agronomy

MDPI

Article

The Quality and Productivity of Strawberry (*Fragaria* × *ananassa* Duch.) Improved by the Inoculation of PGPR *Bacillus velezensis* BS89 in Field Experiments

Vladimir K. Chebotar [1,*], Elena P. Chizhevskaya [1], Nikolai I. Vorobyov [1], Veronika V. Bobkova [2], Lyubov V. Pomyaksheva [2], Yuriy V. Khomyakov [3] and Sergey N. Konovalov [2]

[1] All-Russia Research Institute for Agricultural Microbiology, Podbel'skogo Shosse 3, Pushkin, 196608 St. Petersburg, Russia
[2] Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery, Zagorievskaya Str. 4, 115598 Moscow, Russia
[3] Agrophysical Scientific Research Institute, Grazhdansky pr. 14, 195220 St. Petersburg, Russia
* Correspondence: vladchebotar@rambler.ru; Tel.: +7-9217401753

Abstract: Efficient plant-growth-promoting rhizobacteria (PGPR) used as biofertilizers and biological control agents are promising substitutes for minimizing the application of synthetic agrochemicals in crop production. We studied the effect of PGPR strain *Bacillus velezensis* BS89 alone and in combination with three forms of nitrogen fertilizers (ammonium nitrate, carbamide, and ammonium sulfate) on the productivity of two strawberry varieties in three-year field experiments. We first showed that the application of PGPR *Bacillus velezensis* BS89 on strawberries demonstrated the same effect as the application of nitrogen fertilizers. Use of the strain BS89 increased the chlorophyll content in plant leaves by 2.7–6.8%, and also increased the yield of berries by 6.7–36.4% for cv. Rusich and 7.5–19.3% for cv. Troitskaya depending on the form of nitrogen fertilizer. The best results in the yield of strawberry plants of the cv. Rusich were achieved in the variant BS89 + ammonium nitrate (41.9–57.4%), and the cv. Troitskaya—in the BS89 + carbamide variant (8.1–38.8%). Three-year use of strain BS89 for cv. Rusich resulted in an increase of runner's weight by 212.1%, and also the weight of the roots by 120%, thereby significantly improving the mineral nutrition of plants. This is mainly associated with the plant growth-promoting activity of *Bacillus velezensis* BS89, which was able to produce a high amount of IAA—494.1 µg/mL. We believe that the PGPR strain BS89 can be successfully used for growing strawberries. However, each variety requires careful selection of the composition of nitrogen fertilizers and analysis of the compatibility of fertilizers and the PGPR strain.

Keywords: PGPR; *Bacillus velezensis*; strawberry; field experiments; fertilizers; yield increase; IAA production

Citation: Chebotar, V.K.; Chizhevskaya, E.P.; Vorobyov, N.I.; Bobkova, V.V.; Pomyaksheva, L.V.; Khomyakov, Y.V.; Konovalov, S.N. The Quality and Productivity of Strawberry (*Fragaria* × *ananassa* Duch.) Improved by the Inoculation of PGPR *Bacillus velezensis* BS89 in Field Experiments. *Agronomy* **2022**, *12*, 2600. https://doi.org/10.3390/agronomy12112600

Academic Editor: Junhui Zhou

Received: 2 October 2022
Accepted: 20 October 2022
Published: 22 October 2022

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an important crop that is mainly grown in temperate climates. Its worldwide production is more than 7.7 million tons per year, and this number is growing [1]. Strawberry fruits are rich in substances that are beneficial for human health, including antioxidant agents such as anthocyanins, other phenolic compounds, vitamins, and sugars. Modern strawberry cultivation provides high yields and good-quality fruit but requires the extensive use of chemical fertilizers, which not only disrupt the natural balance of the soil but also decrease economic efficiency [2]. The indiscriminate use of chemical fertilizers causes air, water, and soil pollution and is a hazard to human health. Alternative production systems that are more environmentally friendly and conducive to soil health are urgently needed [3]

Plant growth-promoting rhizobacteria (PGPR) inhabit the plant rhizosphere and rhizoplane and interact with root exudates and form strong associations with plants, which may

be symbiotic or non-symbiotic [4–8]. PGPR are promising candidates for applications in agriculture as biofertilizers, pesticides, and phytoremediation agents [6–8]. They can also be used in the production of inoculants to promote plant growth and biocontrol [9,10]. Certain PGPR employs not one but an entire array of mechanisms of plant growth stimulation, including nitrogen fixation; the synthesis of phytohormones, such as indole-3-acetic acid (IAA) and organic acids; HCN production; the release of enzymes, such as soil dehydrogenase, phosphatase, and nitrogenase; the antagonism of pathogenic fungi; the production of siderophores; increasing the solubilization of phosphates; and the induction of systemic resistance [11–13].

The study of plant–microbial associations have long attracted the attention of scientists interested in the fundamental aspects of coexistence and interactions between different organisms, but there is also a practical side to this research. Microorganisms beneficial for plants may be used in the environmentally oriented production of agricultural products [14]. A plant-associated microorganism is an evolutionarily verified component of a complex plant–microbial system, whose action extends beyond the scope of an individual plant [15] and that has a significant impact on the biological structure and functioning of the entire system [16].

The rhizosphere, a narrow soil space around the roots of growing plants, is a hotspot of microbial activity in the soil [17]. PGPR living in the rhizosphere aggressively colonizes roots and facilitates plant growth. PGPR, is a heterogeneous group of bacteria, including a wide range of genera, such as *Acinetobacter, Aeromonas, Agrobacterium, Allorhizobium, Arthrobacter, Azoarcus, Azorhizobium, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Caulobacter, Chromobacterium, Delftia, Enterobacter, Flavobacterium, Frankia, Gluconacetobacter, Klebsiella, Mesorhizobium, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Rhizobium, Serratia, Streptomyces*, and *Thiobacillus*, is a vital component of the soil microbiome [18]. PGPR influences the overall health of plants by enhancing nutrient intake, protecting plants from phytopathogens, and promoting resistance to various abiotic stressors [19–21]. Different PGPR strains can increase crop yield, exhibit biocontrol, enhance resistance to foliar pathogens, and improve the emergence of seedlings [22–25]. Efficient PGPR used as biofertilizers and biological control agents are promising substitutes for minimizing the application of synthetic agrochemicals in crop production [26–28].

The economic and social importance of strawberry cultivation is unquestionable. This berry crop has high nutritional requirements, especially with respect to nitrogen. The inoculation of microorganisms may be expected to contribute to the growth of strawberry plants through the colonization of their root system. However, the effect of PGPR on strawberry yield and quality has been poorly studied due to the difficulties associated with field experiments. We found only a few publications connected to this topic. For example, it was demonstrated that strawberries reacted positively to inoculation with a combination of three bacteria: *Azospirillum brasilense* Ab-V5, *Burkholderia cepacia* CCMA 0056, and *Enterobacter cloacae* CCMA 1285. More significant reactions from the point of view of plant growth were observed with the combined inoculation of three bacterial strains plus the application of 50% of the recommended dose of nitrogen fertilizer [29]. Also combined application of PGPR strains SY55 *Rhizobium* sp., SK63 *Bacillus* sp. and SY48 *Herbaspirillum* sp. significantly increased the productivity of strawberries and reduced the use of chemicals [30]. Similar results were obtained when using PGPR strains MHA75 *Pseudomonas* sp., RCA3 *Bacillus* sp., and SYB101 *Bacillus* sp. [31,32]. Therefore, the available data indicate that the application of PGPR strains, both alone as biofertilizers and in combination with chemical fertilizers, can substantially increase the yield and quality of strawberries.

