains found to have the best nitrogen fixation and phosphorus solubilizing properties were selected for use in the experiment. Some biochemical characteristics of the bacterial strains used in this experiment, the location where they were isolated, and the host plants were shown in Table 1.

Table 1. Bacterial isolates used in the study and some biochemical properties of them [36].

Isolate No	MIS Diagnosis Result	SIM	Location (in Turkey)	Host	Nitrogen	Phosphate
RK-79	*Pantoea agglomerans*	0.762	Erzurum	Apple	+	+
TV-12E	*Paenibacillus polymyxa*	0.551	Van	Poaceae	S+	+
TV-17C	*Bacillus subtilis*	0.677	Van	Raspberry	S	W+
TV-6D	*Bacillus megaterium*	0.750	Van	Poaceae	+	+
TV-42A *	*Pseudomonas putida*	0.113	Van	Poaceae	W+	W+
TV-91C	*Bacillus megaterium*	0.474	Van	Poaceae	+	W+
TV-113C	*Kluyvera cryocrescens*	0.688	Van	Garlic	+	+
RK-92	*Pantoea agglomerans*	0.889	Erzurum	Pear	+	S

(SIM—similarity index; S—strong +, strong nitrogen fixation property; W—wWeak +, weak nitrogen fixation property; +—positive) *: This isolate produces a siderophore.

Preparation of bacterial solutions: The growth and development of bacterial strains and checking the purity of bacterial strains were carried out using the methods of Turan et al. [37], Parlakova Karagöz et al. [38], and Kaymak et al. [39]. Rhizobacteria isolates were chosen based on differences in colony morphology, including elevation and colony form. A loop full of bacterial culture from the growing fresh cultures was taken from the surface of the agar medium and inoculated into the liquid nutrient medium containing Nutrient Broth (NB), which was prepared in the fermenter and sterilized in an autoclave at 121 °C for 20 min. These cultures were grown in a horizontal shaker incubator for 24 h at 26 °C [38]. The preparation of bacterial formulations was carried out using the methods of Parlakova Karagöz et al. [38] and Kaymak et al. [39]. Each bacterial isolate was prepared separately in the carrier liquid at 250 mL. The carrier liquid contains water, various organic substances (seaweed, whey, and herbal extracts), and various substances (carboxymethylcellülose, calcium carbonate, glycerin, magnesium sulfate) that protect and homogenize the bacterial isolate in its content.

In order to determine the best bacterial formulation, 3 PGPR strains with similar PGPR properties were selected. Four different bacterial formulations were created by mixing the bacterial isolates prepared into the liquid carrier in triplicate combinations: (BI) formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), (BII) formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), (BIII) formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), and (BIV) formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D). One-fortieth (kg/L) of sugar was added to the prepared bacterial suspension and kept for one night by mixing well. The absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and properly diluted to 1×10^8 CFU mL^{-1} in sdH_2O. The prepared bacterial formulation was diluted 10 times with chlorine-free well water when applied to the plants [38].

Practices of plant nutrition used in the cultivation of stock mother plants: The roots of poinsettia cuttings were dipped in the prepared liquid microbial solution for 15 min, and the roots were inoculated with the bacterial mixture [40]. Rooted cuttings being kept in a sterile liquid medium with added sugar for the same time were used as a control. For the stage of growing the stock mother plants in pots, 10 poinsettia-rooted cuttings were used for each application. Pots were sterilized with a 20% sodium hypochlorite solution. As stated above, one rooted cutting was planted in each of the 3.5 L (21.5 × 17.7 cm) plastic pots filled with a growing medium that had been prepared by mixing sterile peat and pumice as the volume in a ratio of 2:1 under greenhouse conditions (day and night temperatures were 27 and 22, respectively, and the average relative humidity was 70–75%) [40]. In cases of need, cultural processes were carried out on the stock mother plants. In the second year, the relevant bacterial formulations were freshly prepared for these plants and applied to the pots for irrigation. Apart from bacterium formulation administrations, no other fertilizer was applied to the stock mother plants until they were two years old (time passed to obtain cuttings). The stock mother plants were 90–100 cm tall, had never been pruned

in the previous two years, and had three to five terminal branches ideal for propagation (Figure 1a).

Initial nutrient contents of bract leaf of stock mother plants: At the time of cuttings collection (Figure 1a), bract leaf samples were taken from stock mother plants of each bacterial formulation and the control group. To determine the mineral nutrient contents of bract leaf, fresh leaves were oven dried at 68 °C for 48 h and then ground in a mortar and passed through a 1-mm sieve. The concentrations of macro-elements (K, P, Ca, and Mg) and micro-elements (Fe, Cu, and Mn) were determined by the method of Mertens [41]. Tissue K, P, Ca, Fe, Mg, Mn, and Cu were determined using an inductively coupled plasma spectrophotometer (Optima 2100 DV, ICP/OES; PerkinElmer, Waltham, Massachusetts, USA) [42]. The N content was determined using the Kjeldhal method [43].

