agronomy

MDPI

Article

Bacillus methylotrophicus Could Improve the Tolerance and Recovery Ability of the Tomato to Low-Temperature Stress and Improve Fruit Quality

Guobin Li [1,2,3,†], Tieli Peng [1,2,3,†], Feng Qu [1,2,3], Junzheng Wang [1,2,3], Yanghao Long [1] and Xiaohui Hu [1,2,3,*]

[1] College of Horticulture, Northwest A&F University, Xianyang 712100, China
[2] Key Laboratory of Protected Horticultural Engineering in Northwest, Ministry of Agriculture, Xianyang 712100, China
[3] Shaanxi Protected Agriculture Research Centre, Xianyang 712100, China
* Correspondence: hxh1977@nwafu.edu.cn
† These authors contributed equally to the work.

Abstract: Low-temperature stress seriously affects the growth, development, yield, and quality of tomato production. *Bacillus methylotrophicus* is an important plant growth promoting rhizobacteria (PGPR). However, the role of *B. methylotrophicus* under low-temperature stress is poorly understood. Accordingly, the effects of *B. methylotrophicus* ('VL-10′) on tomato cold stress (15 °C/8 °C, 12 h/12 h, and day/night) were studied. *B. methyltrophicus* 'VL-10′ was added into the substrate at the time of sowing, and the plants were treated at a low temperature for 2 weeks after 40 days of growth. We found that the low temperature reduced the spatial distribution of the aboveground and underground sections of tomatoes and the leaf SPAD and photochemical efficiency of PS II (Fv/Fm). After low-temperature stress, the tomato flowering was delayed, the vitamin C and lycopene content in fruit decreased, and the nitrate content increased. However, inoculated with *B. methyltrophicus* 'VL-10′ during sowing promoted the growth of tomato seedlings, enhanced the native defense ability of the tomatoes, and effectively reduced the cold shock response of the roots to cold damage and the adverse effects of low temperature on leaf SPAD and Fv/Fm. After the cultivation at normal temperature, the inoculat *B. methyltrophicus* 'VL-10′ could rapidly regain plant growth levers, and eliminate the delay of low temperature on flowering. TOPSIS analysis showed that the nutritional quality of tomatoes could be effectively improved by inoculation with *B. methyltrophicus* 'VL-10′ regardless of normal cultivation or low-temperature stress.

Keywords: tomato; low temperature; *Bacillus methylotrophicus*; growth; fruit quality

Citation: Li, G.; Peng, T.; Qu, F.; Wang, J.; Long, Y.; Hu, X. *Bacillus methylotrophicus* Could Improve the Tolerance and Recovery Ability of the Tomato to Low-Temperature Stress and Improve Fruit Quality. *Agronomy* **2023**, *13*, 1902. https://doi.org/10.3390/agronomy13071902

Academic Editors: Wei Hu, Zhiguo Zhou and Wenqing Zhao

Received: 19 June 2023
Revised: 12 July 2023
Accepted: 17 July 2023
Published: 19 July 2023

1. Introduction

Plants are subject to a variety of environmental challenges, one of which is low temperature, which has a significant negative effect on plant growth [1,2]. Low temperatures can cause damage to plant DNA and proteins, membrane lipid peroxidation, and a series of physiological and metabolic disturbances that inhibit growth and vitality [3]. Low temperatures for crops can lead to poor germination, weakened seedling vigor, reduced vegetative growth and stunted flowering and fruiting, resulting in severe yield loss and crop death [4–7]. The tomato (*Solanum lycopersicum* L.) is an important vegetable crop that is widely cultivated all over the world, but low temperatures severely impair tomato vegetative and reproductive growth [8,9]. Tomatoes frequently experience low-temperature stress in early spring, which poses a huge threat to China's efficient tomato production [10].

Plants have developed a series of mechanisms to increase their tolerance to low-temperature stress, such as increasing antioxidant enzyme activity to reduce oxidative damage to cells caused by reactive oxygen species (ROS) and accumulating more low molecular weight solutes, including soluble sugars, proline and polyamines to protect themselves

from chilling [11–13]. The plants themselves have a limited regulatory capacity; the application of beneficial microorganisms as a biological agent is an environmentally friendly biotechnological approach for sustainable agricultural practices. Plant growth promoting rhizobacteria (PGPR) are a class of beneficial bacteria related to the plant rhizobacteria that improve plant performance and the ability of the plant to cope with stress through either direct or indirect mechanisms [14–16]. The primary mechanisms of PGPR for promoting plant growth mainly include nitrogen fixation, increased nutrient availability in the rhizosphere, root development and spatial distribution, and enhanced interaction between PGPR and the plant [17]. In terms of stress, the mechanisms of PGPR include changing soil pH to increase nutrient availability in adverse environments, chelating toxic compounds, aggregating beneficial bacteria, and secreting substances that inhibit pathogens [18]. At present, PGPR has been widely proven to promote plant tolerance to salinity, drought and lack of nutrients [19,20]. However, reports on chilling damage applications are still rare and need further study [20,21].

PGPR include *Pseudomonas*, *Enterobacter*, *Bacillus*, *Variovorax*, *Klebsiella*, *Burkholderia*, *Azospirillum*, *Serratia*, and *Azotobacter* [22], with *Bacillus* being more typical in enhancing plant tolerance to cold stress [23,24]. The genes related to membrane transport, cold shock proteins, antioxidant enzymes and osmotic regulation are abundantly expressed in PGPR under low temperature environments. These metabolic activities activate various signaling pathways through rhizosphere contacts to improve the plant's response to cold stress [25–27]. For example, grape plants were inoculated with *Burkholderia phytofirmans* PsJN at a low temperature, greatly improving the cold tolerance of grape seedlings and the contents of various phenols; proline and starch were significantly increased after PsJN inoculation [23]. Inoculation with *Pseudomonas* strains could significantly increase shoot and root biomass and reduce the K^+/Na^+ ratio and electrolyte leakage in inoculated wheat plants, thereby improving the ability of wheat to overcome low-temperature stress [28]. Inoculation with a mixture of beneficial microflora including *Bacillus* spp. was found to increase tomato tolerance to cold temperatures [29,30]. However, the role of *Bacillus* alone in improving the cold tolerance of vegetable crops is unclear.

Bacillus methylotrophicus is a new species of *Bacillus*, which is isolated from the root soil of rice under traditional tillage [31]. *B. methylotrophicus* can produce two non-volatile stereoisomers 3S, 4R-acetyl butanediol (3S, 4R-ABD, 1) and 3R, 4R-acetyl butanediol (3R, 4R-ABD, 2) in crop roots. When the two compounds exist in the rhizosphere soil at a volume ratio of 1:2, they can effectively promote the root elongation of maize and rice, thus improving the growth of these crops [32] and improving the drought tolerance of rice [33]. *B. methyltrophicus* is active over a wide temperature range [34], but whether it can improve plant tolerance to low temperatures remains unclear. This study aims to explore the role of *B. methylotrophicus* in tomato cold damage. We inoculated the substrates with *B. methylotrophicus* strain 'VL-10′ and measured the plant morphology, each organ biomass, root growth, and leaf SPAD of the tomato plants after low-temperature stress. The regulatory effect of *B. methylotrophicus* on the cold tolerance of tomato plants under low-temperature stress was also analyzed. The analysis on the flowering phenology, fruit yield and quality after low-temperature stress demonstrated that the strain could improve the tolerance of tomatoes to low-temperature stress and had a positive effect on improving the fruit quality.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Tomato Jinpeng No. 1 cultivars (cold sensitive) were from Xi'an Jinpeng Seed Co., LTD (Xi'an, China). Seeds were germinated in a Petri dish lined with two layers of wet filter paper at 28 °C, and the germinated seeds were sown in 50-well plates filled with substrate containing peat, vermiculite and perlite (2:1:1, *v*/*v*/*v*). *Bacillus methyltrophicus* 'VL-10′ was added into the substrate of the experimental group at a concentration of 5×10^8 CFU/g. All tomato seedlings were cultivated in climate chambers with normal

conditions (25 °C/18 °C and 12 h day/12 h night), relative humidity of 60% and photosynthetic photon flux density of 300 μmol·m^{-2}·s^{-1}. On the 30th day after sowing, the seedings were individually transplanted into a plastic pot (5 cm × 5 cm × 8 cm) and continued to grow for 10 days. Thereafter, low temperature was applied, and the control plant grew in a normal state. The growth environment for low-temperature treatment was 15 °C/8 °C (12 h day/12 h night), the relative humidity was 60%, and the photosynthetic photon flux density was 300 μmol·m^{-2}·s^{-1} [35,36]. After 14 days of low-temperature treatment (54 days of growth), all plants were transferred to a glass greenhouse for normal management. The four treatment combinations set up in this experiment are listed in Table 1:

Table 1. Inoculation and plant growth status for the four treatments.