In our work, we used the PGPR strain *Bacillus velezensis* BS89 (previously identified as *Bacillus subtilis* Ch13) [22]. This strain was originally isolated from the roots of winter wheat plants (cv. Lira). BS89 has the ability to promote plant growth and produce a mix of auxins, hydrolytic enzymes, and vitamins. The genome analysis of strain BS89 revealed the presence of gene clusters responsible for the synthesis of plant growth-promoting

metabolites (IAA and volatiles), numerous hydrolytic enzymes, vitamins, siderophores, and antimicrobial compounds (surfactin, fengycin, bacilysin, macrolactin, difficidin, bacillaene, and plantazolicin) [33]. However, the effectiveness of the strain *B. velezensis* BS89 in long-term field experiments on strawberries has not been studied before. We assume that in such experiments, the plant growth promotion activity of the strain BS89 could be noticeable, especially against the background of full-fledged nitrogen nutrition of plants. In our study, we used two cultivars of strawberry (*Fragaria* × *ananassa* Duch.)—Rusich and Troitskaya, which were very responsive to the application of nitrogen fertilizers but differed in their resistance to the low temperatures and time of ripeness. As nitrogen fertilizers were used ammonium nitrate, carbamide, and ammonium sulfate.

The aim of our research was to study the effect of the PGPR strain *Bacillus velezensis* BS89 used in combination with various forms of nitrogen fertilizers on the productivity and quality of two varieties of strawberries in prolonged (three-year) experiments.

2. Materials and Methods

2.1. Bacterial Strain

The strain *Bacillus velezensis* BS89 was stored in a freezer (Sanyo, Moriguchi, Japan) at −80 °C as cell suspensions from a single colony in 20% glycerol. Potato-dextrose agar (PDA; Sigma Aldrich, St. Louis, MO, USA) plates were used for passages. Strain BS89 was cultured in liquid potato-dextrose broth (PDB, Sigma, St. Louis, MO, USA) for 2 days at 28 °C on a rotary shaker with 200 rpm to get a final concentration of bacterial suspension 1.25×10^8 cfu/mL.

2.2. Plants

Two cultivars of strawberry (*Fragaria* × *ananassa* Duch.)—Rusich and Troitskaya were used in the study. Their characteristics are as follows:

Cv. Rusich: middle-late, provenance Russian Federation, Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery, in State Register from 2002, universal purpose, average winter hardiness;

Cv. Troitskaya: late, provenance Russian Federation, Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery, in State Register from 2006, universal purpose, high winter hardiness.

2.3. Soils

According to the Unified Soil Classification System (USCS), the soil from the experimental field was characterized as lean clay (LC) cultivated soil on the clay loam mantle of the Moscow Region (Leninskiy District, Izmailovo Settlement). The initial nutrient parameters of the LC soil and the contents of nitrate nitrogen and exchangeable ammonium in the soil in the experiment are presented in Tables S1 and S2 (Supplementary Materials).

2.4. Plot Experiments

The plot experiments were carried out in the Izmailovo research division of the Federal Horticultural Research Center for Breeding, Agrotechnology, and Nursery. The dimensions of the experimental plot were 2.5 m × 1.0 m, and the accounting plot was 2.0 m × 1.0 m. Drip irrigation equipment ("Netafim", Tel Aviv and "Metzerplas", Metzer, Israel) was used for the field experiments. A water jet pump (MixRite, Nahsholim, Israel) was used for the metered supply of nitrogen fertilizers to the drip irrigation system. A tensiometer (Irrometer, Riverside, CA, USA) was used to control the soil moisture regime. The leaves were collected for analysis in the third decade of June. Plant productivity was assessed using the weight method during the berry-picking period, from the third decade of June to the second decade of July on average (taking into account the weather conditions).

The nitrogen fertilizers applied were as follows: ammonium nitrate ($N\text{-}NO_3$, 17%; $N\text{-}NH_4$, 17%), urea ($N\text{-}NH_2$, 46%), ammonium sulfate ($N\text{-}NH_4$, 21%), potassium monophosphate (P_2O_5, 50%; K_2O, 33%), and potassium sulfate (K_2O, 46%).

Experimental design:

1. Control (background without fertilizers) (A0B0);
2. Fertigation: ammonium nitrate (Naa) (A1B0);
3. Fertigation: carbamide (Nm) (A2B0);
4. Fertigation: ammonium sulfate (Na) (A3B0);
5. Fertigation: ammonium nitrate (Naa) + *Bacillus velezensis* BS89 (A1B1);
6. Fertigation: carbamide (Nm) + *Bacillus velezensis* BS89 (A2B1);
7. Fertigation: ammonium sulfate (Na) + *Bacillus velezensis* BS89 (A3B1);
8. *Bacillus velezensis* BS89 (fertigation) (A0B1) cell suspension 1.25×10^8 cfu/mL;

Plants were planted in single lines according to a 90 × 20 scheme. The plot area was 0.9 × 2.0 m, and ten plants per plot were planted. The experiment was conducted in three replications. Two factors were taken into account: the form of nitrogen application (four gradations) and the application of *Bacillus velezensis* BS89 (two gradations).

Strawberry plants were planted on 29–30 May 2017. Before planting, the soil was treated with phosphorus and potassium fertilizers in the top layer at a rate of P35K50 per hectare. The macro-elements introduced in 2017–2020 are listed in Table 1.

Table 1. Macroelements introduced during the study period (variants 2–7) and *Bacillus velezensis* BS89 (variants 5–8) under one plant per season.

NPK and PGPR	2017		2018		2019		2020	
	kg/ha	Under One Plant, g	kg/ha	Under One Plant, g	kg/ha	Under One Plant, g	kg/ha	Under One Plant, g
N	70	1.27	40	0.73	80	1.45	50	0.91
P_2O_5	30	0.55	30	0.55	50	0.91	30	0.55
K_2O	50	0.91	30	0.55	70	1.27	50	0.91
Bacillus velezensis BS89	0	0	22	40.0	16.2	30.0	19.25	35.0

The plants were watered at a rate of 300 cm^3 of water per plant on average per day. The soil moisture was sustained at 80% HB (HB is the lowest moisture capacity, i.e., the maximum amount of moisture that the soil can retain, with all the excess water during precipitation or irrigation draining into deeper layers) before and during harvesting, and at a level of 70% HB after harvesting. *Bacillus velezensis* BS89 was applied to the tank mixture at a rate of 5 mL of bacterial suspension (1.25×10^8 cfu/mL) per 1 L of water.

The yield of berries was determined by weighing. For the chemical analysis, berries were sampled at the beginning of mass ripening, the leaves were sampled immediately after harvesting, and the soil was sampled after runner formation.

Agrotechnical care of plants was carried out according to the standard technology.

2.5. Analyses

2.5.1. Analysis of Bacterial Phytohormones

Bacillus velezensis BS89 was cultured in liquid potato-dextrose broth (PDB, Sigma, St. Louis, MO, USA) for 4 days at 28 °C in 500 mL flasks placed in a rotary shaker (180 rpm). The concentrations of indole IAA, *trans-Zeatin (tZ)*, and Gibberellic acids (GA) in the bacterial suspension were determined using a VARIAN 212 LC high-performance liquid chromatograph with a mass-selective detector (Varian 500 MS system, Palo Alto, CA, USA). Detection of IAA was carried out using ESI− (electrospray) ion at 174 m/z. The detection of tZ and GA was carried out using ESI+ for ions at 220 m/z and 345 m/z, respectively. To determine phytohormones, 50 mL of liquid culture (and 50 mL sterile liquid medium, used as a control) was taken and centrifuged at a speed of 3000–5000 rpm for 5 min. The supernatant was drained into a dividing funnel. The precipitate was shaken twice with 30 mL of distilled water and centrifuged after combining the supernatant in a dividing funnel. The combined supernatant in the dividing funnel was acidified with

a 10% solution of acetic acid to a pH of 2, after which phytohormones were extracted three times with 10 mL of ethyl acetate. The upper ethyl acetate layer was drained through anhydrous sodium sulfate and evaporated until dry on a rotary evaporator at a temperature of no more than 40 °C. The extraction was performed three times. Chromatography was carried out in the gradient mode (phase A, methanol + 0.1% formic acid; phase B, deionized water + 0.1% formic acid). The chromatographic system used a Cosmosil C18 4.6 ID 150 mm column (Nacalai Tesque Inc., Kyoto, Japan). The chromatograph was calibrated using the SIGMA-ALDRICH internal standards for pure hormone substances. The identification of hormones was carried out in the mass–mass mode.