Preparation of apical cuttings: From disease-free and medium-sized bushes, 10–15 cm long and 1.0–1.5 cm thick, uniform apical cuttings with at least 2–3 nodes were prepared from stock mother plants by considering the application groups (Figure 1b). All apical cuttings contained a leaf, which has been reduced by 25% from its original size (Figure 1b,c). An oblique cut was made from the base of each cutting with a sharp razor blade to expose the maximum absorbent surface for effective rooting. It was dipped in sterile perlite powder to stop the sap of the plant from flowing due to the cut made from the cutting base. They were kept in perlite powder for five minutes (Figure 1c). The cuttings, whose sap water release stopped, were taken from the BA dose application.

Preparation of IBA concentrations and application to poinsettia cutting bottoms: IBA was dissolved in 1% potassium hydroxide (KOH) (CAS No:1310-58-3, Sigma-Aldrich, Atasehir, Istanbul, Turkey) [44] and then diluted with water for dose adjustment. The IBA doses used in the study were 0 (control), 1000, 1500, and 2000 mg L^{-1} IBA. Before treatments, all cuttings were sterilized with 25% $NaHCO_3$ for 5 min [45]. Cuttings in the control group were treated with sterile water. The bases of the cuttings were dipped in the IBA solution for 10 s for each treatment (Figure 1d); then placed in 10 cm-deep containers filled with sterile perlite (coarse agricultural perlite: 0.0–5.0 mm) (Figure 1e,f). The cuttings were rooted under greenhouse conditions in a shading environment with 50% shade [44] (Figure 1f). To enhance the average relative humidity of 70–75% derived from the greenhouse environment measurement, the cuttings were misted twice a day from the top. The application groups and application codes used in this study's application of various IBA doses to rooted cuttings taken from stock mother plants treated with various bacterial formulations are listed in Table 2.

Table 2. Treatments made to the cuttings in the experiment and the contents of the treatments.

Treatments	Bacteria Formulations (Used to Provide Plant Nutrition to Stock Mother Plants)	Contents of Treatments (Applied in Cutting Rooting)
Control-0	No bacterial formulation was applied to these application groups.	Control (No bacteria + IBA application)
Control-1000		0 mg L^{-1} + IBA 1000 mg L^{-1}
Control-1500		0 mg L^{-1} + IBA 1500 mg L^{-1}
Control-2000		0 mg L^{-1} + IBA 2000 mg L^{-1}
BI + 0	BI: Bacteria formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79)	Bacteria formulation 1 (No IBA application)
BI + 1000		Bacteria formulation 1 + IBA 1000 mg L^{-1}
BI + 1500		Bacteria formulation 1 + IBA 1500 mg L^{-1}
BI + 2000		Bacteria formulation 1 + IBA 2000 mg L^{-1}
BII + 0	BII: Bacteria formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C)	Bacteria formulation 2 (No IBA application)
BII + 1000		Bacteria formulation 2 + IBA 1000 mg L^{-1}
BII + 1500		Bacteria formulation 2 + IBA 1500 mg L^{-1}
BII + 2000		Bacteria formulation 2 + IBA 2000 mg L^{-1}

Table 2. *Cont.*

Treatments	Bacteria Formulations (Used to Provide Plant Nutrition to Stock Mother Plants)	Contents of Treatments (Applied in Cutting Rooting)
BIII + 0	BIII: Bacteria formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C)	Bacteria formulation 3 (No IBA application)
BIII + 1000		Bacteria formulation 3 + IBA 1000 mg L^{-1}
BIII + 1500		Bacteria formulation 3 + IBA 1500 mg L^{-1}
BIII + 2000		Bacteria formulation 3 +IBA 2000 mg L^{-1}
BIV + 0	BIV: Bacteria formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D)	Bacteria formulation 4 (No IBA application)
BIV + 1000		Bacteria formulation 4 + IBA 1000 mg L^{-1}
BIV + 1500		Bacteria formulation 4 + IBA 1500 mg L^{-1}
BIV + 2000		Bacteria formulation 4 + IBA 2000 mg L^{-1}

2.3. Experimental Design, Data Collection, and Analysis

The experiments were repeated twice. A two-factor (bacterial formulations and IBA doses) experimental design with 20 treatments set up in a randomized block design was used for the study. The 20 treatments were put to the test (Table 2). Each treatment was performed three times, for a total of 60 experimental/treatment units. Each experimental unit contained ten cuttings, for a total of 600 cuttings/polybag. The experiment was terminated 90 days after the cuttings were planted. Number of rooted cuttings (NR), number of mean roots (NAR: each rooted cutting was determined and the mean of recurrence was determined), mean root length (ARL: The length of the roots from each cutting was measured in mm with a caliper and determined as the average of repetitions), number of newly formed leaves (NFL: the number of leaves of the shoots emerging from each cutting was determined and the average of repetitions was determined), and number of mean shoots (NAS: each cutting producing a shoot was determined and the mean of recurrence was determined) parameters were measured and evaluated.