Treatment Name	Inoculate	Plant Growth Process
Control (CK)	no	Growth at 25 °C/16 °C for 40 d + 25 °C/16 °C for 14 d + normal temperature cultivation
B	yes	Growth at 25 °C/16 °C for 40 d + 25 °C/16 °C for 14 d + normal temperature cultivation
CK + LT	no	Growth at 25 °C/16 °C for 40 d + 15 °C/8 °C for 14 d + normal temperature cultivation
B + LT	yes	Growth at 25 °C/16 °C for 40 d + 15 °C/8 °C for 14 d + normal temperature cultivation

2.2. Plant Morphology and Biomass Determination

From the 40th day of plant growth, the number of leaves, plant height and stem diameter were measured every 7 days until the 89th day after sowing. The fresh and dry weight of the tomato organs and root morphological indexes were recorded and analyzed on the 40th day (before low-temperature treatment), 54th day after sowing (at the end of low-temperature treatment), and 61st day after sowing (7 days after the end of low-temperature treatment), with 9 replicates for each treatment. A single plant is a repeat. The stem diameter and plant height (from the stem base to the growing point) were measured with digital explicit vernier calipers. The root morphology was scanned with EPSON Chops V700N (EPSON China Co., Ltd., Beijing, China) scanner and analyzed with WinRHIZO PRO 2012 (Regent Instruments Inc., Québec City, QC, Canada) to obtain the root length (RL), root surface area (RA), root avgDiam (RD), root volume (RV), root tips (RT), root forks (RF), and root data of the different diameter classes. After the root scanning was completed, the roots, stems and leaves were placed in envelopes, kept at 105 °C for 30 min and dried at 65 °C to constant weight, and the dry weight of each part was measured.

2.3. Root Development Strategy

Based on the obtained root morphological data and root dry weight (RDW), the following indexes of roots were calculated:

Specific root length (SRL): SRL = RL/RDW;
Specific root surarea (SRA): SRA = RA/RDW;
Root tips density (RTD): RTD = RT/RL;
Root branch density (RBI): RDWI = RF/RL;
Root tissue density (RTID): RTID = RDW/RV;
Root fineness (RFN): RFN = RL/RV.

The root morphological indexes were divided into 5 grades according to the root diameter (RD), Grade 1 (G1): RD $\leq$ 0.50 mm, G2: 0.50 mm < RD $\leq$ 1.00 mm, G3: 1.00 mm < RD $\leq$ 1.50 mm, G4: 1.50 mm < RD $\leq$ 2.00 mm, and G5:2.00 mm < RD, G1-G4 are the absorbing roots, and G5 denotes the transmitting roots to analyze the root morphological indicators in different diameter levels and the functional divisions [37].

2.4. Leaf Chlorophyll Relative Content and Damage Estimation

From the 40th day to the 89th day from sowing, the relative chlorophyll content of the first, second and third leaves at the upper end of the tomato morphology was measured every 7 days with a SPAD-502 PLUS chlorophyll meter (Konica Minolta Inc., Tokyo, Japan). Fv/Fm was measured with the Open FluorCam FC 800-O and FluorCam 7 software (PSI, Brno, Czech Republic), referring to previously reported methods [38], and the leaves were left in dark for 30 min before measuring.

2.5. Flowering Statistics of the Tomato

This collection takes place in the morning during the flowering period, defining the budding stage as S0 when the flowering buds are visible. An initial flowering stage (S1) is defined when 10% of the flowers on an inflorescence are open. The blooming stage (S2) is defined when 60% of the flowers in an inflorescence are blooming. The end flowering stage is defined (S3) when 90% of the flowers on an inflorescence wither. A Kaplan–Meier curve and a Cox proportional hazards model were used for further analysis of the flowering time [39].

2.6. Determination and Analysis of Fruit Yield and Quality

The fruit yields of the first and the second inflorescence of tomatoes in each group were counted, and the arithmetic mean value of these fruit weights was taken as the single fruit weight of each treatment. We selected fruits with similar maturity and fruit size in each treatment, and took the middle pulp to determine the quality. The quality indicators measured include the total soluble sugar (TSSC), vitamin C (VC), total soluble solids (TSS), sugar-acid ratio (SAR), nitrate nitrogen, lycopene and titratable acid. The nitrate nitrogen was measured by using the 3,5-dinitrosalicylic acid (DNS) method [40]. The TSSC was measured through anthrone colorimetry, and the VC concentrations were determined via molybdenum blue colorimetry [41]. The TSS and SAR were determined with a digital PAL-Easy ACID3 tonic system (ATAGO Co., Ltd., Fukaya-shi, Japan). Lycopene was extracted with a solution containing hexane, acetone, and ethanol (2: 1: 1, $v/v/v$), and the absorbance was measured at 485 nm with a spectrophotometer (UV-2500, Shimadzu Corporation, Kyoto, Japan). The tomato fruit quality of each treatment was comprehensively evaluated using the technique for order preference by similarity to an ideal solution (TOPSIS), in which nitrate nitrogen and titratable acid were set as negative indicators [42].

2.7. Statistical Analysis

Excel 2016 (Microsoft Corp., Redmond, WA, USA) was used for data sorting, Excel 2016, Word 2016 (Microsoft Corp., USA) and Origin 2022 (OriginLab Corp., Northampton, MA, USA) were used to draw graphs, and ANOVA LSD significance analysis was performed with SPSS 26.0 (IBM Crop., Armonk, NY, USA). The data are expressed as "mean ± standard deviation".

3. Results

3.1. B. methylotrophicus Improves the Growth of Tomato Seedlings at Low Temperature

Low temperature seriously affected the growth and development of tomatoes. On the 14th day (the 54th day after sowing) of low-temperature treatment, the number of leaves on the CK + LT plant was significantly less than that of the CK plant by 0.95 times. This significant effect continued until the 89th day after sowing. Given that the inoculum promoted an increase in the number of tomato leaves, the tomato under the B treatment always had the largest number of leaves. The number of leaves in the B/B + LT plant was 1.25 times that of the CK/CK + LT plant. The number of leaves of the B + LT plant during the low-temperature treatment period (40–54th d) was still not less than that of the CK plant (Figure 1a and Table S1).

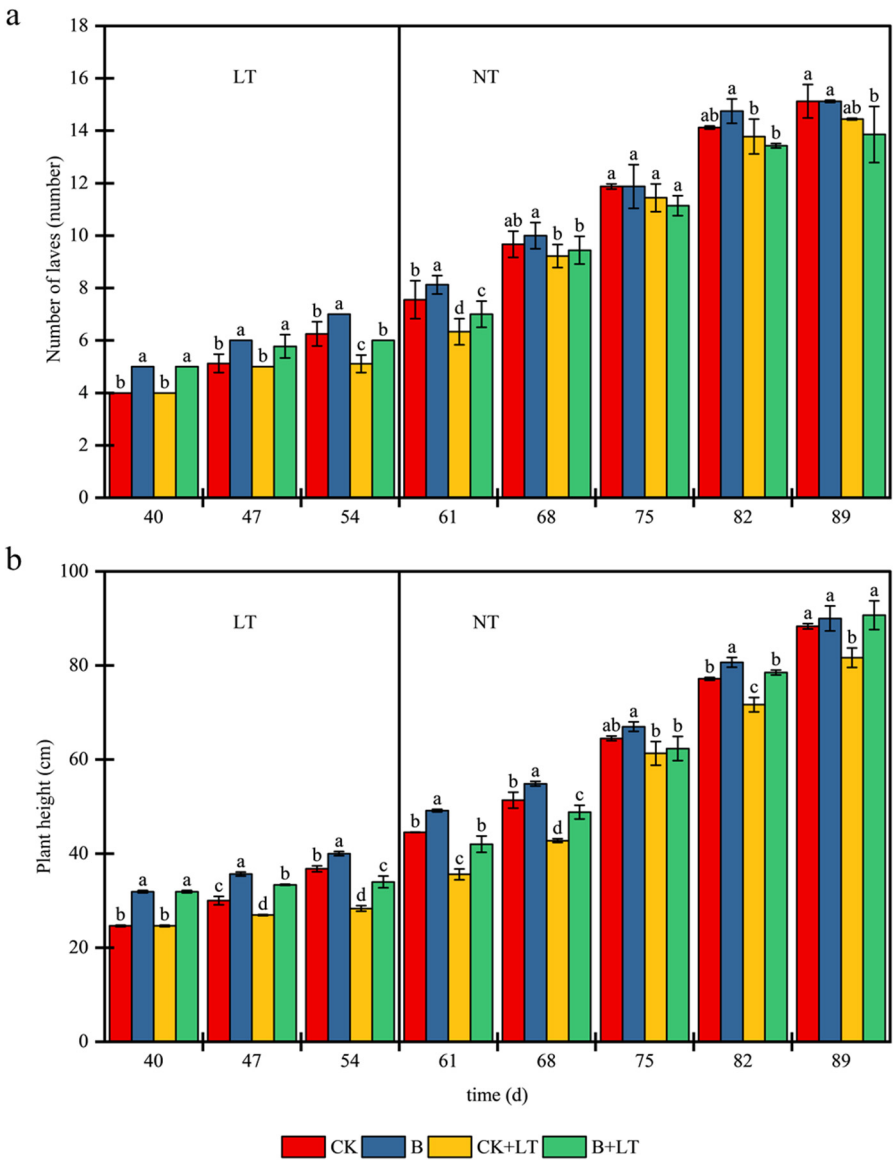

Figure 1. Effects of *B. methylotrophicus* inoculation on tomato growth at low temperature. (**a**) Effects of *B. methylotrophicus* inoculation on the number of leaves on the tomato plant. (**b**) Effects of *B. methylotrophicus* inoculation on the height of the tomato plant. LT represents the period of low-temperature treatment, and NT denotes the period of normal temperature cultivation. The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

On the 7th day of low-temperature treatment (the 47th day after sowing), the growth rate of plant height of the CK + LT/B + LT plant was significantly lower than that of CK/B plant. At the end of the low-temperature treatment (the 54th day after sowing), the plant height of tomato plants in CK + LT and B + LT treatments increased by 15.02% and 6.47%, respectively, which was lower than 49.25% and 25.39% in the CK and B treatments compared with before the low-temperature treatment (40th day after sowing) (Figure 1b and Table S1). From the 47th day to the 89th day after sowing, the plant height of CK + LT was lower than those of other treatments due to low temperature. However, the plant height of the B + LT treatment was higher than that of the CK treatment on the 82nd day after sowing, and increased to the same level as the B treatment on the 89th day. This height was significantly higher than those of CK and CK + LT treatments, indicating that

inoculation was beneficial to the recovery of tomato growth after low-temperature stress (Figure 1b and Table S1).