2.5.2. Method for Determining the Content of Chlorophyll

The method consisted of the quantitative determination of the chlorophyll in the raw mass of leaves using a spectrophotometer. A total of 0.1 g of leaves were crushed and placed in a porcelain mortar, and 2–3 drops of 96% ethanol and laboratory quartz sand were added. The mixture was then ground. Gradually, ethanol was added as the grinding continued. Thereafter, 25 mL of the extract was filtered through a filter with a white ribbon into measuring cylinders, with care being taken to wash off the pigment with alcohol. After filtration, the ethanol concentration was adjusted to 96%. If the solution was turbid, it was filtered again. The amount of chlorophyll (a + b), in mg/g of the raw mass of leaves, was determined using a Photometer Photoelectric CFK-3 ("Zagorsk Optical-Mechanical Plant", Zagorsk, Russia) with a red light filter, with a wavelength of 670 nm. A scale was previously constructed, and the optical density of a series of standard solutions with standard concentrations was measured. On the basis of the results, a calibration curve was constructed.

When working with chlorophyll, a Getry solution was used as a standard solution (28.5 mL of a 1% solution of $CuSO_4 \times 5H_2O$, 50 mL of a 2% solution of $K_2Cr_2O_7$, and 10 mL of a 2N solution of NH_4OH; the concentration of the resulting Getry solution corresponded to 85 mg/L of chlorophyll).

2.5.3. Methods for Determining Macro- and Meso-Elements

The determination of soil pH_{KCl} was performed utilizing the potentiometric method on a laboratory ionomer I-160MI ("Measuring Equipment" LLC, Moscow, Russia) using a glass electrode to determine the activity of hydrogen ions and a silver chloride reference electrode according to ISO 10390:2005 [34], with KCl as an extract.

The content of alkaline hydrolysable nitrogen in the soil was measured according to Kornfield to ISNT [35] by a titrimetric method. The method is based on the hydrolysis of organic soil compounds with a 1N sodium hydroxide solution, and the determination of the ammonia released (taking into account the exchange of ammonium) using the micro-diffusion method. This utilizes modified cast Conway dishes to absorb ammonia with a solution of boric acid and titrate it with sulfuric acid.

The content of nitrate nitrogen in the soil was estimated using the colorimetric method with disulfophenolic acid according to Grandvalle; the ammonium nitrogen content was estimated using the colorimetric method with Nessler reagent [36].

The exchange of calcium and magnesium in the soil was determined by complexometric titration with Trilon B [37].

The contents of labile phosphorus in the soil were determined using 0.2N HCl on a Photometer Photoelectric CFK-3-01 ("Zagorsk Optical-Mechanical Plant", Zagorsk, Russia) according to Bray's method with hydrochloric acid [37,38]. The contents of labile potassium in the soil were determined using a flame photometer FPA-2 ("Zagorsk Optical-Mechanical Plant", Zagorsk, Russia) [38], respectively.

The NPK in the leaves and berries was determined as follows: The samples were prepared according to the method in [39]; this involved salting in a mixture of sulfuric and hydrochloric acids. The phosphorus content in the leaves was determined on a Photometer Photoelectric CFK-3-01 ("Zagorsk Optical-Mechanical Plant", Zagorsk, Russia), and the

potassium content was determined on a flame photometer, FPA-2 ("Zagorsk Optical-Mechanical Plant", Zagorsk, Russia).

The contents of calcium and magnesium in the leaves were determined complexometrically with Trilon B; the nitrogen content was determined using the Kjeldahl method [38,39].

2.5.4. Estimation of the Root and Runner Weight of Strawberry Plants

At the end of the third year of fruition, before the experiment was concluded, we studied the vegetative productivity of the cv. Rusich strawberry plants by weighing the root systems and the tops of the plants in the experimental and control variants. The roots in the soil mass (5–15 cm depth; 0.28 dm^3 volume) at different distances from the drip tape (5, 15, and 25 cm) in the row of plantations were studied.

2.5.5. Statistical Analysis

For statistical analysis, we used a two-factor analysis of variance (ANOVA) of the dependence of four variables (chlorophyll content in leaves and strawberry yields 2018–2020) on two independent factors (nitrogen fertilizers and PGPR *Bacillus velezensis* BS89) and Duncan's multiple range test to determine the significance of differences between the mean values.

2.6. Climate Conditions in 2017–2020

In 2017, the weather conditions in the first half of the growing season were abnormal for the Moscow Region; i.e., they were characterized by a low temperature, high humidity, and rainfall above the long-term average (in April and June, it was twice the long-term average). As a result, the strawberry plants started to flower a week later than usual, and the fruit ripened 3 weeks later than usual. Experimental plants were planted in May 2017, and their establishment took place under unfavorable weather conditions. In the second half of the growing season, the air temperature and precipitation values corresponded to the climatic norms, which facilitated the formation of flowering buds and the establishment of the following year's yield.

In 2018, the weather conditions were favorable for plant development. The low monthly average temperatures in February and March did not contribute to plant mortality. The temperatures during the growing season did not differ from the average norm, while the lower rainfall in June and August was compensated for by additional watering.

In 2019, the weather conditions in the first half of the growing season differed from the long-term average regarding precipitation; i.e., from mid-May to mid-June, only 4 mm of precipitation fell in the area of the experiment, and drought was observed. The temperature corresponded to the climatic norm. The soil moisture was maintained at the required level with the help of drip watering.

The winter period of 2019–2020 was one of the warmest in recent decades. In December, there was little precipitation (mostly rain). In January and February 2020, the amount of precipitation was within the norm, but due to the high temperatures, no stable snow cover formed. During the spring period, the weather conditions were normal, but as there was no snowmelt, a deficit of moisture was observed in the soil in April.

In May, June, and July, the precipitation was twice as high as is normal for these periods (more than 150 mm per month). The temperatures remained within the norm but dropped lower than normal in May, contributing to fungal infections of the berry and fruit crops. On 19 June 2020, a weather collapse was observed; i.e., in the morning and early afternoon, the temperature remained at +28 °C; at 2 pm, hail began falling, with hailstones the size of hazelnuts, and the temperature dropped to +15 °C within 15 min. Strawberries and other berry crops, which were in the flowering, fruit formation, and ripening phases, were damaged both mechanically by the hail and due to the sharp drop in temperature and the subsequent development of diseases.

3. Results

3.1. Bacterial Phytohormones Production

We used the PGPR strain *Bacillus velezensis* BS89 which has the ability to promote plant growth and produce a mix of auxins, hydrolytic enzymes, and vitamins as was shown in our previous study [22,33]. The genome analysis of strain BS89 revealed the presence of gene clusters responsible for the synthesis of plant growth-promoting metabolite IAA [33]. That is why we estimated the production of phytohormones by the strain BS89. The concentrations of IAA, tZ, and GA in the bacterial suspension of *Bacillus velezensis* BS89 were presented in Table 2. It was demonstrated that strain BS89 produced a large amount of IAA, but do not able to produce Gibberellic acids and trans-Zeatin.

Table 2. The concentrations of IAA, tZ, and GA in the bacterial suspension of *Bacillus velezensis* BS89.

Sample	IAA, μg/mL	GA, μg/mL	tZ, μg/mL
Bacillus velezensis BS89	494.1 ± 17.3	0	0

It was demonstrated that strain BS89 produced a large amount of IAA, but do not able to produce Gibberellic acids and trans-Zeatin.

3.2. Yield of Berries of Strawberry Plants and Chlorophyll Content in the Plant Leaves

In this study, field experiments, lasting 3 years, assessed the effect of the PGPR *Bacillus velezensis* BS89, used alone and in combination with nitrogen fertilizers, on the productivity of two strawberry varieties (cv. Rusich and cv. Troitskaya). The qualitative and quantitative parameters of the strawberry plants (the fruit yield, chlorophyll content in the plant leaves, and contents of chemical elements (N, K, P, Ca, and Mg) in the plant leaves, Tables S3 and S4) were recorded. Since the chlorophyll content in the plant leaves of two strawberry cultivars varied to a lesser extent every year (Tables S5 and S6) we decided to present the results of chlorophyll content as an average of three years in Table 3.