All statistical analysis was performed using SPSS Statistics (v. 20 for Windows; IBM Corporation, Armonk, NY, USA). Multivariate analysis of variance (MANOVA) was used to detect significant differences between groups. The means were separated by Duncan's multiple range test ($p < 0.05$). The principal component analysis (PCA) and biplot graphs were used to display the correlation between the various parameters and their relationship with the different treatments and were performed by JMP version 7.0 (SAS Institute, Cary, NC, USA). Additionally, an online heat mapper software (http://www.heatmapper.ca/ (accessed on 1 January 2023)) was used as the heat mapper of all treatments. Heat mapper analysis is an analysis method that classifies the means of the parameters measured in the study according to color and provides general information about the applications to the researchers. According to the analysis, as the numerical value of the applications increases, the color changes from red to green. The black color represents the mean.

3. Results

3.1. Rooting Capacity of Poinsettia Cuttings

In the present study, analysis of variance revealed that the rooting capacity of poinsettia cuttings varied significantly depending on cuttings bacteria formulation treatments, IBA treatment (concentration), and interactions between these factors (Table 3).

Cuttings taken from poinsettia stock mother plants grown with different bacterial formulations were treated with different IBA doses for rooting, and these cuttings showed different levels of root morphological responses (Table 4). The highest value of the number of rooted cuttings (NR) was for cuttings treated with BIV (8.42), which was 1.74 times higher than the control. The BIV treatment was in the same statistical group as the BIII and BII treatments. While the nutritional status of the stock mother plants differed according to the number of rooted cuttings parameter, IBA applications to the rooting of the cuttings did not make a statistically significant difference (Table 4). In the experimental group, which did not apply any bacterial formulation, NR was increased by 100.30% compared with the

0 mg L^{-1} IBA dose in response to the IBA 2000 mg L^{-1} treatment. In the experimental group that applied the BI bacterial formulation, NR was increased by 14.26% and 19.00% compared with the control, in response to IBA 1500 mg L^{-1} or IBA 2000 mg L^{-1} treatment, respectively. Moreover, the BI bacteria formulation prepared with *Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79 increased in NR. In the experimental group that applied the BII bacterial formulation, NR was not affected by IBA treatments. In the experimental groups that applied the BIII and BIV bacteria formulations, NR was adversely affected by the increase in IBA dose applications (Table 4).

Table 3. Analysis of variance of the effect of bacterial formulation treatments and the concentration of exogenous IBA application on the root development parameters.

Source of Variance	Number of Rooted Cuttings (NR)	Number of Mean Roots (NAR)	Mean Root Length (ARL) (mm)	Number of Newly Formed Leaves (NFL)	Number of Mean Shoots (NAS)
Bacteria Formulation Treatments (A)	24.31 ***	3.36 ***	111.05 ***	2.55 ***	0.48 ***
IBA doses (B)	1.17 ^{ns}	0.54 ***	15.15 ***	1.90 ***	0.13 *
A × B	5.74 ***	0.93 ***	28.23 ***	2.78 ***	0.32 ***

ns: not significant; * $p < 0.05$; *** $p < 0.001$.

Table 4. Root growth as affected by treatment with different IBA doses of cuttings taken from poinsettia stock mother plants grown with the use of different bacterial formulations as biofertilizers.

Bacteria Formulations		Control	BI	BII	BIII	BIV	Mean
				Mean ± SD [x]			
				Number of rooted cuttings (NR)			
IBA Doses	0	3.33 ± 0.58 b **	7.00 ± 1.00 a **	8.00 ± 1.00 ^{ns}	8.33 ± 0.58 a *	9.67 ± 0.58 a **	7.27 ± 2.31 ^{NS}
	1000	4.67 ± 0.58 b	4.33 ± 0.58 b	7.67 ± 1.53	8.00 ± 1.00 ab	9.33 ± 0.58 a	6.80 ± 2.18
	1500	4.67 ± 0.58 b	8.00 ± 1.00 a	7.00 ± 1.00	9.00 ± 1.00 a	7.33 ± 0.58 b	7.20 ± 1.66
	2000	6.67 ± 1.15 a	8.33 ± 0.58 a	8.33 ± 0.58	6.67 ± 0.58 b	7.33 ± 1.15 b	7.47 ± 1.06
	Mean	4.83 ± 1.40 C ***	6.92 ± 1.78 B	7.75 ± 1.06 A	8.00 ± 1.13 A	8.42 ± 1.31 A	
				Number of mean roots (NAR)			
IBA Doses	0	0.36 ± 0.16 c ***	1.66 ± 0.12 a ***	0.59 ± 0.09 c **	0.90 ± 0.15 bc **	2.45 ± 0.30 a ***	1.19 ± 0.81 A ***
	1000	0.96 ± 0.09 b	0.26 ± 0.08 c	0.87 ± 0.04 b	0.97 ± 0.08 b	2.88 ± 0.12 a	1.19 ± 0.92 A
	1500	0.92 ± 0.05 b	0.50 ± 0.17 b	0.82 ± 0.09 b	0.74 ± 0.13 c	0.97 ± 0.10 c	0.79 ± 0.20 B
	2000	1.26 ± 0.08 a	0.18 ± 0.06 c	1.08 ± 0.09 a	1.20 ± 0.03 a	1.64 ± 0.50 b	1.07 ± 0.54 A
	Mean	0.88 ± 0.35 B ***	0.65 ± 0.63 C	0.84 ± 0.20 B	0.95 ± 0.20 B	1.99 ± 0.81 A	
				Mean root length (ARL) (mm)			
IBA Doses	0	10.66 ± 0.57 c ***	16.33 ± 1.53 a ***	14.67 ± 0.58 a ***	10.67 ± 0.58 b ***	18.67 ± 1.53 ^{ns}	14.20 ± 3.39 A ***
	1000	17.67 ± 0.58 a	17.00 ± 1.00 a	9.33 ± 0.58 b	10.33 ± 0.58 b	18.00 ± 1.00	14.47 ± 4.00 A
	1500	15.67 ± 0.58 b	11.00 ± 1.00 b	7.67 ± 0.58 c	9.00 ± 1.00 c	18.00 ± 2.65	12.27 ± 4.25 C
	2000	16.33 ± 0.58 b	8.00 ± 1.00 c	10.33 ± 0.58 b	14.33 ± 0.58 a	17.00 ± 1.00	13.20 ± 3.67 B
	Mean	15.08 ± 2.81 B ***	13.08 ± 4.03 C	10.50 ± 2.75 D	11.08 ± 2.15 D	17.92 ± 1.56 A	