From the 40th day to the 75th day after sowing, the stem diameter of the tomato plant in each treatment only increased by 4.36–17.61%, and the stem diameter slowly increased. However, the stem diameter of the treatment with inoculants was still thicker than that of the treatment without inoculants. However, the stem diameter of the tomato plant significantly increased from the 75th day to the 82nd day after sowing, increasing by 17.08–25.53%, which indicates that the stem diameter of the tomato plant would increase significantly after it entered the flowering stage (Figure S1 and Table S1).

3.2. B. methylotrophicus Promotes Biomass Accumulation in Tomato at Low Temperature

Low temperatures decrease plant metabolism and growth. At the end of the low-temperature treatment (day 54 after sowing), the dry matter accumulation of roots, stems, leaves and whole plant in the CK + LT treatment increased by 17.92%, 121.00%, 53.55% and 71.46%, the B + LT treatment increased by 40.28%, 128.38%, 82.05% and 86.29%, the CK treatment increased by 88.41%, 178.54%, 145.98% and 139.80%, and the B treatment increased by 59.33%, 133.14%, 108.61% and 86.30%, respectively. These results indicated that inoculation could promote dry matter accumulation in tomatoes under low-temperature stress (Figure 2a–d). During the low temperature period, the growth rate of fresh weight of the tomato seedlings in each treatment was consistent with the change of dry weight, indicating that *B. methylotrophicus* can promote the biomass accumulation of tomatoes at low temperatures (Figure 2).

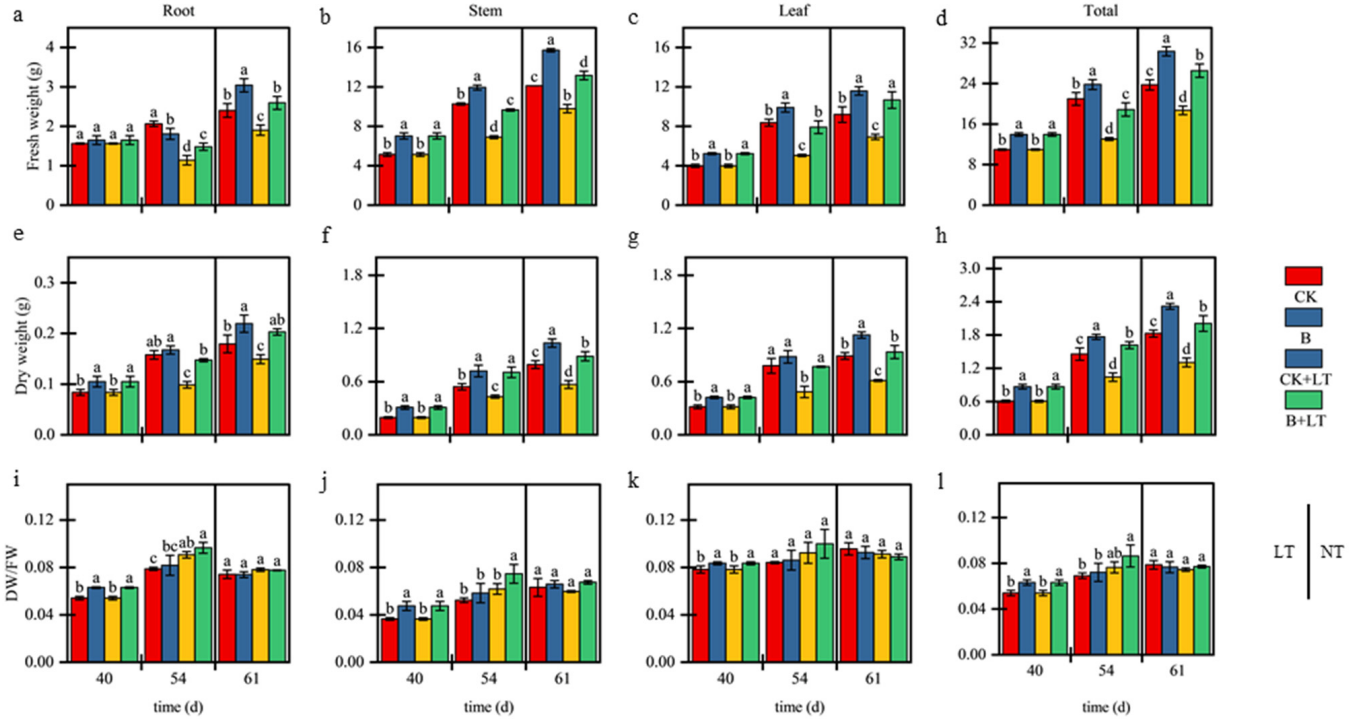

Figure 2. Biomass of the different parts of the tomato seedlings. (**a**–**d**) Dry weight of the different organs of the tomato seedlings before and after low-temperature treatment. (**e**–**h**) Fresh weight of the different organs of the tomato seedlings before and after low-temperature treatment. (**i**–**l**) Ratio of the dry weight to fresh weight (DW/FW) of the different organs of the tomato seedlings before and after low-temperature treatment. The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

3.3. *B. methylotrophicus* Can Alleviate the Damage to Tomato Roots Caused by Low Temperature

Low-temperature slows down the development of new roots, which greatly reduces the root length, root surface area, lateral root number, root volume and other indicators, and hinders the transport of nutrients from the roots to the shoots [43]. Before the low-temperature treatment (40th day after sowing), only the RA and RT of tomatoes between treatments had significant differences. The RA and RT of the tomato under the B/B + LT treatment were larger than those under the CK/CK + LT treatment, while other root morphological indexes had no significant differences (Figure 3b,g). At the end of the low-temperature treatment (54th day after sowing), the RL, RA, SRL, SRA, RT, RF, RBI, and RV of the CK + LT and B + LT treatments were significantly lower than those of the CK and B treatments, but the RTID of the CK + LT and B + LT treatments was significantly increased (Figure 3). The RFN of the CK + LT plant was significantly higher than those of the other treatments, indicating that low temperature affected the increase in root diameter (Figure 3h). The effect of low temperature on the CK + LT plant was greater than that of the B + LT plant, and the RA, RD and RV of the B + LT plant were significantly higher than those of the CK + LT plant (Figure 3b,i,k).

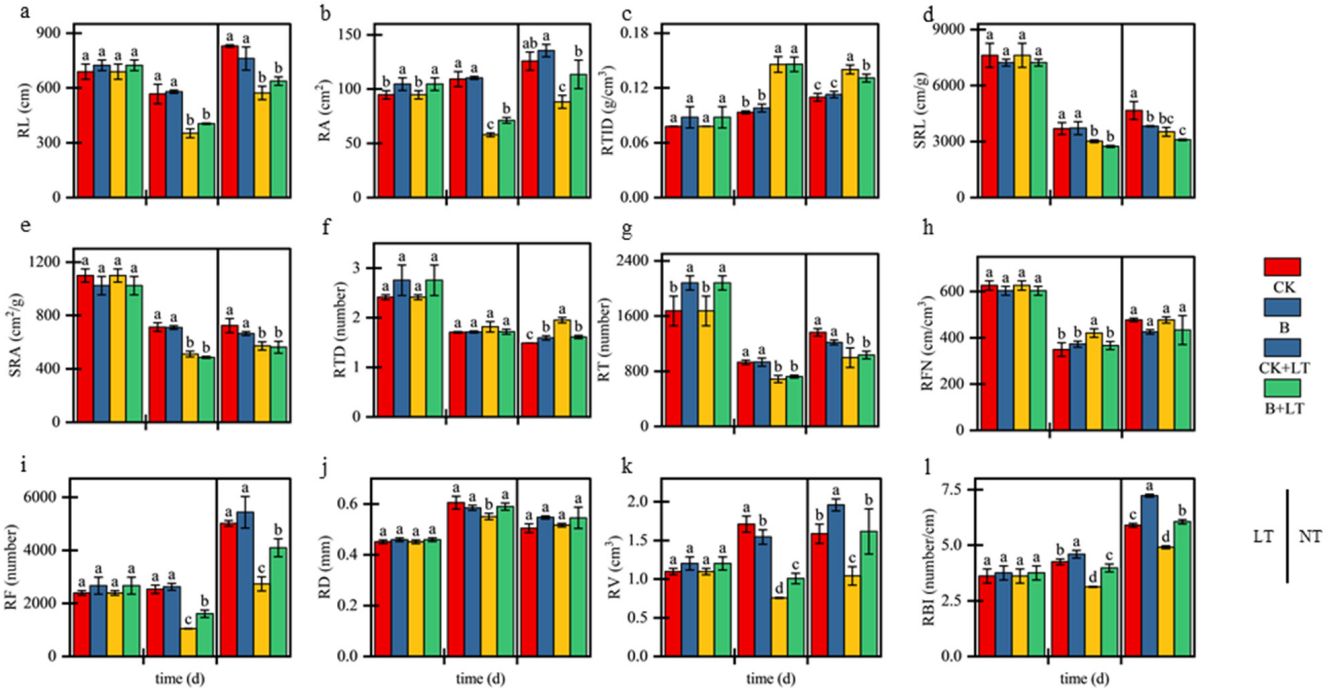

Figure 3. Effect of inoculated *B. methylotrophicus* on tomato root development. (**a**–**l**) Show the root index of each treatment on different days after sowing. The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

The root development of the CK + LT and B + LT plants partially recovered at 7 days after the end of low temperature (61st day after sowing). Only the RD of the CK + LT plant recovered to the CK plant level. However, the RA, RBI and RV of the B + LT plant recovered to the same level as the CK plant. In addition, the CK + LT plant still maintained a high RTID due to the low temperature, hence the root recovery of the B + LT plant was better than that of the CK + LT plant (Figure 3).