Table 3. Chlorophyll content in the plant leaves of two strawberry cultivars (average of three years).

Experimental Variant	Cv. Rusich	Cv. Troitskaya
Without fertilizers	14.6 ^{a}	14.8 ^{a}
Fertigation Naa	14.6 ^{a}	15.0 ^{a}
Fertigation Nm	14.7 ^{a}	15.6 ^{a}
Fertigation Na	15.4 ^{b}	16.3 ^{b}
Without fertilizers + BS89	15.6 ^{b}	15.2 ^{a}
Fertigation Naa + BS89	15.8 ^{b}	15.5 ^{a}
Fertigation Nm + BS89	15.5 ^{b}	16.4 ^{b}
Fertigation Na + BS89	15.3 ^{ab}	16.6 ^{b}
Standard error (SE)	±0.6	±0.6
F1.Fertigation	$p < 0.87$ *	$p < 0.026$
F2.BS89	$p < 0.009$	$p < 0.035$
F1.Fertigation ∗ F2.BS89	$p < 0.29$ *	$p < 0.016$

Different letters indicate a significant difference between the means at the probability level of $p < 0.05$. * Unreliable influence of F1-factor and F1 ∗ F2-multifactor on chlorophyll content

The use of nitrogen fertilizers on strawberry cv. Rusich did not have a significant effect on the chlorophyll content in the plant leaves, with the exception of the variant Fertigation Na (an increase of 5.5%), and the use of strain BS89 increased the chlorophyll content by 6.8% compared to the control. The combined application of nitrogen fertilizers and PGPR strain *Bacillus velezensis* BS89 increased the chlorophyll content by 5.4–8.2%, with the exception of the variant Fertigation Na, but the increase was not statistically significant (Table 3). The Troitskaya variety was more responsive to the use of nitrogen fertilizers. Thus,

the chlorophyll content in the leaves increased by 1.4–10.1%. The use of strain BS89 was comparable to the effect of nitrogen fertilizers—the increase in chlorophyll content in the plant leaves was 2.7%. The increase in chlorophyll content with the combined application of BS89 and nitrogen fertilizers was statistically significant at 1.8–5.1%. Data on the effects of both nitrogen fertilizers and PGPR strain *Bacillus velezensis* BS89 on the yield of berries of strawberry plants are presented in Tables 4 and 5.

Table 4. Yield of berries of cv. Rusich strawberry plants and ANOVA data.

Experimental Variant	Yield, 2018, g/plant	Yield, 2019, g/plant	Yield, 2020, g/plant
Without fertilizers	37.1 ^{a}	110 ^{a}	150 ^{a}
Fertigation Naa	39.0 ^{a}	108 ^{a}	155 ^{a}
Fertigation Nm	40.0 ^{a}	121 ^{a}	180 ^{ab}
Fertigation Na	53.8 ^{b}	140 ^{b}	195 ^{b}
Without fertilizers + BS89	47.0 ^{b}	150 ^{b}	160 ^{a}
Fertigation Naa + BS89	59.9 ^{c}	170 ^{c}	220 ^{bc}
Fertigation Nm + BS89	48.5 ^{b}	149 ^{b}	200 ^{b}
Fertigation Na + BS89	50.7 ^{b}	153 ^{b}	175 ^{a}
Standard error (SE)	±6.0	±15	±20
F1.Fertigation	$p < 0.035$	$p < 0.31$ *	$p < 0.026$
F2.BS89	$p < 0.002$	$p < 0.00003$	$p < 0.035$
F1.Fertigation ∗ F2.BS89	$p < 0.026$	$p < 0.07$	$p < 0.016$

Different letters indicate a significant difference between the means at the probability level of $p < 0.05$. * Unreliable influence of F1-factor and F1 ∗ F2-multifactor on strawberry yield.

Table 5. Yield of berries of cv. Troitskaya strawberry plants and ANOVA data.

Experimental Variant	Yield, 2018, g/plant	Yield, 2019, g/plant	Yield, 2020, g/plant
Without fertilizers	41.5 ^{a}	146.6 ^{a}	160.0 ^{a}
Fertigation Naa	49.5 ^{ab}	165.0 ^{b}	180.9 ^{a}
Fertigation Nm	43.3 ^{a}	163.3 ^{b}	192.4 ^{b}
Fertigation Na	54.9 ^{b}	180.0 ^{c}	195.4 ^{b}
Without fertilizers + BS89	49.5 ^{ab}	165.0 ^{b}	172.0 ^{a}
Fertigation Naa + BS89	52.5 ^{b}	175.0 ^{bc}	190.0 ^{ab}
Fertigation Nm + BS89	60.1 ^{bc}	196.2 ^{d}	208.0 ^{c}
Fertigation Na + BS89	66.0 ^{c}	199.4 ^{d}	210.0 ^{c}
Standard error (SE)	±7.0	±15	±12
F1.Fertigation	$p < 0.015$	$p < 0.008$	$p < 0.0002$
F2.BS89	$p < 0.036$	$p < 0.001$	$p < 0.018$
F1.Fertigation ∗ F2.BS89	$p < 0.41$ *	$p < 0.27$ *	$p < 0.97$ *

Different letters indicate a significant difference between the means at the probability level of $p < 0.05$. * Unreliable influence of F1 ∗ F2-multifactor on strawberry yield.

In 2018, the use of nitrogen fertilizers on strawberry cv. Rusich increased the yield of berries by 5.1–45.0%, and the use of the BS89 strain increased the yield of berries by 26.7%, which was comparable to the effectiveness of nitrogen fertilizers. An even more significant effect on the strawberry yield was the application of the BS89 strain together with nitrogen fertilizers (except for the variant of Fertigation Na)—an increase of 21.3–53.6%. The same effect was observed on cv. Troitskaya. Thus, the increase in the yield of berries with the use of nitrogen fertilizers was 4.3–32.2%, and with the use of strain BS89 19.3%. The combined

use of nitrogen fertilizers and strain BS89 increased the yield of berries by 6.1–38.8%, but the increase was not statistically significant (Table 5).

In 2019, the use of nitrogen fertilizers on cv. Rusich increased the yield of berries by 10.0–27.3% (except for the variant of Fertigation Naa), and the use of the BS89 strain increased the yield of berries by 36.4%. With the joint application of nitrogen fertilizers and strain BS89, the yield of berries increased by 9.2–57.4%. The same effect was observed on cv. Troitskaya. The strawberry yield increased by 11.4–22.8% when applying nitrogen fertilizers, and by 12.6% when applying strain BS89. With the joint application of nitrogen fertilizers and strain BS89, the yield of berries increased by 6.0–20.1%, but the increase was not statistically significant (Table 5).

In 2020, the yield of strawberry cv. Rusich when applying nitrogen fertilizers increased by 3.3–30.0%, and when applying strain BS89 by 6.7%. With the joint application of nitrogen fertilizers and strain BS89, the yield of strawberries increased by 11.1–41.9% (except for the variant of Fertigation Na). The use of nitrogen fertilizers at cv. Troitskaya increased the yield of berries by 13.1–22.1% and the use of strain BS89 increased the yield by 7.5%. With the combined use of nitrogen fertilizers and strain BS89, the yield of strawberries increased by 5.0–8.1%, but the increase was not statistically significant (Table 5). Thus, the results of three-year experiments showed that strain PGPR *Bacillus velezensis* BS89 has had a significant growth-stimulating effect, comparable with the efficiencient use of nitrogen fertilizers.

The effect of nitrogen fertilizers (F1) on the berries yield of cv. Rusich was statistically significant in the years 2018 and 2020, while the effect of PGPR BS89 (F2) was statistically significant for all three years (Table 4). The effect of both factors (F1 $*$ F2) was statistically significant for all three years.

