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Evaluation of the Effects of Two Plant Growth-Promoting Bacterial Strains (*Bacillus tequilensis* and *Bacillus subtilis*) on Tomato Growth, Physiology, and Nitrogen Utilization Efficiency at Various Nitrogen Levels

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ABSTRACT

Several microorganisms that encourage plant growth possess the capacity to redox atmospheric dinitrogen, which can be employed in sustainable crop production to reduce the need for nitrogen (N) fertilizer. Tomatoes receive a lot of N fertilizer application (Lycopersicon esculentum). Therefore, applying PGPB, a nitrogen-fixing microbial inoculant, will help reduce the use of inorganic fertilizer on tomato plants. This study aimed to test the hypothesis of whether the application of PGPB will reduce the amount of nitrogen fertilizer required by tomato plants. Therefore, the microbial inoculants used were Bacillus subtilis (UPMB10) and Bacillus tequilensis (UPMRB9) at different N fertilizer levels. To lower the nitrogen fertilizer requirement for tomato plants, a glasshouse pot experiment was carried out with the chosen microbial inoculants and nitrogen fertilizers at varied levels (0% N, 50% N, and 100% N). The pot experiment's findings indicated that microbial inoculation increased plant growth, dry weight, physiology, tissue N concentrations, and bacterial population in rhizosphere soil when N fertilization was present. Additionally, the findings revealed that reducing 50% of the prescribed N fertilizer through microbial inoculation does not affect tomato plant growth, nutrition, and development. Future field research must ascertain whether these inoculants may be used as biofertilizers in agriculture nutrient management techniques.

Keywords: Nitrogen, PGPB, NUE, physiology, bacterial population

INTRODUCTION

Tomato (*Lycopersicum esculentum)* is among the crucial vegetables globally, which is significant in nutrition and economy and regarded as a defensive crop of vegetables. It has a wide production span and provides a large yield quickly. Over 180 million tonnes of tomatoes were produced worldwide in 2018, making it significant in the world (FAOSTAT, 2021). It is a vegetable widely consumed worldwide due to its high levels of b-carotene, flavonoids, lycopene, and vitamins (Ahmed *et al*., 2018). Its importance is increased by the presence of the

carotenoid lycopene, which has anti-cancer, anti-cardiovascular disease, and anti-aging characteristics (Al-Amri, 2013). As an intensively grown crop, tomatoes use a wide array of pesticides and other chemical inputs to guarantee successful crop yields (Engindeniz, 2006). In 2050, the world's population is expected to reach 9.1 billion, according to the Food and Agriculture Organization of the United Nations (FAO) (Godfray *et al*., 2010), and there is a need to produce enough for the population.

Agriculture now heavily relies on artificial fertilizers and pesticides to meet the rising worldwide demand for food. Fertilizers sold commercially, particularly N fertilizers, are necessary to maintain worldwide agriculture and meet food demands for a fast-expanding global population with few land resources (Hu and Desai, 2004; Wit and McClure, 2004). The nitrogen utilization efficiency (NUE) of crops is another factor that influences optimal crop yields. In general, NUE is extremely low (33%) (Giambastiani, 2007) because of different processes in the soil and aspects of the environment (Honeycutt, 1998). Since leaching loss is the primary (50%) method of N loss in the zones with frequent and heavy rains, the plant cannot utilize the complete amount of N provided to the soil. Increasing tomato's nitrogen utilization efficiency (NUE), however, is becoming more critical due to current worries about crop and environmental sustainability. The employment of bacteria that promote plant growth (PGPB) is a suggested substitute for conventional agrochemicals (Chandra *et al*., 2018). PGPB can enhance plant growth and health in several ways. Indole acetic acid levels, which are similar to phytohormones, are impacted by direct advantages on plant metabolism (Etesami *et al*., 2015) and enhance nutrients are easily accessible by, for instance, producing siderophores (Gull and Hafeez, 2012), the fixing of nitrogen, or solubilization of phosphate (Midekssa *et al*., 2016). Plants' resistance to unfavorable environmental circumstances can also be increased by PGPB, such as dryness and excessive salinity, by controlling the mechanisms that produce hormones; the following, via affecting the ACC deaminase production (Jiménez-Gómez *et al*., 2020), which lessens difficulty producing ethylene (Danish and Zafar-ul-Hye, 2019). An investigation of sugar beet plants (*Beta vulgaris)* revealed that application with three PGPBs (*Bacillus pumilus*, *Chryseobacterium indologene*, and *Acinetobacter johnsonii*) led to increased dry mass and plant height and enhanced higher amount of chlorophyll, which boosted the production of carbohydrates (Shi *et al*., 2010). Stefan *et al*. (2013) evidenced that, in comparison to control plants, runner bean plants (*Phaseolus coccineus*) inoculated with two siderophores and IAA-producing PGPB strains had higher chlorophyll contents and photosynthetic rates. This study aimed to assess the impact of *Bacillus spp*. as a plant growth-promoting bacteria on tomato plant development under different levels of nitrogen fertilization.