On the 40th, 54th and 61st days after sowing, the RL distribution of each treatment was mainly concentrated on the fine absorbing roots with RD < 1.00 mm (G1–G2), and the RV was mainly distributed on the transporting root with RD > 2.00 mm (G5). The RA was 54.99–78.07% distributed on G1–G2, and 11.35–24.45% was distributed on G5 (Figure 4).

On the 40th day after sowing, the (G1–G2)/G5 values (absorbing roots/transporting root) of tomato RL and RV in the CK/CK + LT plant were significantly higher than those in the B/B + LT plant, indicating that the CK/CK + LT plant was more inclined to retain nutrients absorbed by roots at this time to promote root development. The B/B + LT plant was more inclined to choose the root development strategy of balancing between aboveground and underground parts (Figure 4a). On the 54th day after sowing, the (G1–G2)/G5 values of RL, RA and RV of all treatments significantly decreased compared with those on the 40th day. The nutrients of the aboveground and underground parts were gradually balanced in all four treatments. However, the reduction of the CK + LT treatment at the low temperature was much less than those of the other treatments. The (G1–G2)/G5 values of RL, RA, and RV of the CK + LT plant were higher than those of the other treatments, and were 1.21, 1.19 and 1.37 times higher than those of the CK plant, respectively. The (G1–G2)/G5 values of RL, RA, and RV of the B + LT plant were smaller than those of the CK plant and had no significant difference with the B plant, suggesting that low temperature greatly inhibited the development of transporting roots, whilst the inoculum could alleviate the inhibition of low temperature on the growth of transporting roots (Figure 4b). After 7 days of normal temperature growth, the tomato plants in all treatments continued to balance the development strategy of the aboveground and underground parts, which was consistent with the trend on the 40th day; B/B + LT preferred to choose the balanced strategy, and the effect of low temperature on the (G1–G2)/G5 value disappeared (Figure 4c).

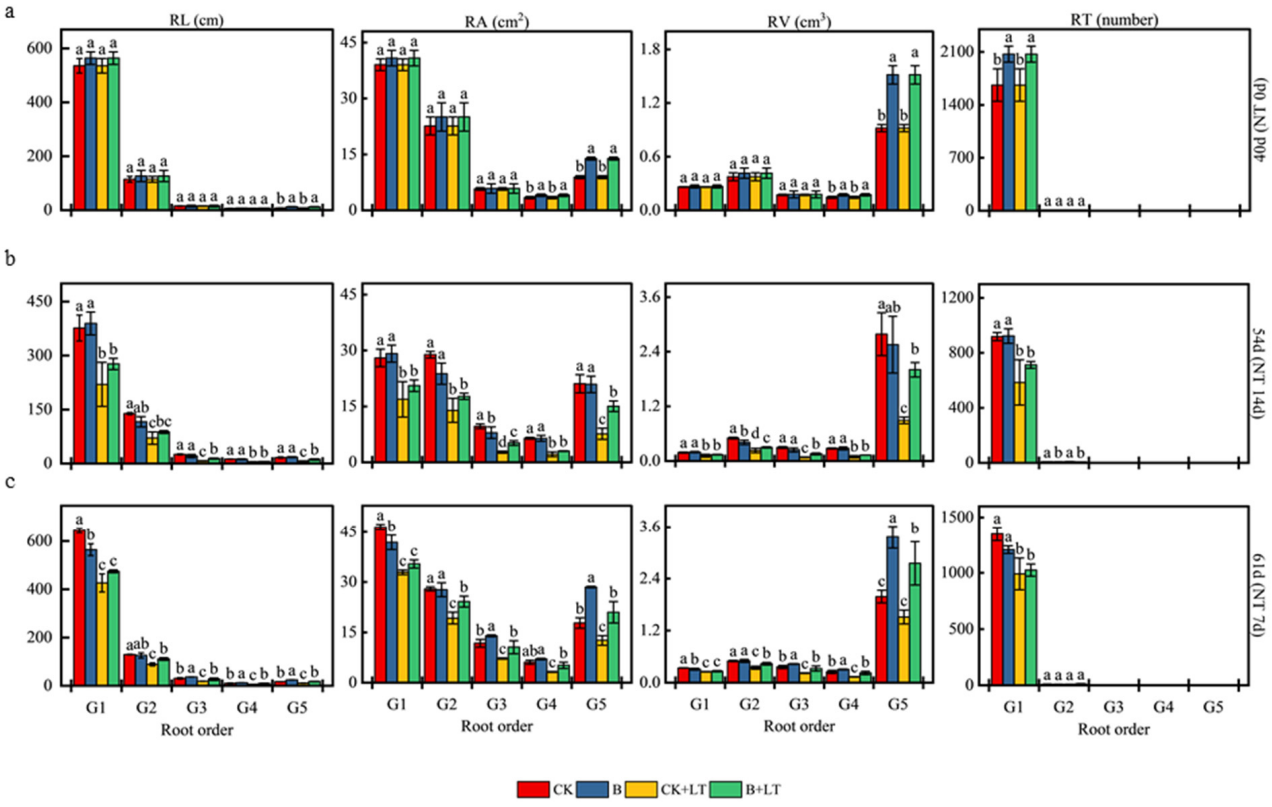

Figure 4. Effect of inoculated *B. methylotrophicus* on root traits with different diameters of tomato seedlings at low temperature. (**a**) Root traits with different diameters before low-temperature treatment (40th d). (**b**) Root traits with different diameters after 14 days of low-temperature treatment (54th d). (**c**) Root traits with different diameters after 7 days of normal temperature cultivation (61st d). The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

In conclusion, low-temperature treatment can lead to an accelerated senescence of tomato roots and delay the further development of new roots, which leads to the conservative strategy of root development and nutrient transport, and the plant growth process is delayed. However, inoculation can significantly reduce this effect.

3.4. B. methylotrophicus Can Reduce the Damage of Low Temperature on Tomato Leaves

On the 47th day after sowing (after 7 days of low-temperature treatment), the SPAD value of the leaves of the CK + LT/B + LT plants decreased by 6.42%/6.17% compared with that of the CK/B plant, and decreased by 15.94% and 13.3% on the 54th day, respectively. The negative effect of low temperature on the leaf SPAD value of the CK + LT plant was much greater than that of the B + LT plant. From the 47th day to the 75th day, the SPAD value of the CK + LT leaves was much lower than that of the other treatments, whilst the second and third leaves of the B + LT plant were lower than those of the CK plant only at the 54th and 61st days (Figure 5a). The SPAD value in the three upper leaves of the tomato morphology was significantly decreased by the low-temperature treatment, and the effect on mature leaves was greater than that on the new leaves. Moreover, the longer the exposure time to low-temperature stress, the greater the decline of the SPAD value of the leaves (Table S2). Accordingly, low temperatures will reduce the relative content of chlorophyll in tomato leaves and form long-term negative effects. Inoculation of *B. methylotrophicus* can improve the SPAD value of tomato leaves and reduce the negative effects of low-temperature stress on the SPAD value of leaves.

The maximum photochemical efficiency of PS II (Fv/Fm) is an important criterion for measuring whether the leaves are damaged. If the Fv/Fm is less than 0.76, then the plant leaves are damaged. On the 7th day of low-temperature stress (47th day after sowing), the Fv/Fm values of the CK + LT and B + LT plant leaves were both lower than 0.76, indicating that the leaves suffered from low-temperature damage, and the damage degree of the CK + LT plant leaves was significantly higher than that under the B + LT treatment. On the 14th day of low-temperature stress (54th day after sowing), the Fv/Fm values of the CK + LT and B + LT plant leaves recovered to a small extent, but the CK + LT plant leaves were still damaged, whilst the Fv/Fm values of the B + LT plant leaves recovered to the normal level (0.76) (Figure 5b). The result showed that the inoculant promotes the rapid adaptation of plants to low temperatures.