Cv. Troitskaya was also responsive to the application of both the BS89 strain and nitrogen fertilizers (Table 5). Data from two-factor analysis demonstrated that the effect of nitrogen fertilizers (F1) and PGPR BS89 (F2) were statistically significant for all three years (Table 5), but not the effect of both factors (F1 $*$ F2). It is clear that *Bacillus velezensis* BS89 had a growth-stimulating effect; however, it is not clear which properties were responsible for this result.

3.3. Root and Runner Weight of cv. Rusich Strawberry Plants

At the end of the third year of fruition, before the experiment was concluded, we studied the vegetative productivity of the cv. Rusich strawberry plants. The results of the root and runner weight of cv. Rusich strawberry plants are presented in Figures 1 and 2.

The results of our study showed that the increase in the root weight of strawberry plants after three years equaled 50.9–120% in all the variants, except the carbamide. Thus, the highest increase in the root weight of strawberry plants was observed in the variant with the application of the strain BS89 −120%, followed by the variant BS89+ Fertigation Na −105% and the variant with BS89+ Fertigation Naa −51% comparing with the untreated control, respectively. Moreover, the increase in runner weight (an index of future productivity) was 97.1–212% in all the variants, except the carbamide. We observed the same trend. The highest increase in runner weight was observed in the variant with the application of the strain BS89 −212%, followed by the variant BS89+ Fertigation Na −159% and the variant with BS89+ Fertigation Naa −97% compared with the untreated control, respectively.

We can conclude that the application of *Bacillus velezensis* BS89 without nitrogen fertilizers and with nitrogen fertilizers has a significant effect on the productivity of both strawberry varieties used in our study, increasing productivity by 20.9–57.4 for cv. Rusich and 5.0–38.8% for cv. Troitskaya. This is mainly associated with the growth-stimulating activity of *Bacillus velezensis* BS89, which increased the root mass of cv. Rusich strawberry plants several-fold during the three years of plant growth, thus increasing the mineral nutrition area of the plants. On the other hand, the increase in the runner weight of cv

Rusich strawberry plants indicates a significant influence of *Bacillus velezensis* BS89 on the generative abilities of strawberry plants.

However, we cannot unambiguously conclude that *Bacillus velezensis* BS89 has only growth-stimulating abilities, because a number of biochemical and biometric parameters of the strawberry plants did not significantly change (Tables S2 and S3).

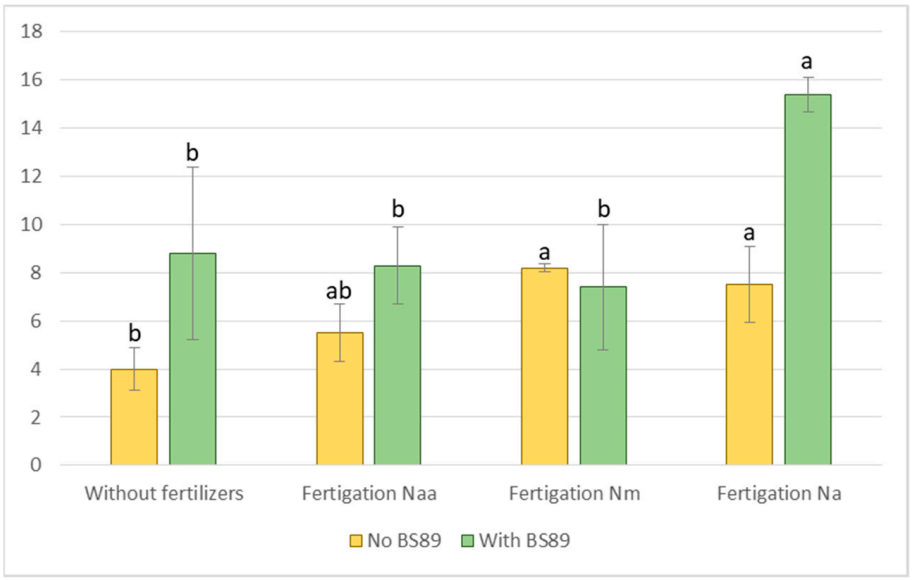

Figure 1. The effect of nitrogen fertilizers and PGPR *Bacillus velezensis* BS89 on the root weight of cv. Rusich strawberry plants, October 2020 (root weight, grams). The bars are means of three replications per variant. Bars show ± SE, and different letters show a significant difference at the $p \leq 0.05$ level, as determined by Duncan's multiple tests.

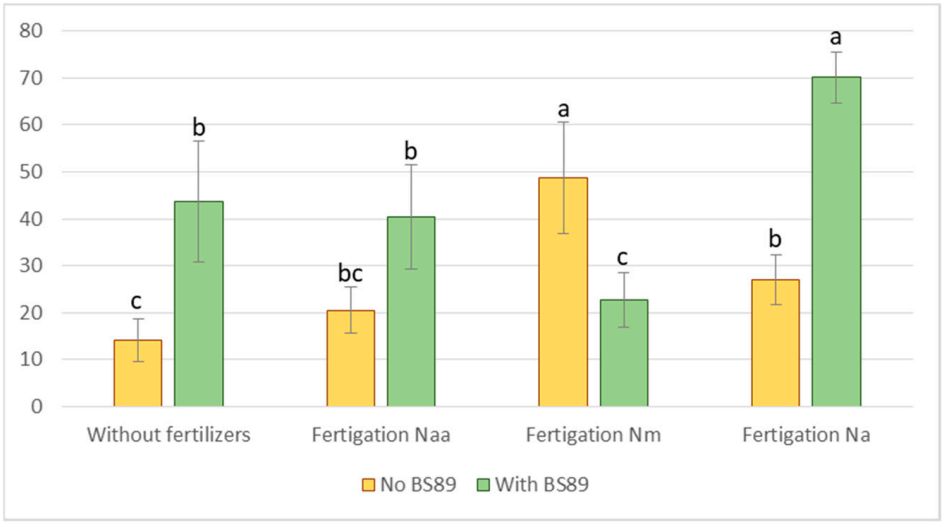

Figure 2. The effect of nitrogen fertilizers and PGPR *Bacillus velezensis* BS89 on runner weight of cv. Rusich strawberry plants, October 2020 (runner weight, grams). The bars are means of three replications per variant. Bars show ± SE, and different letters show a significant difference at the $p \leq 0.05$ level, as determined by Duncan's multiple tests.

4. Discussion

The use of PGPR in agricultural production is embarrassed by a lack of understanding of the mechanism of their action: it is unclear how PGPR promotes an increased plant yield. A classical explanation is that phytohormones stimulate the rate of plant biomass accumulation [18–20]. Without phytohormones, it is said, plants grow sluggishly and

slowly. It is obvious, however, that plants always interact with rhizosphere microflora, whether we use effective PGPR or not. The reason behind the successful interaction of plants with bacteria must lie elsewhere [40–42].

In this paper, we conducted an experiment with growing strawberry plants on nitrogen fertilizers and PGPR *Bacillus velezensis*. When studying the literature on this issue, we found that few papers have been published on the effect of PGPR and nitrogen fertilizers on the productivity and quality of strawberries. We have not found data on conducting three-year field experiments with PGPR and nitrogen fertilizers on strawberries. Therefore, we believe that the data we have obtained will be of interest to researchers working in this area. In our experiments, we have shown that the use of the strain BS89 increased the yield of berries, the weight of roots and runners of strawberries, as well as the content of chlorophyll in the leaves of plants.

Strain BS89 is able to produce a high amount of IAA −494.1 μg/mL. We compared this amount with other PGPR *Bacillus subtilis*, which were able to produce auxins. So, the IAA production was 13.0–25.5 μg/mL [40], from 0.75 to 21.3 μg/mL [41], twenty isolates had concentrations of auxin more than 100 μg/mL [42], from 137.81 μg/mL to 162.93 μg/mL depending on condition of cultivation [43]. The addition of tryptophan to the nutrient medium and prolonged cultivation can increase the production of IAA [42–44]. However, the strain BS89 synthesized a large amount of IAA without the additional introduction of tryptophan into the nutrient medium, which makes it possible to consider it a superproducer of IAA.