[x]: SD: Standard Deviation, Note: Different letters in the same column indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. NS; ns—non-significant (at $p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Cuttings from stock mother plants grown and inoculated with BIV showed a great increase in the number of mean roots (NAR) (126.14% compared to the control). While the NAR of cuttings treated with various IBA doses was statistically significant, nothing of these differences was statistically significant when compared to control plants, with the exception of IBA 1500 mg L^{-1}. NAR increased in response to IBA 2000 mg L^{-1} treatment in the experimental groups that included control, BI, and BIII compared with the 0 mg L^{-1} IBA dose. In the experimental groups that applied BI and BIV bacteria formulations, NAR was adversely affected by the increase in IBA dose applications (Table 4).

The mean root length parameter was affected by the bacterial formulations with which the stock mother plants were treated. The highest mean root lengths were obtained from

cuttings taken from stock mother plants grown with BIV application. The mean root length of the cuttings in the BIV treatment increased by 18.83% compared to the mean root length of the cuttings in the control treatment. The mean root length values decreased with increasing IBA doses. In the experimental group, which applied BIII bacterial formulations, ARL increased by 34.30% compared with the control, in response to IBA 2000 mg L^{-1} treatment (Table 4).

The data presented in Table 5 show that the number of newly formed leaves (NFL) of poinsettia cuttings significantly increased in IBA compared to the untreated cuttings. Overall, the doses of the IBA were increased in the NFL. In addition, NFL data obtained from cuttings taken from rootstock plants treated with BIV application were determined as the highest values. In the application of the 2000 mg L^{-1} IBA dose to the cuttings taken from the stock mother plants grown without adding the bacterial formulation, more newly formed leaves were obtained. It was determined that 1500 doses of IBA application can promote the rooting of cuttings taken from plants treated with BI formulation. With this application, the number of newly formed leaves increased by 89% when compared to the control application. It was determined that 1500 doses of IBA application can promote the rooting of cuttings taken from plants treated with BI formulation. It was concluded that the rooting of cuttings taken from plants treated with the BIII formulation increased with 1500 and 2000 doses of IBA. Control, 1000, and 1500 IBA doses had a statistically significant effect on the quantity of newly produced leaves in cuttings derived from plants treated with the BIV formulation. In addition, a decrease in the number of newly formed leaves was determined in the cuttings in the 2000 IBA dose applications (Table 5).

Table 5. The effect of different IBA doses on the shoot growth of cuttings taken from poinsettia stock mother plants grown by using different bacterial formulations as biofertilizers.

Bacteria Formulations		Control	BI	BII	BIII	BIV	Mean
				Mean ± SD [x]			
				Number of newly formed leaves (NFL)			
IBA Doses	0	0.33 ± 0.58 b *	0.33 ± 0.58 c ***	2.33 ± 0.58 a **	1.00 ± 0.00 b **	2.67 ± 0.58 a **	1.33 ± 1.11 B ***
	1000	1.00 ± 0.00 ab	2.00 ± 0.00 b	0.00 ± 0.00 c	0.33 ± 0.58 c	2.00 ± 0.00 a	1.07 ± 0.88 B
	1500	0.33 ± 0.58 b	3.00 ± 0.00 a	1.33 ± 0.58 b	1.83 ± 0.29 a	2.67 ± 0.58 a	1.83 ± 1.06 A
	2000	1.67 ± 0.58 a	2.00 ± 0.00 b	2.33 ± 0.58 a	2.00 ± 0.00 a	0.67 ± 0.58 b	1.73 ± 0.70 A
	Mean	0.83 ± 0.72 D ***	1.83 ± 1.03 AB	1.50 ± 1.09 BC	1.29 ± 0.75 C	2.00 ± 0.95 A	
				Number of mean shoots (NAS)			
IBA Doses	0	0.19 ± 0.02 b *	0.70 ± 0.06 b **	0.50 ± 0.50 [ns]	1.25 ± 0.08 a ***	0.92 ± 0.09 a **	0.71 ± 0.42 A *
	1000	0.57 ± 0.24 a	0.74 ± 0.08 b	0.42 ± 0.30	0.20 ± 0.06 d	0.78 ± 0.25 ab	0.54 ± 0.28 B
	1500	0.18 ± 0.01 b	1.35 ± 0.34 a	0.61 ± 0.09	0.76 ± 0.08 b	0.60 ± 0.10 b	0.70 ± 0.42 A
	2000	0.39 ± 0.10 ab	0.75 ± 0.08 b	0.88 ± 0.08	0.58 ± 0.09 c	0.16 ± 0.01 c	0.55 ± 0.27 B
	Mean	0.33 ± 0.20 C ***	0.89 ± 0.32 A	0.60 ± 0.31 B	0.70 ± 0.40 B	0.61 ± 0.32 B	