3.5. B. methylotrophicus Can Accelerate the Growth Process of Tomato

After 7 days of growth in a greenhouse (61st day after sowing), the first panicle of the B treatment plant entered the budding stage (S0), followed by the B + LT and CK plants, and the CK + LT plant entered S0 last (Figure 6). The first panicle flowers of the CK + LT plant entered S3 significantly later than those of the CK plant, and the time spent in each flowering stage was longer than that of the CK plant. In the B + LT plant, only the second panicle flowers took more time to enter S2 than in the CK plant, and no significant difference was observed in other flowering times between the B + LT plant and the CK plant. The time for the B + LT plant to enter S0 and S1 was significantly less than the CK + LT plant. The probability of the first panicle flower of the B + LT plant entering S0 was also greater than that of the CK plant (1.66 times that of the CK plant at the same period). The time to complete the flowering period of each panicle of the B treatment was less than that of the CK plant, and the probability of the first panicle entering S0 was 4.61 times that of the CK plant in the same period (Figure S2 and Table S3). Consequently, the low-temperature treatment delayed the time of tomato flowering in this study, whilst the inoculant could make tomato flowering earlier and alleviate the effect of low temperature. This may be related to the fact that low temperature inhibits the growth process of the tomato, whilst the inoculum can accelerate its growth process.

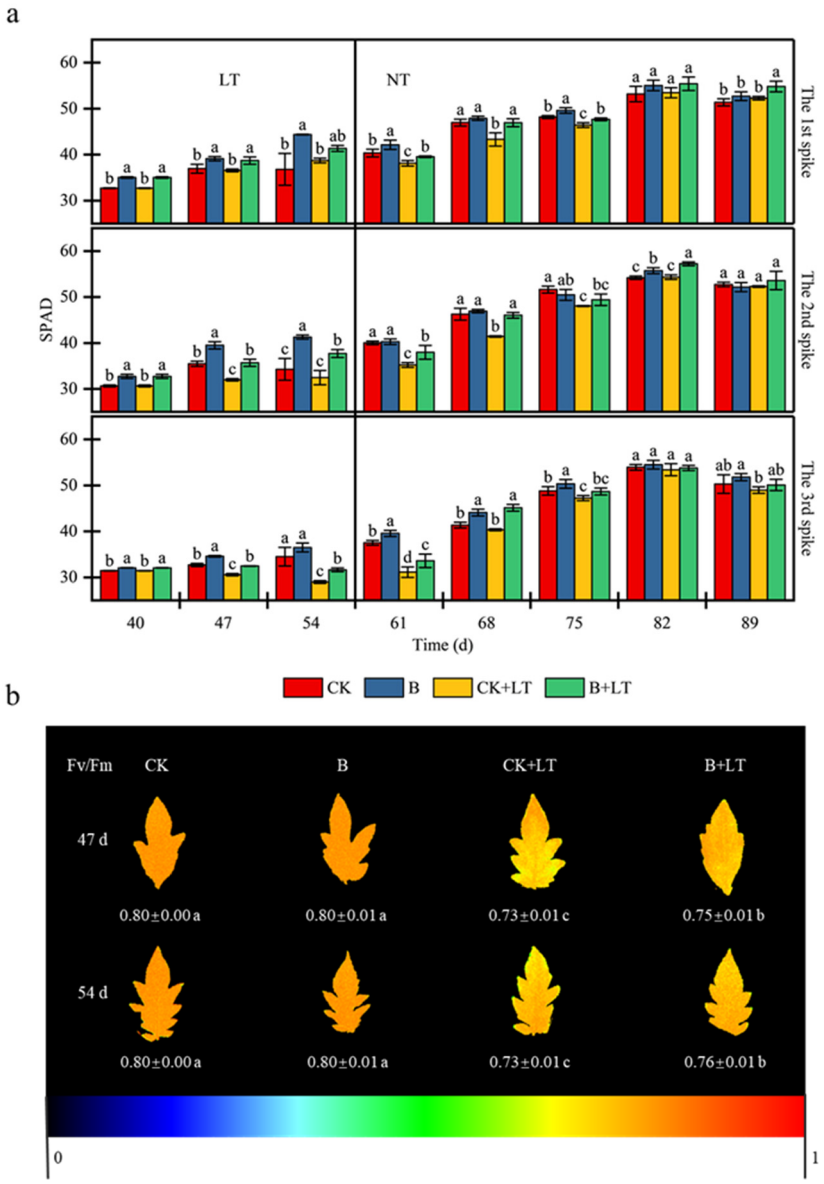

Figure 5. Effects of inoculated *B. methylotrophicus* on the SPAD value and Fv/Fm of leaves. (**a**) SPAD values of leaves of different treatments. (**b**) Fv/Fm values of leaves of different treatments during low temperature. The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

Evident differences can be observed in the seedling age and the effective accumulated temperature of the tomato panicles when they entered S0 under different treatments. The difference in the physiological seedling age was mainly caused by the inoculation of bacteria, which made the B/B + LT plants enter S0 when the physiological seedling age was smaller than for the CK/CK + LT plants. Meanwhile, the low temperature had no significant effect on the physiological seedling age. The calendar seedling age and the effective accumulated temperature are affected by low-temperature treatment and inoculum. Inoculation of bacteria shortened the time for the tomato to enter S0, whilst low temperature delayed the time for the tomato to enter S0 (Table 2). Low temperatures will reduce the effective accumulated temperature value of tomatoes before they enter the budding stage, which will delay the growth of tomatoes and the time at which they enter each flowering stage. The inoculant reduces the effective accumulated temperature and the

physiological seedling age required for the tomato to enter the budding stage, allowing the tomato to flower earlier.

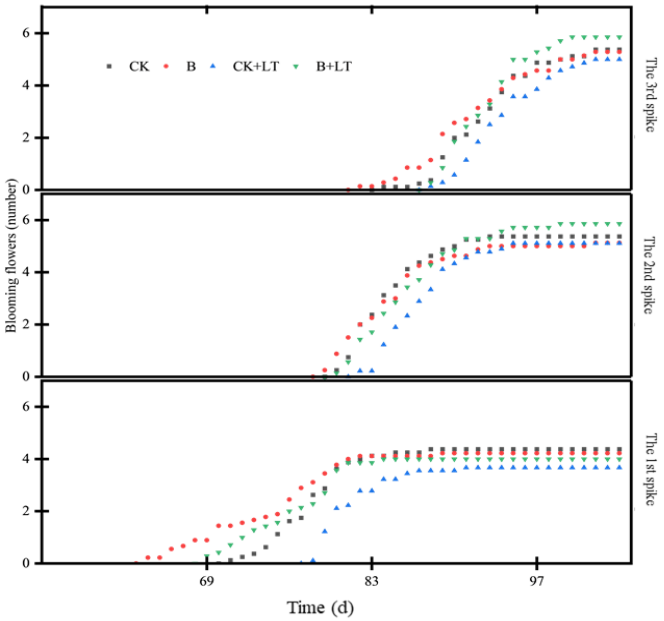

Figure 6. Dynamics of the number of blooming flowers with time under different treatments. The various shapes and colors represent the number of flowering plants of different treatments on the day, and the average value of 9 plants is counted.

Table 2. The seedling age and effective cumulative temperature required for tomatoes to reach budding stage under different treatments.

Spike	Treatment	Calendar Seedling Age (d)	Physiological Seedling Age (Number of Leaves)	Effective Cumulative Temperature (°C)
1st	CK	64.25 ± 0.03 b	8.29 ± 0.08 ab	654.12 ± 22.52 a
	B	62.13 ± 0.03 c	8.16 ± 0.03 b	626.88 ± 18.06 b
	CK + LT	67.11 ± 0.01 a	8.65 ± 0.03 a	568.22 ± 7.60 c
	B + LT	62.57 ± 0.04 c	7.59 ± 0.09 c	515.71 ± 25.87 d
2nd	CK	70.63 ± 0.02 b	10.38 ± 0.08 ab	692.55 ± 16.30 a
	B	68.88 ± 0.03 c	9.59 ± 0.10 c	667.81 ± 17.52 b
	CK + LT	73.00 ± 0.00 a	10.81 ± 0.04 a	602.00 ± 3.45 c
	B + LT	70.29 ± 0.03 bc	9.94 ± 0.06 bc	563.97 ± 21.40 d
3rd	CK	78.25 ± 0.01 b	12.96 ± 0.05 ab	739.02 ± 13.02 a
	B	77.86 ± 0.01 b	12.98 ± 0.05 ab	724.83 ± 12.87 a
	CK + LT	81.86 ± 0.04 a	13.29 ± 0.05 a	660.14 ± 22.71 b
	B + LT	78.14 ± 0.02 b	12.37 ± 0.07 b	611.48 ± 17.99 c

Note: The data are expressed as the mean ± standard error of the three independent biological replicates. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

3.6. B. methylotrophicus Can Improve Tomato Fruit Quality

The order of weight of a single fruit from large to small was B + LT > CK + LT > B > CK. The single fruit fresh weights of B + LT, CK + LT and B plants were 18.66%, 17.42% and 17.34% higher than that of CK, respectively. Moreover, the single fruit dry weights of B + LT, CK + LT and B plants were 21.10%, 18.65% and 18.60% higher than that of CK, respectively. The dry weight of a single fruit in B + LT treatment was significantly different from the CK treatment. This finding showed that low-temperature treatment and addition of inoculants could increase in tomatoes (Figure 7).