Auxin plays an important role in the establishment and maintenance of beneficial plant—PGPR interaction. For instance, auxin-producing PGPR strains PNS-1 *Aeromonas punctata*, 90–166 *Serratia marcescens*, and Sp245 *Azospirillum brasilense* stimulate growth and induce morphological changes in *Arabidopsis thaliana* [45,46]. At the same time, the auxin over-producing strain of *Burkholderia cepacia* has a greater stimulating effect on rice plants than strains with negligible auxin production [47]. This evidence suggests that IAA synthesis may be the primary cause of the stimulatory effect of some PGPR strains on host plants. Interestingly, high concentrations of auxin synthesized by nonpathogenic strains of rhizobacteria (for example I-3 *Enterobacter* sp.) may have an inhibiting effect on plants [48]. Therefore, to achieve a stimulating effect on the host plant, the amount of the auxin produced by the strain should correspond with the optimum for a given species under given environmental conditions.

We supposed that high production of IAA by the strain BS89 can improve and enhance the root system development of strawberry plants. Auxins are responsible for the division, extension, and differentiation of plant cells and tissues and are known to increase the rate of xylem and root formation [49]. All these processes i.e., initiation, development, emergence, and elongation of lateral roots are regulated by the biosynthesis, transport, and signaling of auxins [50,51]. An increase in the number of auxins can cause increased formation of roots in an amount-dependent manner [52]. It was reported that inoculation with IAA-producing *Bacillus* strains enhances root length as well as the number of lateral roots [53–55]. IAA-producing bacteria control endogenous IAA levels in plant roots by regulating auxin-responsive genes, which change the root architecture [55]. The results of our study showed that the increase in the root weight of strawberry plants after three years equaled 50.9–120% in all the variants, except the carbamide (Figure 1).

The positive effect of PGPR inoculation has been reported on aboveground as well as belowground plant parts, although, more attention has been given to the above-ground part due to the economic importance of the aerial parts as food and fodder [56]. The primary function of the roots is to provide anchorage as well as support to the aboveground biomass and uptake water and nutrients (macro and micro) from the soil for plant growth. Roots also play important role in nutrient cycling by providing organic matter and by influencing the activity of soil microbial communities. PGPR-mediated modulations of root traits can be advantageously explored in the future for improving the efficiency of agroecosystems.

PGPR able to induce desired root traits for harnessing the soil resources can be a way toward sustainable agricultural production [57].

It has also been reported that IAA-producing bacilli are able to increase the chlorophyll content in plant leaves [54]. Similarly, in our study, inoculation with *Bacillus velezensis* BS89 resulted in an increase in runner weight (Figure 2), and chlorophyll content (Table 3).

Three-year-long field experiments on the effect of strain BS89 on the productivity of two strawberry varieties showed that this strain could significantly affect the productivity of plants increasing it by 6.7–36.4% (Tables 4 and 5). The same effect was obtained by other researchers on strawberries of the cv. Chandler when used three PGPR strains (MHA75 *Pseudomonas* sp., RCA3 *Bacillus* sp., and SYB101 *Bacillus* sp.) either alone or in combination with fertilizers [32]. Another study was carried out with cv. Festival strawberry and three PGPR strains in a 1:1:1 ratio [30]. The application of bacteria in strawberry cultivation decreased the use of chemicals and increased productivity on average by 32.97%.

Regarding the mechanism of positive action of microorganisms on the berries yield, we can propose the following. It is likely that in the early stages of plant root, stem, and leaf formation, microorganisms provide the plants with everything necessary for the formation of leaves with increased chlorophyll content. This action benefits not only the plants but also the rhizosphere plant growth-promoting microorganisms. Since the chlorophyll content of the plant leaves increases, the flow of carbon into the plant during CO_2 conversion increases too, and so does the flow of organic acids and sugars from the plant into the rhizosphere, where the microorganisms feed on these substances. Thus, this microbial-plant interaction is beneficial both for the plants and for the microorganisms, which is a prerequisite for the progressive evolutionary development of microbial-plant biosystems [25–28].

It should be noted that one must carefully choose the application rates and the composition of nitrogen fertilizers to be used together with PGPR *Bacillus velezensis* BS89. In our experiments, we have shown that the combined use of strain BS89 and nitrogen fertilizers has always increased the yield of berries compared to the use of nitrogen fertilizers, with the exception of the variant with ammonium sulfate on cv. Rusich. Probably in this case cv. Rusich did not react to the combined use of strain BS89 and ammonium sulfate. The best results in terms of cv. Rusich strawberry plant yield were achieved in the variant with the use of PGPR *Bacillus velezensis* BS89 together with the nitrogen fertilizer "Fertigation Naa"−41.9–57.4% of untreated. The best results in terms of cv. Troitskaya strawberry plant yield were achieved in the variant with the use of BS89 together with the "Fertigation Nm"−9.9–38.8%. Probably each strain requires the selection of a certain composition of nitrogen fertilizers. However, before that, it should be studied the compatibility of nitrogen fertilizer and PGPR strain.

5. Conclusions

We first showed that the application of PGPR *Bacillus velezensis* BS89 on strawberries in three-year field trials demonstrated the same effect as the application of nitrogen fertilizers. Application of PGPR strain BS89 alone, increased the yield of strawberries by 6.7–36.4% for cv. Rusich and 7.5–19.3% for cv. Troitskaya. This is mainly associated with the plant growth-promotion activity of *Bacillus velezensis* BS89, which was able to produce a high amount of IAA −494.1 µg/mL and increased the runners and roots weight of cv. Rusich strawberry plants several-fold during the three years of plant growth, thus increasing the mineral nutrition area of the plants. Our results demonstrated that it is very important to select the best combination of PGPR with nitrogen fertilizer. Thus, the best results in terms of cv. Rusich strawberry plant yield were achieved in the variant with the use of BS89 together with the ammonium nitrate −41.9–57.4% of untreated and in terms of cv. Troitskaya in the variant with the use of BS89 together with the carbamide −8.1–38.8%. Probably each strain requires the selection of a certain composition of nitrogen fertilizers. Only such an integrated approach will make it possible to effectively use PGPR for growing strawberries.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12112600/s1. Table S1: The initial nutrient parameters of the lean clay (LC) cultivated soil on the clay loam mantle of the Moscow Region (Leninskiy District, Izmailovo Settlement). Table S2: The contents of nitrate nitrogen and exchangeable ammonium in the soil in the experiment, mg/kg, 2018–2020. Table S3: Contents of chemical elements in the leaves of strawberries of cv. Rusich in experimental variants (% of 1 g weight of plant leaves). Table S4: Contents of chemical elements in the leaves of strawberries of cv. Troitskaya (% of g weight of plant leaves). Table S5: Chlorophyll content in plant leaves of strawberries of cv. Rusich by year (2018–2020), mg/g of raw mass. Table S6: Chlorophyll content in plant leaves of strawberries of cv. Troitskaya by year (2018–2020), mg/g of raw mass.

Author Contributions: Conceptualization, V.K.C. and S.N.K.; Methodology, S.N.K.; Software, N.I.V.; Validation, V.K.C. and N.I.V.; Formal Analysis, V.V.B. and L.V.P.; Investigation, E.P.C. and Y.V.K.; Resources, V.V.B. and L.V.P.; Data Curation, S.N.K.; Writing—Original Draft Preparation, V.K.C. and E.P.C.; Writing—Review & Editing, V.K.C. and E.P.C.; Visualization, N.I.V.; Supervision, V.K.C. and S.N.K.; Project Administration, S.N.K. and V.K.C.; Funding Acquisition, V.K.C. All authors have read and agreed to the published version of the manuscript.