[x]: SD—standard deviation. Note: different letters in the same column indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. ns—non-significant (at $p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The maximum number of mean shoots (NAS) (0.89) was determined by the cuttings taken from poinsettia stock mother plants fertilized with BI bacterial formulations. The maximum number of mean shoots (NAS) (0.71) was obtained from the cuttings with no IBA application. The maximum number of mean shoots (NAS) (1.35) was found in the IBA 1500 mg l treatment in the cuttings taken from poinsettia stock mother plants fertilized with BI bacterial formulations. The highest value of NAS was the cuttings treated with BI+1500 treatment, which was 1.93 times highest than the control. The effect of IBA dose applications in the experimental groups that used the BII bacteria formulation was not statistically significant in terms of NAS parameters. However, NA was adversely affected by the increase in IBA dose applications in the experimental groups that apply BIII and BIV bacteria formulations (Table 5).

In the current study, N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu nutrient contents in the bract leaf of stock mother plants changed significantly among the bacteria formulations (Table 6). The highest nitrogen content of 5.73% was determined in the bract leaves of the BIV application. In this application, an increase in the nitrogen concent of 81.33% was observed when compared to the control. In terms of P, Ca, and Fe contents, higher values were obtained from the BIV application when compared to the control application. While the BIII application had the highest K content, the BII application was in the same statistical group as this application. Mg and Cu contents of stock mother plants treated with bacterial formulations were not found to be statistically significant compared to the control treatment. While Mn content increased in BIII and BI applications when compared to control, Zn content decreased in BIII, BII, and BI applications (Table 6).

Table 6. Initial mineral nutrient contents of the bract leaf of poinsettia stock mother plants.

Bacteria Formulation Treatments	Initial Nutrient Contents of Bract Leaf								
	N (%)	mg kg^{-1}							
		P	K	Ca	Mg	Fe	Mn	Zn	Cu
Control	3.16 c ***	2958.40 c ***	23,406.65 c ***	7900.27 b ***	5044.40 ^{ns}	205.96 b **	41.21 b *	48.18 a ***	12.05 ^{ns}
BI	4.76 b	4270.97 b	27,176.26 ab	10,797.56 a	4720.05	233.52 a	44.55 a	33.99 c	11.78
BII	4.77 b	4337.38 b	28,493.00 a	10,833.58 a	4592.27	234.24 a	41.55 b	36.83 c	12.82
BIII	4.81 b	4544.35 a	28,726.76 a	10,653.20 a	4528.03	225.96 a	44.60 a	37.45 c	11.62
BIV	5.73 a	4639.67 a	25,946.64 b	11,155.99 a	4798.28	233.57 a	43.60 ab	42.81 b	12.07
F	54.157	208.788	19.190	27.279	1.963	6.255	4.756	21.737	1.076
Sig.	0.000	0.000	0.000	0.000	0.176	0.009	0.021	0.000	0.418

Different letters in the same column indicate significant differences ($p < 0.05$) based on Duncan's multiple range test. ns—non-significant (at $p > 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2. Principal Component Analysis

According to the results of the biplot analysis (Figure 2a), the applications were divided into 4 groups, and the C-1000, C-2000, C-1500, BI 1000, and BIII-2000 applications were in the same group as the C-0 application (the group without bacterial formulation or IBA). On the other hand, it has been determined that BIV-0 and BIV-1000 applications are different from all other applications and C-0 applications (Figure 2a). It was determined that the results of the biplot analysis showed parallelism with the general means of the bacterial formulation applications given in Tables 4 and 5.