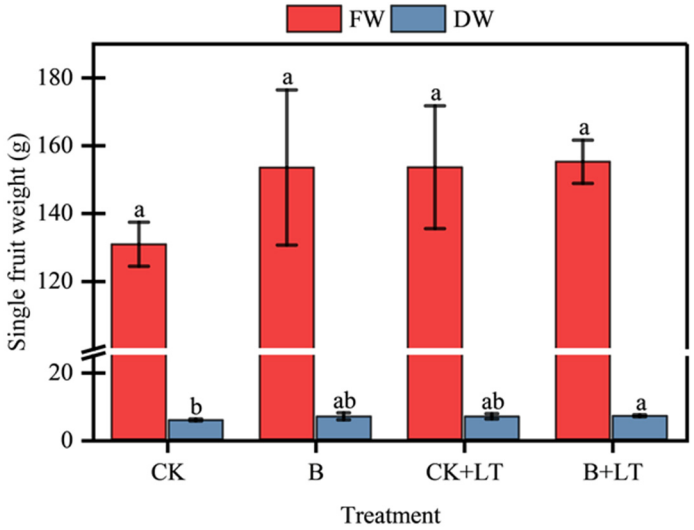

Figure 7. Single fruit weight of tomatoes under different treatments. FW represents the fresh fruit weight and DW denotes the dry fruit weight. The different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level using LSD's multiple range test.

In comparison with CK, the low-temperature treatment increased the nitrate nitrogen, soluble solid content and SAR in the CK + LT fruit and decreased the content of vitamin C, TSSC and titratable acid. The nitrate nitrogen, soluble solid content, SAR, vitamin C and TSSC content in the fruit were decreased in the B + LT treatment compared with those in the B treatment, and the titratable acid content was increased. Low-temperature treatment will result in a significant decrease in lycopene (Table 3) [7,44]. In the nutritional quality of tomato fruit, except for lycopene, the other six nutritional quality indicators were affected by the interaction between low-temperature treatment and inoculants (Table 3). In the comprehensive evaluation of the tomato fruit quality by the TOPISS method, nitrate nitrogen and titratable acid content were set as negative indicators. The comprehensive analysis showed that the quality of the tomato fruit was the best under treatment B, and the fruit evaluation of the CK + LT treatment was the lowest (Table S4). The inoculants could improve the nutritional quality of fruit under different temperatures.

Table 3. Nutritional quality of fresh tomatoes under different treatments.

Treatment	Nitrate Nitrogen (NO_3^--N mg·g^{-1})	Vitamin C (μg/100 g)	Lycopene (μg/g)	Total Soluble Sugars (%)	Soluble Solids (%)	Titratable Acids (%)	Sugar to Acid Ratio
CK	0.58 ± 0.04 b	33.84 ± 0.04 a	24.41 ± 1.57 a	3.57 ± 0.26 b	3.80 ± 0.10 d	5.75 ± 0.30 a	8.27 ± 0.36 c
B	0.80 ± 0.11 a	33.64 ± 0.06 b	21.60 ± 2.78 a	4.63 ± 0.41 a	4.60 ± 0.10 b	5.42 ± 0.05 a	10.15 ± 0.26 a
CK + LT	0.86 ± 0.08 a	33.61 ± 0.07 b	16.62 ± 2.19 b	3.49 ± 0.08 b	4.80 ± 0.10 a	5.56 ± 0.23 a	9.95 ± 0.51 ab
B + LT	0.62 ± 0.06 b	33.63 ± 0.03 b	15.66 ± 0.17 b	3.63 ± 0.15 b	4.00 ± 0.10 c	5.68 ± 0.22 a	9.45 ± 0.13 b
PGPR	0.05	8.68 *	2.84	16.46 **	0.00	11.11 *	11.97 **
LT	1.25	15.45 **	37.69 ***	13.16 **	12.00 **	0.11	5.97 *
PGPR × LT	27.83 **	14.16 **	0.69	9.45 *	192.00 ***	7.11 *	35.49 ***

Note: "*", "**" and "***" stand for $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively, and the different letters on the bars indicate the significant differences between treatments at the $p < 0.05$ level on the same days using LSD's multiple range test.

4. Discussion

Low-temperature stress can seriously affect the growth, spatial distribution, yield and quality of horticultural crops. In the early stage of low-temperature stress, plants can activate their own defense through a series of intracellular and intercellular reactions to alleviate the damage, but prolonged low temperature will seriously harm plants [8,45]. In the tomato, the morphological distribution of the shoots and roots is inhibited, the

photosynthetic capacity decreases, the flowering period is delayed, and the inflorescence node position increases [46,47]. Therefore, innovative strategies are required to improve tomato tolerance to low-temperature stress, lessen damage and prevent yield loss.

A vast resource of microorganisms found in the plant rhizosphere may be exploited to develop beneficial microorganisms that will boost plant growth and stress resistance. This study discovered that low temperatures resulted in the slow root development and inhibited aboveground growth in tomato. The activity of PGPR in the plant rhizosphere can promote the solubilization of soil phosphates and the production of siderophores and plant hormones. These factors can promote the development of plant roots, and the absorption area of water and mineral nutrients increase with the increase in root surface area [32]. In this study, the value of fine absorbing roots/transporting roots inoculated with an inoculum and treated at low temperature (B + LT) was always at the same level as those of normal temperature control (B). Meanwhile, the whole plant fresh weight (compared with B) relative decline was less than that of the CK + LT treatment (compared with the CK). The result showed that the inoculation of *B. methylotrophicus* can effectively alleviate the growth inhibition of low temperature on the plant and the influence of chilling injury on root growth and promote root recovery after low temperatures. Some beneficial microorganisms and root symbiosis can promote soybean root development, improve soybean cold tolerance and expand the soybean planting range [48]. In the rhizosphere, PGPR adhere to plant roots and contribute to complex processes, and some secretions may promote plant growth or activate a series of metabolic pathways to improve plant response to cold stress. Some studies showed that inoculation with PGPR promotes the expression of the cold stress transcription factor *CBF1* in tomatoes [30], but the specific metabolic pathways are still unclear, which is worthy of further research in the future.

Under low-temperature stress, the accumulation of reactive oxygen species (ROS) can cause damage to plant leaves, and PGPR can alleviate the damage to leaves by improving the antioxidant capacity of leaves [30]. The change of SPAD value can reflect the damage condition of leaves. The SPAD value of leaves will decrease under low-temperature stress [49]. The SPAD value of the CK + LT plant leaves significantly decreased on the 7th day of low-temperature treatment, and the negative effect of low temperature on the SPAD value lasted until the 21st day after the end of the low-temperature treatment. The inoculum could continuously maintain the SPAD value of leaves. In the research of Caradonia et al., the consortium of inoculum *Funneliformis mosseae* and *Paraburkholderia graminis* alleviated the damage of low temperature to tomato leaves and increased the efficiency of photosystem II [29]. In our study, inoculation with *B. methylotrophicus* alone achieved the same effect. During the treatment period, the SPAD values of the mature leaves and the new leaves of the B + LT plant were always higher than that of the CK + LT plant and recovered to the level of the B treatment on the 14th day after the end of the low-temperature treatment. The inoculant can effectively reduce the damage of low temperatures on the maximum photochemical efficiency of PS II (Fv/Fm) and enhance the adaptability of leaves to low temperature. These data indicated that the inoculant could effectively alleviate the damage to the photosynthetic system of tomato leaves caused by low temperatures, and enhance the adaptability and recovery ability of the photosynthetic system to low temperature.

Low temperature at the seedling stage of the tomato can cause tomato growth retardation and delay flowering [46,50]. PGPR inoculation can promote the vegetative growth of plants and lead to early flowering. PGPR have been applied to regulate the growth and promote the flowering of some ornamental plants [14,51]. In this experiment, the time of the CK + LT plant to enter each flowering stage was later than that of the normal temperature control plant (CK). Meanwhile, the time of the B + LT plant to enter each flowering stage was not significantly different from that of the CK. The time of the B treatment to enter the budding stage was significantly earlier than that of the CK. The result showed that the inoculation of *B. methylotrophicus* also has a positive effect on improving the delay of plant flowering caused by low-temperature stress. However, no difference was observed in the time and effective accumulated temperature required for each treatment to complete the

whole flowering process. Hence, the difference in the flowering time in the experiment was mainly due to the growth rate in the vegetative growth stage. Low-temperature stress significantly reduced not only the effective accumulated temperature required for the tomato to reach the budding stage but also the growth rate of the tomato, resulting in a delay in the flowering time under the combined effect. Inoculation could reduce the demand for an effective accumulated temperature of tomato reaching the budding stage and promote its growth. Meanwhile, PGPR has been found to promote the expression of flowering genes [52]. After low-temperature stress, the time of flower bud emergence of each panicle of the B + LT plant had no significant difference with that of the B treatment, and entered the bud emergence stage earlier than that of the CK + LT and CK plants. Inoculants can effectively alleviate the delay of low temperature on tomato flowering by regulating the coordination of plant growth and flowering.

Low temperatures will reduce the quality of tomato fruit, reduce the content of vitamin C, soluble solids, soluble sugar, lycopene content and sugar-acid ratio in the fruit, and increase the content of titratable acid [47,53]. Studies have found that inoculation of PGPR in the roots can improve the yield and fruit quality of strawberries, cucumbers and other crops, which may be related to the promotion of nutrient absorption and utilization by PGPR [54–56]. In this experiment, the low-temperature treatment was carried out before the flowering stage, and the contents of vitamin C and lycopene in the CK + LT fruits were lower than those in the CK fruits. Meanwhile, the nitrate nitrogen, soluble solids and sugar–acid ratio were higher than those in CK. No difference was observed in the titratable acid content in fruits of all treatments. In this experiment, the soluble total sugar, soluble solids and sugar–acid ratio in the fruit of the B treatment were better than those of the CK. However, the vitamin C content slightly decreased, and the nitrate nitrogen content increased in the B treatment. The B + LT treatment could effectively reduce the accumulation of nitrate nitrogen in fruits compared with the CK + LT treatment. The TOPISIS method was used to comprehensively evaluate the fruit quality. The comprehensive score of the B treatment was higher than that of the CK treatment, and the score of the B + LT treatment was higher than that of the CK + LT treatment, indicating that the inoculant improved the tomato fruit quality regardless of whether it suffered from low temperature.