Funding: Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2022-320 dated 20 April 2022 on providing a grant in the form of subsidies from the Federal budget of the Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author. The complete genome sequences were deposited in National Center for Biotechnology Information (NCBI) under the accession number MPHE00000000. The version described in this paper is the first version (MPHE00000000.1). Raw sequences were deposited in the Sequence Read Archive under the accession number SRR1296299.

Acknowledgments: The research was performed using equipment of the Core Centrum "Genomic Technologies, Proteomics and Cell Biology" in ARRIAM.

Conflicts of Interest: The authors declare that they have no competing interest in this paper and the study as a whole.

References

1. FAO. FAOStat. Available online: http://www.fao.org/faostat/en/#data/ (accessed on 25 December 2016).
2. Martinsson, M.; Kwast, A.; Cieslinski, G.; Treder, W. Impact of production systems and fertilizer application on yield and quality of strawberries. *Acta Hortic.* **2006**, *708*, 59–64. [CrossRef]
3. Ali, S.; Moon, Y.-S.; Hamayun, M.; Khan, M.A.; Bibi, K.; Lee, I.-J. Pragmatic role of microbial plant biostimulants in abiotic stress relief in crop plants. *J. Plant Interact.* **2022**, *17*, 705–718. [CrossRef]
4. Kloepper, J.W.; Schroth, M.N. Plant growth promoting rhizobacteria on radishes. In Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, Angers, France, 27 August–2 September 1978; pp. 879–882.
5. Ahmad, F.; Ahmad, I.; Khan, M. Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res.* **2008**, *163*, 173–181. [CrossRef] [PubMed]
6. Compant, S.; Clément, C.; Sessitsch, A. Plant growth-promoting bacteria in the rhizo and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* **2010**, *42*, 669–678. [CrossRef]
7. Pishchik, V.; Mirskaya, G.; Chizhevskaya, E.; Chebotar, V.; Chakrabarty, D. Nickel stress-tolerance in plantbacterial associations. *PeerJ* **2021**, *9*, e12230. [CrossRef]
8. Etesami, H.; Beattie, G.A. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Front. Microbiol.* **2018**, *9*, 148. [CrossRef] [PubMed]
9. Harman, G.E.; Obregón, M.A.; Samuels, G.J.; Lorito, M. Changing models for commercialization and implementation of biocontrol in the developing and the developed world. *Plant Dis.* **2010**, *94*, 928–939. [CrossRef]
10. Jiao, X.; Takishita, Y.; Zhou, G.; Smith, D.L. Plant associated rhizobacteria for biocontrol and plant growth enhancement. *Front. Plant Sci.* **2021**, *12*, 634796. [CrossRef]
11. Barriuso, J.; Solano, B.R.; Fray, R.G.; Camara, M.; Hartmann, A.; Gutierrez Mahero, F.J. Transgenic tomato plants alter quorum sensing in plant growth-promoting rhizobacteria. *Plant Biotech. J.* **2008**, *6*, 442–452. [CrossRef]

12. Santoyo, G.; Moreno-Hagelsiebb, G.; Orozco-Mosquedac, M.d.C.; Glick, B.R. Plant growth-promoting bacterial endophytes. *Microb. R.* **2016**, *183*, 92–99. [CrossRef]
13. Moon, Y.-S.; Ali, S. Isolation and identification of multi-trait plant growth-promoting rhizobacteria from coastal sand dune plant species of Pohang beach. *Folia Microbiol.* **2022**, *67*, 523–533. [CrossRef] [PubMed]
14. Chebotar, V.K.; Malfanova, N.V.; Scherbakov, A.V.; Ahtemova, G.A.; Borisov, A.Y.; Lugtenberg, B.; Tikhonovich, I.A. Endophytic bacteria in microbial preparations thar improve plant development (Review). *Appl. Biochem. Microbiol.* **2015**, *51*, 283–289. [CrossRef]
15. Clay, K.; Holah, J. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **1999**, *285*, 1742–1745. [CrossRef] [PubMed]
16. Omacini, M.; Chaneton, E.J.; Ghersa, C.M.; Müller, C.B. Symbiotic fungal endophytes control insect host-parasite interaction webs. *Nature* **2001**, *409*, 78–81. [CrossRef]
17. De la Fuente Cantó, C.; Simonin, M.; King, E.; Moulin, L.; Bennett, M.J.; Castrillo, G.; Laplaz, L. An extended root phenotype: The rhizosphere, its formation and impacts on plant fitness. *Plant J.* **2020**, *103*, 951–964. [CrossRef] [PubMed]
18. Basu, A.; Prasad, P.; Das, S.N.; Kalam, S.; Sayyed, R.Z.; Reddy, M.S.; El Enshasy, H. Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: Recent developments, constraints, and prospects. *Sustainability* **2021**, *13*, 1140. [CrossRef]
19. Backer, R.; Rokem, J.S.; Ilangumaran, G.; Lamont, J.; Praslickova, D.; Ricci, E.; Subramanian, S.; Smith, D.L. Plant growth promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci.* **2018**, *9*, 1473. [CrossRef]
20. Parray, J.A.; Jan, S.; Kamil, A.N.; Qadri, R.A.; Egamberdieva, D.; Ahmad, P. Current perspectives on plant growth-promoting rhizobacteria. *J. Plant Growth Regul.* **2016**, *35*, 877–902. Available online: https://link.springer.com/article/10.1007/s00344-016-9583-4 (accessed on 1 March 2016). [CrossRef]
21. Khan, M.A.; Asaf, S.; Khan, A.L.; Adhikari, A.; Jan, R.; Ali, S.; Imran, M.; Kim, K.-M.; Lee, I.-J. Halotolerant rhizobacterial strains mitigate the adverse effects of NaCl stress in soybean seedlings. *BioMed Res. Int.* **2019**, *2019*, 9530963. [CrossRef]
22. Chebotar, V.K.; Makarova, N.M.; Shaposhnikov, A.I.; Kravchenko, L.V. Antifungal and phytostimulating characteristics of *Bacillus subtilis* Ch-13 rhizospheric strain, producer of bioprepations. *Appl. Biochem. Microbiol.* **2009**, *45*, 419–423. [CrossRef]
23. Sagar, A.; Sayyed, R.Z.; Ramteke, P.W.; Sharm, S.; Marraiki, N.; Elgorba, A.M.; Syed, A. ACC deaminase and antioxidant enzymes producing halophilic *Enterobacter* sp. PR14 promotes the growth of rice and millets under salinity stress. *Physiol. Mol. Biol. Plants* **2020**, *26*, 1847–1854. [CrossRef] [PubMed]
24. Kalam, S.; Basu, A.; Ankati, S. Plant root-associated biofilms in bioremediation. In *Biofilms in Plant and Soil Health*; Ahmad, I., Husain, F.M., Eds.; John Wiley & Sons, Ltd.: Chichester, UK, 2017; pp. 337–355.
25. Etesami, H.; Maheshwari, D.K. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicol. Environ. Saf.* **2018**, *156*, 225–246. [CrossRef]
26. Anli, M.; Baslam, M.; Tahiri, A.; Raklami, A.; Symanczik, S.; Boutasknit, A.; Ait-El-Mokhtar, M.; Ben-Laouane, R.; Toubali, S.; Ait Rahou, Y.; et al. Biofertilizers as strategies to improve photosynthetic apparatus, growth, and drought stress tolerance in the date palm. *Front. Plant Sci.* **2020**, *11*, 516818. [CrossRef]
27. Dong, L.; Li, Y.; Xu, J.; Yang, J.; Wei, G.; Shen, L.; Ding, W.; Chen, S. Biofertilizers regulate the soil microbial community and enhance *Panax ginseng* yields. *Chin. Med.* **2019**, *14*, 20. [CrossRef]
28. Atieno, M.; Herrmann, L.; Nguyen, H.T.; Phan, H.T.; Nguyen, N.K.; Srean, P.; Than, M.M.; Zhiyong, R.; Tittabutr, P.; Shutsrirung, A.; et al. Assessment of biofertilizer use for sustainable agriculture in the Great Mekong Region. *J. Environ. Manag.* **2020**, *275*, 111300. [CrossRef] [PubMed]
29. De Andrade, F.M.; de Assis, T.P.; Souza, T.P.; Guimarães, P.H.S.; Martins, A.D.; Schwan, R.F.; Moacir, P.; Joyce, D. Beneficial effects of inoculation of growth-promoting bacteria in strawberry. *Microbiol. Res.* **2019**, *223–225*, 120–128. [CrossRef] [PubMed]
30. Bayramoğlu, Z.; Selvi, T.; Eşitgen, A.; Dönmez, M.F. The effect of bacterial applications on resource utilization ın strawberry (*Fragaria* × *ananassa* Duch.) production. *J. Agric. Nat.* **2020**, *23*, 1308–1313. [CrossRef]
31. Anuradha; Goyal, R.K.; Sindhu, S.S. Response of strawberry (*Fragaria* × *ananassa* Duch.) to PGPR inoculation. *Bangladesh J. Bot.* **2020**, *49*, 1071–1076. [CrossRef]
32. Anuradha; Goyal, R.K.; Sindhu, S.; Godara, A. Effect of PGPR on strawberry cultivation under greenhouse conditions. *Indian J. Hort.* **2019**, *76*, 400–404. [CrossRef]
33. Chebotar, V.K.; Voshol, G.P.; Malfanova, N.V.; Chizhevskaya, E.P.; Zaplatkin, A.N.; Komarova, O.V.; Baganova, M.E.; Lazarev, A.M.; Balakina, S.V. Draft genome sequence of plant growthpromoting *Bacillus velezensis* BS89. *Microbiol. Resour. Announc.* **2021**, *10*, e01294-20. [CrossRef]
34. *ISO 10390:2005*; Soil Quality—Determination of pH. 2nd ed. ISO: Geneva, Switzerland, 2005; p. 7.
35. Khan, S.A.; Mulvaney, R.L.; Hoeft, R.G. A simple soil test for detecting sites that are nonresponsive to nitrogen fertilization. *Soil Sci. Soc. Am. J.* **2001**, *65*, 1751–1760. [CrossRef]
36. *ISO 14255:1998*; Soil Quality—Determination of Nitrate Nitrogen, Ammonium Nitrogen and Total Soluble Nitrogen in Air-Dry Soils using Calcium Chloride Solution as Extractant. ISO: Geneva, Switzerland, 1998; p. 14.
37. Motsara, T.N.; Roy, R.N. Guide to Laboratory Establishment for Plant Nutrient Analysis. In *Fertilizer and Plant Nutrition Bulletin*; No. 19; Food and Agriculture Organization of the United Nations: Rome, Italy, 2008; p. 206. ISBN 978-92-5-105981-4.