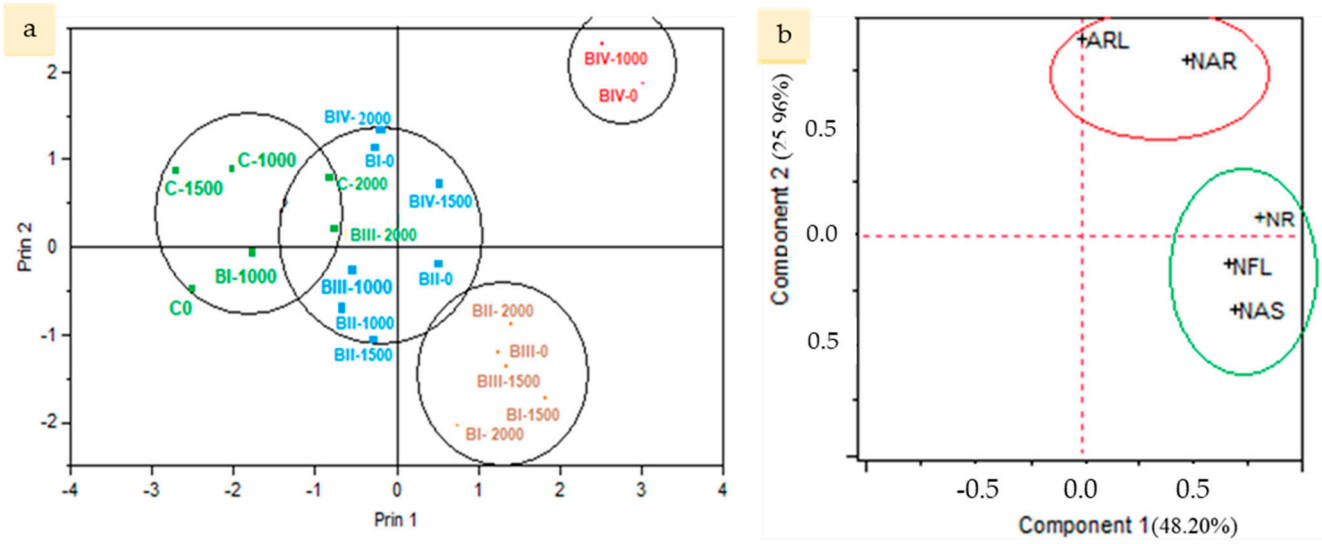

Figure 2. Score plot grouping of treatments based on principal component scores (**a**); principal component analysis plot f rooting parameters of poinsettia cuttings (**b**).

The data from the rooting parameters were used for principal component analysis (PCA) (Figure 2b). This method specifies mathematical criteria for quantifying similarities between samples or sets. The PCA plot revealed that the rooting parameters were made up of two distinct components (Figure 2b). Whereas the first component comprised NR, NFL, and NAS applications, the second component included ARL and NAR applications. The first two main components accounted for 74.16% of the total variation (Figure 2b).

According to the study we have conducted, the C-0 application has turned red in all parameters, and it has been observed that this application has the lowest values. BIV-0 and BIV-1000 applications, on the other hand, became prominent applications by obtaining the highest values by taking colors in green and green tones in all parameters (Figure 3). These results were compatible with the biplot analysis (Figure 3). Heat mapper analysis showed that the same groups were obtained from PCA (Figure 3).