5. Conclusions

Low temperatures will slow down the growth of the tomato, reduce the growth rate of the leaf number and shrink the distribution range of roots, increase the proportion of fine roots, decrease the relative chlorophyll concentration, weaken the photosynthetic capacity, delayed flowering, decreased vitamin C and lycopene content in fruit, and increase the content of nitrate nitrogen in fruit. However, inoculation with 5×10^8 CFU/g *B. methylotrophicus* could increase tomato growth rate by about 10%, alleviate the cold shock reaction of the root system to low temperatures, reduce the negative effects of low temperatures on the relative chlorophyll content and Fv/Fm of leaves. Moreover, the low-temperature tolerance and recovery ability of tomatoes during the vegetative growth period were enhanced, and the flowering time was 3–9 d earlier. Inoculation with *B. methylotrophicus* also reduced the content of nitrate nitrogen in fruit by about 27% after low-temperature stress and improved the fruit quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071902/s1. Table S1: Response of the number of leaves (NL), plant height (PH) and stem diameter (SD) of tomato to inoculating PGPR and low temperature. Table S2: Response of the SPAD and Fv/Fm of tomato leaves to inoculating PGPR and low temperature. Table S3: Cox proportional hazards model was used to determine the likelihood of the tomato plants reaching a particular developmental stage under different treatments. Table S4: Different processing TOPSIS analysis decision matrix and score ranking. Figure S1. Effects of *B. methylotrophicus* inoculation on the stem diameter of tomato. LT represents the period of low temperature treatment and NT denotes the period of normal temperature cultivation. Figure S2. Time it takes for tomatoes

to reach each flowering stage under different treatments, with that flowering stage completed when the survival function is 0.

Author Contributions: G.L.: analysis the data and wrote the manuscript. T.P.: performed the experiments and analyzed the data. F.Q.: performed the experiments. J.W.: performed the experiments. Y.L.: performed the experiments. X.H.: designed the experiments and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Key R&D Program of China (2019YFD1001900), China Agriculture Research System (CRAS-23-D06), Technological Innovative Research Team of Shaanxi Province (2021TD-34), and Key Research and Development Program of Shaanxi Province in China (2022ZDLNY03-11) and the Scientific Startup Foundation for Doctors of Northwest A&F University (Z1090122021).

Data Availability Statement: All data supporting the findings of this study are available within the paper and within the Supplementary Data published online.

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

References

1. Willis, C.G.; Ruhfel, B.; Primack, R.B.; Miller-Rushing, A.J.; Davis, C.C. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17029–17033. [CrossRef] [PubMed]
2. Ding, F.; Ren, L.; Xie, F.; Wang, M.; Zhang, S. Jasmonate and Melatonin Act Synergistically to Potentiate Cold Tolerance in Tomato Plants. *Front. Plant Sci.* **2021**, *12*, 763284. [CrossRef] [PubMed]
3. Yadav, S.K. Cold stress tolerance mechanisms in plants: A review. *Agron. Sustain. Dev.* **2010**, *30*, 515–527. [CrossRef]
4. Hussain, H.A.; Hussain, S.; Khaliq, A.; Ashraf, U.; Anjum, S.A.; Men, S.; Wang, L. Chilling and Drought Stresses in Crop Plants: Implications, Cross Talk, and Potential Management Opportunities. *Front. Plant Sci.* **2018**, *9*, 393. [CrossRef]
5. Lu, G.; Wu, F.Q.; Wu, W.; Wang, H.J.; Zheng, X.M.; Zhang, Y.; Chen, X.; Zhou, K.; Jin, M.; Cheng, Z.; et al. Rice LTG1 is involved in adaptive growth and fitness under low ambient temperature. *Plant J.* **2014**, *78*, 468–480. [CrossRef] [PubMed]
6. Luan, W.; Chen, H.; Fu, Y.; Si, H.; Peng, W.; Song, S.; Liu, W.; Hu, G.; Sun, Z.; Xie, D.; et al. The effect of the crosstalk between photoperiod and temperature on the heading-date in rice. *PLoS ONE* **2009**, *4*, e5891. [CrossRef]
7. Mesa, T.; Polo, J.; Arabia, A.; Caselles, V.; Munne-Bosch, S. Differential physiological response to heat and cold stress of tomato plants and its implication on fruit quality. *J. Plant Physiol.* **2022**, *268*, 153581. [CrossRef]
8. Song, J.; Shang, L.; Chen, S.; Lu, Y.; Zhang, Y.; Ouyang, B.; Ye, Z.; Zhang, J. Interactions between ShPP2-1, an F-box family gene, and ACR11A regulate cold tolerance of tomato. *Hortic. Res.* **2021**, *8*, 148. [CrossRef]
9. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants* **2019**, *8*, 34. [CrossRef]
10. Liu, T.; Du, Q.; Li, S.; Yang, J.; Li, X.; Xu, J.; Chen, P.; Li, J.; Hu, X. GSTU43 gene involved in ALA-regulated redox homeostasis, to maintain coordinated chlorophyll synthesis of tomato at low temperature. *BMC Plant Biol.* **2019**, *19*, 323. [CrossRef]
11. Ding, F.; Wang, C.; Xu, N.; Wang, M.; Zhang, S. Jasmonic acid-regulated putrescine biosynthesis attenuates cold-induced oxidative stress in tomato plants. *Sci. Hortic.* **2021**, *288*, 110373. [CrossRef]
12. Krasensky, J.; Jonak, C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* **2012**, *63*, 1593–1608. [CrossRef]
13. Che, Y.; Zhang, N.; Zhu, X.; Li, S.; Wang, S.; Si, H. Enhanced tolerance of the transgenic potato plants overexpressing Cu/Zn superoxide dismutase to low temperature. *Sci. Hortic.* **2020**, *261*, 108949. [CrossRef]
14. South, K.A.; Nordstedt, N.P.; Jones, M.L. Identification of Plant Growth Promoting Rhizobacteria that Improve the Performance of Greenhouse-Grown Petunias under Low Fertility Conditions. *Plants* **2021**, *10*, 1410. [CrossRef] [PubMed]
15. Glick, B.R. Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica* **2012**, *2012*, 963401. [CrossRef]
16. Wang, J.; Qu, F.; Liang, J.; Yang, M.; Hu, X. Bacillus velezensis SX13 promoted cucumber growth and production by accelerating the absorption of nutrients and increasing plant photosynthetic metabolism. *Sci. Hortic.* **2022**, *301*. [CrossRef]
17. Lugtenberg, B.; Kamilova, F. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* **2009**, *63*, 541–556. [CrossRef]
18. Vives-Peris, V.; de Ollas, C.; Gomez-Cadenas, A.; Perez-Clemente, R.M. Root exudates: From plant to rhizosphere and beyond. *Plant Cell Rep.* **2020**, *39*, 3–17. [CrossRef]
19. Gouda, S.; Kerry, R.G.; Das, G.; Paramithiotis, S.; Shin, H.S.; Patra, J.K. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiol. Res.* **2018**, *206*, 131–140. [CrossRef] [PubMed]
20. Rubin, R.L.; van Groenigen, K.J.; Hungate, B.A. Plant growth promoting rhizobacteria are more effective under drought: A meta-analysis. *Plant Soil.* **2017**, *416*, 309–323. [CrossRef]
21. Ha-Tran, D.M.; Nguyen, T.T.M.; Hung, S.H.; Huang, E.; Huang, C.C. Roles of Plant Growth-Promoting Rhizobacteria (PGPR) in Stimulating Salinity Stress Defense in Plants: A Review. *Int. J. Mol. Sci.* **2021**, *22*, 3154. [CrossRef] [PubMed]
22. Kumar, A.; Verma, J.P. Does plant-Microbe interaction confer stress tolerance in plants: A review? *Microbiol. Res.* **2018**, *207*, 41–52. [CrossRef] [PubMed]