38. Bray, R.H.; Kurtz, L.T. Determination of total, organic and available forms of phosphorus in soils. *Soil Sci.* **1945**, *59*, 30–45. [CrossRef]
39. Ginzburg, E.K.; Shcheglova, E.K. Determination of nitrogen, phosphorus and potassium in plant material from a single sample. *Soil Sci.* **1960**, *5*, 100–105.
40. Naeima, Y.M.H. Capability of plant growth-promoting rhizobacteria (PGPR) for producing indole acetic acid (IAA) under extreme conditions. *Eur. J. Biol. Res.* **2018**, *8*, 174–182. [CrossRef]
41. Araújo, F.F.; Henning, A.A.; Hungria, M. Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. *World J. Microbiol. Biotechnol.* **2005**, *21*, 1639–1645. [CrossRef]
42. Chagas, A.F.; de Oliveira, A.G.; de Oliveira, L.A.; dos Santos, G.R.; Chagas, L.F.B.; Lopes da Silva, A.L.; da Luz Costa, J. Production of indole-3-acetic acid by *Bacillus* isolated from different soils. *Bulg. J. Agric. Sci.* **2015**, *21*, 282–287. Available online: http://www.agrojournal.org/21/02-08.pdf (accessed on 1 April 2016).
43. Kumari, S.; Prabha, C.; Singh, A.; Kumari, S.; Kiran, S. Optimization of indole-3-acetic acid production by diazotrophic *B. subtilis* DR2 (KP455653), isolated from rhizosphere of *Eragrostis Cynosuroides*. *Int. J. Pharma Med. Biol. Sci.* **2018**, *7*, 20–27. [CrossRef]
44. Khan, A.L.; Haloa, B.A.; Elyassi, A.; Ali, S.; Al-Hosni, K.; Hussain, J.; Al-Harrasi, A.; Lee, I.-J. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electron. J. Biotechnol.* **2016**, *21*, 58–64. [CrossRef]
45. Spaepen, S.; Bossuyt, S.; Engelen, K.; Marchal, K.; Vanderleyden, J. Phenotypical and molecular responses of *A. thaliana* roots as a result of inoculation with the auxin producing bacterium *Azospirillum brasilense*. *New Phytol.* **2014**, *201*, 850–861. [CrossRef]
46. Tsukanova, K.A.; Chebotar, V.K.; Meyer, J.J.M.; Bibikova, T.N. Effect of plant growth-promoting Rhizobacteria on plant hormone homeostasis. *S. Afr. J. Bot.* **2017**, *113*, 91–102. [CrossRef]
47. Singh, R.K.; Malik, N.; Singh, S. Improved nutrient use efficiency increases plant growth of rice with the use of IAA-overproducing strains of endophytic *Burkholderia cepacia* strain RRE25. *Microbial. Ecol.* **2013**, *66*, 375–384. [CrossRef] [PubMed]
48. Park, J.M.; Radhakrishnan, R.; Kang, S.M.; Lee, I.J. IAA producing *Enterobacter* sp. I-3 as a potent bio-herbicide candidate for weed control: A special reference with lettuce growth inhibition. *Indian J. Microbiol.* **2015**, *55*, 207–212. [CrossRef]
49. Bashan, Y.; de-Bashan, L.E. Chapter two—How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—A critical assessment. *Adv. Agron.* **2010**, *108*, 77–136. [CrossRef]
50. Péret, B.; Li, G.; Zhao, J.; Band, L.R.; Voß, U.; Postaire, O.; Luu, D.-T.; Da Ines, O.; Casimiro, I.; Lucas, M.; et al. Auxin regulates aquaporin function to facilitate lateral root emergence. *Nat. Cell Biol.* **2012**, *14*, 991–998. [CrossRef]
51. De Smet, I. Lateral root initiation: One step at a time. *New Phytol.* **2012**, *193*, 867–873. [CrossRef] [PubMed]
52. Malamy, J.E. Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell Environ.* **2005**, *28*, 67–77. [CrossRef] [PubMed]
53. Raheem, A.; Shaposhnikov, A.; Belimov, A.A.; Dodd, I.C.; Ali, B. Auxin production by rhizobacteria was associated with improved yield of wheat (*Triticum aestivum* L.) under drought stress. *Arch. Agron. Soil Sci.* **2017**, *64*, 574–587. [CrossRef]
54. Mirskaya, G.V.; Khomyakov, Y.V.; Rushina, N.A.; Vertebny, V.E.; Chizhevskaya, E.P.; Chebotar, V.K.; Chesnokov, Y.V.; Pishchik, V.N. Plant development of early-maturing spring wheat (*Triticum aestivum* L.) under inoculation with *Bacillus* sp. V2026. *Plants* **2022**, *11*, 1817. [CrossRef]
55. Ambreetha, S.; Chinnadurai, C.; Marimuthu, P.; Balachandar, D. Plant-associated *Bacillus modulates* the expression of auxin-responsive genes of rice and modifies the root architecture. *Rhizosphere* **2018**, *5*, 57–66. [CrossRef]
56. Bardgett, R.D.; Mommer, L.; De Vries, F.T. Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* **2014**, *29*, 692–699. [CrossRef]
57. Grover, M.; Bodhankar, S.; Sharma, A.; Sharma, P.; Singh, J.; Nain, L. PGPR mediated alterations in root traits: Way toward sustainable crop production. *Front. Sustain. Food Syst.* **2021**, *4*, 618230. [CrossRef]