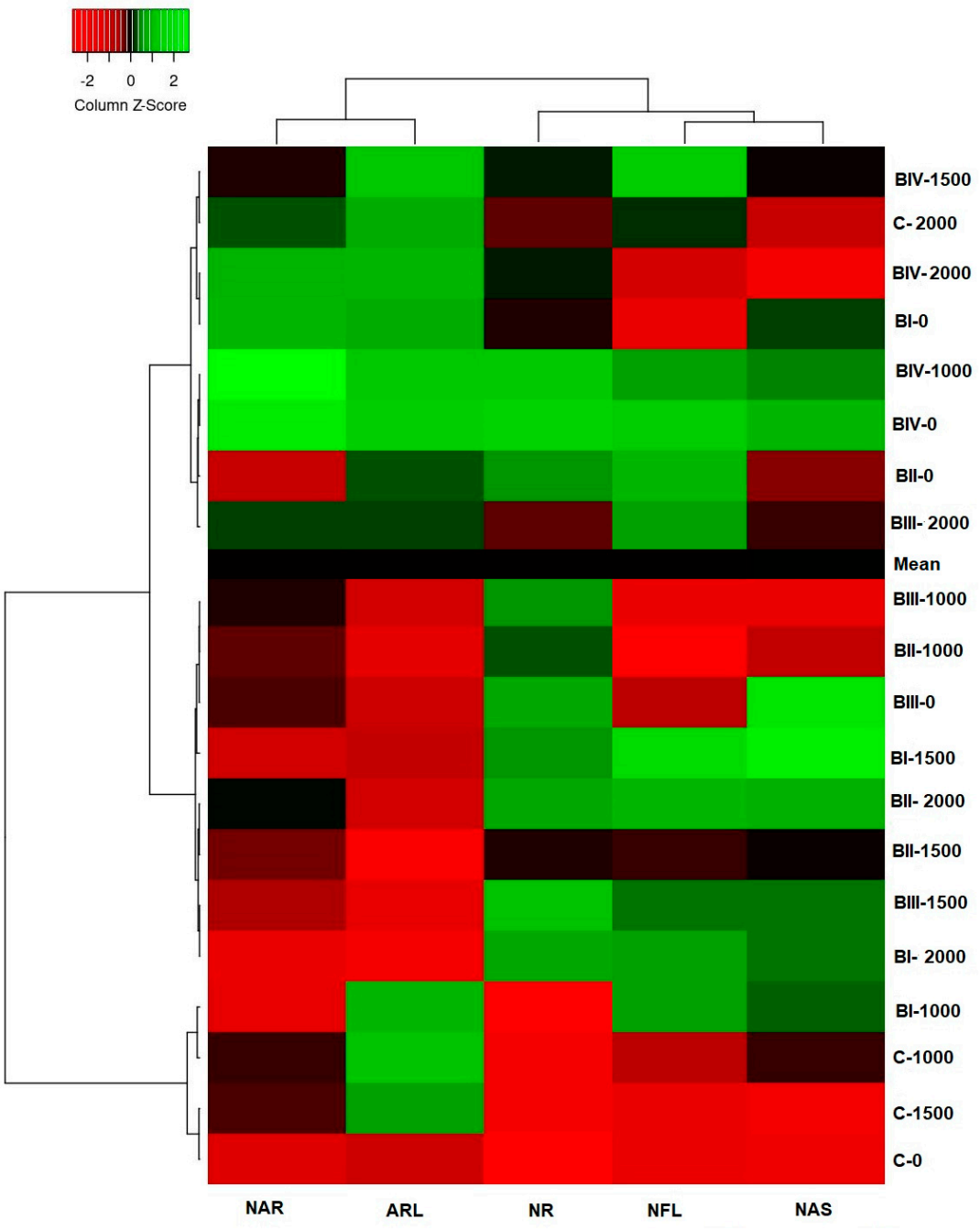

Figure 3. A heat map plot of poinsettia cutting samples and treatments using a color score.

4. Discussion

In poinsettia production, vegetative propagation from mother plants with high plant growth and development characteristics provides an important mechanism for obtaining a high yield and quality. The cuttings used in commercial poinsettia production are generally imported as rootless branches, which plant-growing companies root in their own greenhouses and sell as rooted cuttings. In this context, it is very important for companies to root the propagation material obtained from minimum loss. At the same time, for the producer who buys rooted cuttings, the quality of the cuttings is important in terms of soil compatibility, plant growth, and development performance after planting. Despite the intense control of environmental factors in the modern propagation industry, high economic losses still occur due to insufficient rooting or even rotting of cuttings [8,46,47]. The hypothesis of the study was to use bacterial mixtures prepared with PGPRs as biofertilizers in the cultivation of stock mother poinsettia plants and to determine an effective IBA dose to increase root development and root yield of cuttings prepared from these stock mother plants. The results of this study indicate that cuttings taken from poinsettia stock mother plants fertilized with different bacterial formulations contributed to poinsettia cutting root development by increasing the number of rooted cuttings (NR), number of mean roots (NAR), mean root length (ARL) (mm), and number of newly formed leaves (NFL).

The acceleration of root formation in the cuttings plucked from the mother plant increases their chances of survival, because of their ability to attach themselves to the soil and to absorb water and nutrients from their environment [48]. The physiological state of the mother plant, such as carbohydrate storage and nutrient content, has a great influence on the rooting ability of cuttings [48–50]. The rooting efficiency of cuttings is directly related to the carbohydrate content of stock plants. In other words, root formation efficiency is generally highest when the carbohydrate content of stock plants is high and very poor when carbohydrate storage is low [50]. In the present study, plant growth-promoting rhizobacteria were used in the feeding of parent plants. It is reported that rhizobacteria that promote plant growth accelerate the growth rate of plants, shorten the vegetative period of the plant, and have beneficial effects throughout the entire life cycle of plants [51]. There are also numerous research results reporting that PGPR has a multifunctional effect on the soil-plant system, improving plant tolerance to multiple stresses [52,53]. In addition to these, the treatments of cuttings with PGPR can increase root initiation and increase the quality of rooted cuttings produced in some plants because of the natural auxin production (IAA) by PGPR [54–62]. These effects were generally associated with nutrient availability and phytohormone production [63].

Auxins are known to play a key role in plant reproduction as they regulate cell differentiation and are the most well-known promoters of root formation [64]. Runkle [24] determined that a 2000 mg L^{-1} IBA concentration resulted in an increase in the rooting rate and homogeneity of poinsettia. According to Ramtin et al. [4], the maximum effect on root length, the number of bracts, the number of cyathiums, the number of leaves, the number of roots, and bract diameter was determined at 1000 mg L^{-1} IBA concentration. As a result of our study, the result of the 2000 mg L^{-1} IBA dose (the cuttings taken from the stock mother plants without bacterial formulation application) had a positive effect on the number of rooted cuttings, which was found to be consistent with the results of the previous study. As a result of feeding the main plants with the whole bacterial formulation, a positive increase in the number of rooted cuttings could not be obtained from the application of different IBA doses. However, the rooting number of cuttings taken from the stock mother plants treated with the BIII bacterial formulation decreased in the application of the 2000 mg L^{-1} IBA dose when compared to the control application. The rooting number of cuttings taken from stock mother plants and treated with the BIV bacteria formulation decreased compared to the control in terms of the rooting number between 1500 and 2000 mg L^{-1} IBA applied applications. The explanation for this situation can be made when the N contents of the parent plants are examined. The application with the highest nitrogen content is the BIV bacterial formulation, and the second is the BIII bacterial formulation. Plants showing

excessive nitrogen content have rapid growth, but cuttings from such plants show poor rooting efficiency [48]. Therefore, the balance of low N and high carbohydrates in the stock plant provides better rooting.