23. Ait Barka, E.; Nowak, J.; Clement, C. Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, Burkholderia phytofirmans strain PsJN. *Appl. Env. Environ. Microbiol.* **2006**, *72*, 7246–7252. [CrossRef] [PubMed]
24. Mitra, D.; Díaz Rodríguez, A.M.; Parra Cota, F.I.; Khoshru, B.; Panneerselvam, P.; Moradi, S.; Sagarika, M.S.; Anđelković, S.; Santos-Villalobos, S.d.l.; Das Mohapatra, P.K. Amelioration of thermal stress in crops by plant growth-promoting rhizobacteria. *Physiol. Mol. Plant Pathol.* **2021**, *115*, 101679. [CrossRef]
25. Kaan, T.; Homuth, G.; Mader, U.; Bandow, J.; Schweder, T. Genome-wide transcriptional profiling of the *Bacillus subtilis* cold-shock response. *Microbiology* **2002**, *148*, 3441–3455. [CrossRef]
26. Aguilar, P.; Hernandez-Arriaga, A.; Cybulski, L.; Erazo, A.; de Mendoza, D. Molecular basis of thermosensing a two-component signal transduction thermometer in. *EMBO J.* **2001**, *20*, 1681–1691. [CrossRef]
27. Hoffmann, T.; Boiangiu, C.; Moses, S.; Bremer, E. Responses of *Bacillus subtilis* to hypotonic challenges: Physiological contributions of mechanosensitive channels to cellular survival. *Appl. Environ. Microbiol.* **2008**, *74*, 2454–2460. [CrossRef] [PubMed]
28. Mishra, P.K.; Mishra, S.; Bisht, S.C.; Selvakumar, G.; Kundu, S.; Bisht, J.K.; Gupta, H.S. Isolation, molecular characterization and growth-promotion activities of a cold tolerant bacterium Pseudomonas sp. NARs9 (MTCC9002) from the Indian Himalayas. *Biol. Res.* **2009**, *42*, 305–313. [CrossRef]
29. Caradonia, F.; Francia, E.; Morcia, C.; Ghizzoni, R.; Moulin, L.; Terzi, V.; Ronga, D. Arbuscular Mycorrhizal Fungi and Plant Growth Promoting Rhizobacteria Avoid Processing Tomato Leaf Damage during Chilling Stress. *Agronomy* **2019**, *9*, 299. [CrossRef]
30. Wang, C.; Wang, C.; Gao, Y.-L.; Wang, Y.-P.; Guo, J.-H. A Consortium of Three Plant Growth-Promoting Rhizobacterium Strains Acclimates *Lycopersicon esculentum* and Confers a Better Tolerance to Chilling Stress. *J. Plant Growth Regul.* **2015**, *35*, 54–64. [CrossRef]
31. Madhaiyan, M.; Poonguzhali, S.; Kwon, S.W.; Sa, T.M. *Bacillus methylotrophicus* sp. nov., a methanol-utilizing, plant-growth-promoting bacterium isolated from rice rhizosphere soil. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 2490–2495. [CrossRef] [PubMed]
32. Wang, A.; Hua, J.; Wang, Y.; Zhang, G.; Luo, S. Stereoisomers of Nonvolatile Acetylbutanediol Metabolites Produced *by Bacillus velezensis* WRN031 Improved Root Elongation of Maize and Rice. *J. Agric. Food Chem.* **2020**, *68*, 6308–6315. [CrossRef]
33. Narayanasamy, S.; Thangappan, S.; Uthandi, S. Plant Growth-Promoting Bacillus sp. Cahoots Moisture Stress Alleviation in Rice Genotypes by Triggering Antioxidant Defense System. *Microbiol. Res.* **2020**, *239*, 126518. [CrossRef] [PubMed]
34. Chaprao, M.J.; da Silva, R.; Rufino, R.D.; Luna, J.M.; Santos, V.A.; Sarubbo, L.A. Production of a biosurfactant from *Bacillus methylotrophicus* UCP1616 for use in the bioremediation of oil-contaminated environments. *Ecotoxicology* **2018**, *27*, 1310–1322. [CrossRef] [PubMed]
35. Qiu, Z.; Wang, X.; Gao, J.; Guo, Y.; Huang, Z.; Du, Y. The Tomato Hoffman's Anthocyaninless Gene Encodes a bHLH Transcription Factor Involved in Anthocyanin Biosynthesis That Is Developmentally Regulated and Induced by Low Temperatures. *PLoS ONE* **2016**, *11*, e0151067. [CrossRef] [PubMed]
36. Xu, Z.; Wang, J.; Ma, Y.; Wang, F.; Wang, J.; Zhang, Y.; Hu, X. The bZIP transcription factor SlAREB1 regulates anthocyanin biosynthesis in response to low temperature in tomato. *Plant J.* **2023**, *115*, 205–219. [CrossRef]
37. McCormack, M.L.; Dickie, I.A.; Eissenstat, D.M.; Fahey, T.J.; Fernandez, C.W.; Guo, D.; Helmisaari, H.S.; Hobbie, E.A.; Iversen, C.M.; Jackson, R.B.; et al. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* **2015**, *207*, 505–518. [CrossRef]
38. Perez-Bueno, M.L.; Pineda, M.; Diaz-Casado, E.; Baron, M. Spatial and temporal dynamics of primary and secondary metabolism in *Phaseolus vulgaris* challenged by *Pseudomonas syringae*. *Physiol. Plant* **2015**, *153*, 161–174. [CrossRef]
39. Igwe, A.N.; Quasem, B.; Liu, N.M.; Vannette, R.L. Plant phenology influences rhizosphere microbial community and is accelerated by serpentine microorganisms in *Plantago erecta*. *Fems Microbiol. Ecol.* **2021**, *97*, fiab085. [CrossRef]
40. Li, H.S. *Physiology and Biochemical Principles and Techniques*; Higher Education Press: Beijing, China, 2000; pp. 195–196. (In Chinese)
41. Sundriyal, M.; Sundriyal, R.C. Wild edible plants of the Sikkim Himalaya: Nutritive values of selected species. *Econ. Bot.* **2001**, *55*, 377–390. [CrossRef]
42. Zhu, K.; Zhao, Y.; Ma, Y.; Zhang, Q.; Kang, Z.; Hu, X. Drip irrigation strategy for tomatoes grown in greenhouse on the basis of fuzzy Borda and K-means analysis method. *Agric. Water Manag.* **2022**, *267*, 107598. [CrossRef]
43. Koevoets, I.T.; Venema, J.H.; Elzenga, J.T.; Testerink, C. Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. *Front. Plant Sci.* **2016**, *7*, 1335. [CrossRef]
44. Chen, L.; Pan, Y.; Li, H.; Liu, Z.; Jia, X.; Li, W.; Jia, H.; Li, X. Constant temperature during postharvest storage delays fruit ripening and enhances the antioxidant capacity of mature green tomato. *J. Food Process. Preserv.* **2020**, *44*, e14831. [CrossRef]
45. Ding, Y.; Shi, Y.; Yang, S. Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. *New Phytol.* **2019**, *222*, 1690–1704. [CrossRef]
46. Li, S.; Xia, Y.; Sun, Z. Effects of cold-shock on the growth and flower bud differentiation of tomato seedlings under high temperature stress. *J. Appl. Ecol.* **2016**, *27*, 477–483.
47. Affandi, F.Y.; Pijnenburg, C.; Verdonk, J.C.; Woltering, E.J.; Schouten, R.E. Growth Temperature Influences Postharvest Quality and Cold Tolerance of Green Harvested Dwarf Tomatoes During Storage. *Front. Sustain. Food Syst.* **2022**, *6*, 876597. [CrossRef]
48. Schmidt, J.; Messmer, M.; Wilbois, K.-P. Beneficial microorganisms for soybean (*Glycine max* (L.) Merr), with a focus on low root-zone temperatures. *Plant Soil.* **2015**, *397*, 411–445. [CrossRef]

49. Munoz-Huerta, R.F.; Guevara-Gonzalez, R.G.; Contreras-Medina, L.M.; Torres-Pacheco, I.; Prado-Olivarez, J.; Ocampo-Velazquez, R.V. A review of methods for sensing the nitrogen status in plants: Advantages, disadvantages and recent advances. *Sensors* **2013**, *13*, 10823–10843. [CrossRef]
50. Rajametov, S.N.; Lee, K.; Jeong, H.-B.; Cho, M.-C.; Nam, C.-W.; Yang, E.-Y. Physiological Traits of Thirty-Five Tomato Accessions in Response to Low Temperature. *Agriculture* **2021**, *11*, 792. [CrossRef]
51. Girgin, E.; Sezen, I. The Effects of Rhizobacteria and Chemical Fertilizers on the Development of *Cyclamen persicum*. *Fresenius Environ. Bull.* **2021**, *30*, 9642–9652.
52. Sharma, M.; Charron, J.B.; Rani, M.; Jabaji, S. Bacillus velezensis strain B26 modulates the inflorescence and root architecture of Brachypodium distachyon via hormone homeostasis. *Sci. Rep.* **2022**, *12*, 7951. [CrossRef]
53. Siomos, A.; Tsouvaltzis, P.; Kasampalis, D. Tomato fruit quality in relation to growing season, harvest period, ripening stage and postharvest storage. *Emir. J. Food Agric.* **2020**, *33*, 130–138. [CrossRef]
54. Pirlak, L.; Kose, M. Runner Plant Yield and Quality of Strawberry (Fragaria x ananassa Duch_) Inoculated with Plant Growth Promoting Rhizobacteria (PGPR). *Philipp. Agric. Sci.* **2010**, *93*, 42–46.
55. Zapata-Sifuentes, G.; Hernandez-Montiel, L.G.; Saenz-Mata, J.; Fortis-Hernandez, M.; Blanco-Contreras, E.; Chiquito-Contreras, R.G.; Preciado-Rangel, P. Plant Growth-Promoting Rhizobacteria Improve Growth and Fruit Quality of Cucumber under Greenhouse Conditions. *Plants* **2022**, *11*, 1612. [CrossRef] [PubMed]
56. Mena-Violante, H.G.; Olalde-Portugal, V. Alteration of tomato fruit quality by root inoculation with plant growth-promoting rhizobacteria (PGPR): *Bacillus subtilis* BEB-13bs. *Sci. Hortic.* **2007**, *113*, 103–106. [CrossRef]