As a result of the application of 1500 and 2000 mg L^{-1} IBA doses to the cuttings of the main plants treated with BIV application, the mean root number decreased. This decrease may be related to the high nitrogen content of the cuttings. In addition, another study by Bortoloso Pigatto et al. [65] reported that apical cuttings have higher rates of auxin synthesis and there may be less tissue differentiation. Apical cuttings were also used in the present study. Higher auxin synthesis in cuttings of mother plants treated with BIV application and additionally endogenously high IBA applications may have caused a decrease in the mean root number. As a matter of fact, it is a more important result that a 1000 mg L^{-1} IBA dose can be sufficient for the cuttings of stock mother plants treated with BIV application to increase in NAR.

In terms of mean root length (ARL) (mm), 1000 mg L^{-1} was determined as the most appropriate IBA dose in the control application. The longest roots in cuttings taken from BI and BII applications were determined in the application of a 0 mg L^{-1} IBA dose. Longer roots were obtained from the cuttings taken from the BI application compared to the general averages. This can be explained by the fact that Ca and Fe contents are also higher in the BI application compared to the control, and these elements may have an encouraging effect on root regeneration [66,67] because they are involved in many physiological processes that lead to the formation of new cells in plants. Our results may also be supported by the fact that rhizobacteria isolates found in bacterial formulations have the capacity to promote plant growth, increase nutrient availability and uptake, and support plant health [68].

The stored nutrients in the cutting are activated with the help of growth regulators [7]. This can accelerate cutting in cuttings, increasing the utilization of carbohydrates at the base of the cuttings through better utilization of photosynthesis [69,70]. The increase in the use of carbohydrates at the base of the cuttings can accelerate the sprouting of the cuttings [12]. In the BIV application, the number of newly formed leaves decreased at a dose of 2000 mg L^{-1} IBA compared to the control. Hartman et al. [71] stated that high auxin concentrations may have an inhibitory effect on bud growth, and this effect may be due to apical dominance. The explanation of the decrease in the number of newly formed leaves determined at a dose of 2000 mg L^{-1} IBA in the BIV application in the current study can be made using the results of Hartman et al. [71].

It was determined that a 1000 mg L^{-1} IBA dose applied to cuttings taken from stock rootstock plants without any bacterial formulation application was sufficient to increase the number of mean shoots (NAS). The number of shoots formed in cuttings taken from stock mother plants treated with BI was increased at a 1500 mg L^{-1} IBA dose. The possible reason for the increase in the number of shoots may be better utilization of carbohydrates, nitrogen, and other nutrients with the help of growth regulators. The PGPR can improve nutrient availability and trigger hormone production [63,72], increasing plant enzymatic activity [72]. These effects have been confirmed in a variety of crops, including the poinsettia plant [40]. In a study on *Bursera penicillata* cuttings, it was reported that shoot length and the number of leaves increased with increasing auxin concentration [73]. In addition, Gaudin et al. [74] have stated that PGPR can produce both auxins themselves and increase auxin synthesis in plants.

The influence of PGPR on rooting cutting is already widely recognized and studied [57,58,60,75–79]. The function of bacteria to release inorganic nutrients from organic reserves at a sufficient rate to sustain rapid plant growth is an important mechanism [80]. In terms of nitrogen (N), P, Ca, and Fe contents, higher values were obtained from the BIV application when compared to the control application in the present study. Cultivation of poinsettia stock mother plants with PGPR formulations may have resulted in the rooting of cuttings by enhancing higher N accumulation and more water and nutrient uptake due to these bacteria, which are known to be effective in N_2 fixation [81,82].

5. Conclusions

The data support the following conclusions:

- This study provides positive effects on the nutrition of stock mother plants with PGPR to provide sprout production by cuttings technique;
- It has been concluded that the rooting performance of poinsettia cuttings can be improved with lower IBA dose applications if the stock mother plants from which the poinsettia cuttings will be taken are nutrient-enriched with PGPR bacterial formulations and the carbohydrate content is enriched;
- It has been revealed that lower IBA dose applications can be recommended, especially for rooting cuttings taken from stock mother plants treated with the BIV bacterial formulation;
- The rooting properties of poinsettia cuttings treated with BIV bacterial formulation are adversely affected by the application of high doses of IBA;
- Through biplot and heat mapper analyses, it has been determined that BIV-0 and BIV-1000 applications are different from all other applications and C-0 applications in the rooting performance of poinsettia cuttings;
- Further studies can be carried out to determine rooting performance after the poinsettia stock mother plants were fertilized with PGPR formulations prepared with bacterial isolates tested for auxin properties.

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