MATERIALS AND METHODS

The experiment was conducted as a pot experiment in the glasshouse (03° 00′ 12.6″ N; 101° 47′ 22.4″ E) at Field 15, Agriculture Faculty, UPM, Malaysia. Sandy clay loam soil was collected from a field at UPM, Malaysia. The physical and chemical properties of the soil were pH of 4.91, total N (%) of 0.199, total C (%) of 2.31, P of 39.71 mg/kg, K of 0.23 (me/100g), Ca of 0.31 (me/100g), and Mg of 0.10 (me/100g). Based on soil pH and nutrient levels, DOA (2003) recommended applying 180 kg/ha for N as Urea, 180 kg/ha for P_2O_5 as TSP, and 255 kg/ha for K_2O as MOP. The plastic pot (27 cm x 27 cm) contained 10 kg of soil, and field capacity (FC) water content (23.95%) was used to moisten the soil. Tomato seeds were initially sown in plastic trays; after 3 weeks, seedlings were transplanted in plastic pots. A single seedling was planted in each pot.

Experimental design

The completely randomized design (CRD) method was used with nine (9) treatment combinations, which were replicated three times, each giving a total of 27 experimental units (pots). The treatment combinations used in the experiment are presented in **Table 1**.

Table 1. Description of the treatments used in the study

Treatment	Doses level
B0N0	No Nitrogen and no Bacteria (Control)
B0N50	No Bacteria with 50% Nitrogen applied @ 180 kg/ha in three installations
B0N100	No Bacteria with 100% Nitrogen applied @ 180 kg/ha in three installations
UPMB10N0	*Bacillus subtilis* (10^8 CFU/mL) with no Nitrogen applied
UPMB10N50	*Bacillus subtilis* (10^8 CFU/mL) with 50% Nitrogen applied @ 180 kg/ha in three installations
UPMB10N100	*Bacillus subtilis* (10^8 CFU/mL) with 100% Nitrogen applied @ 180 kg/ha in three installations
UPMRB9N0	*Bacillus tequilensis* (10^8 CFU/mL) with no Nitrogen applied
UPMRB9N50	*Bacillus tequilensis* (10^8 CFU/mL) with 50% Nitrogen applied @ 180 kg/ha in three installations
UPMRB9N100	*Bacillus tequilensis* (10^8 CFU/mL) with 100% Nitrogen applied @ 180 kg/ha in three installations

Fertilizer management

The types of fertilizers used were Urea, TSP, and MOP at the rate of 0.783 g (for 50% N), 1.565 g (for 100% N), 4.13 g, and 3.74 g per pot, respectively. Before 48 hours of seedling transplant, triple super phosphate and MOP were introduced into the soil. The soil received the fertilizer applications, and the initial dose of N was given 7 days after transplanting (DAT). The other two doses were applied at 14-day intervals. The moisture level was maintained by daily manual irrigation. If there was any weed, it was removed by hand. The surrounding of the experimental units (pots) was covered with a net to prevent the plant from pest infestation.

Preparation of inoculums and application to plant

Two promising bacterial isolates, UPMB10 (*Bacillus subtilis*) and UPMRB9 (*Bacillus tequilensis*), were selected for this study. These microorganisms were inoculated into a 250 mL conical flask containing 100 mL of tryptic soy broth (TSB). The flask was incubated after inoculation in an orbital shaking incubator (Model OSI-503 LD; Firstek Scientific, Japan) for 24 hours at 28^{o}C, shaken at 150 rpm. A UV-visible spectrophotometer set the optical density (OD600) of the two strains to 1. Each plant inoculated with 28.57 mL of 108 CFU/mL live inoculants per pot every 14 days intervals till harvest. Distilled water was used for the control.

Total N and C content of soils

The total C, N, and S content in the soils was determined by TruMac CNS Macro Analyzer LECO FP-2000 (LECO Corp. Michigan, USA).

Growth parameters

The plant height was measured at 2-week intervals until the 8 WAT using measuring tape from the soil to the tip of the apical leaf. The number of tomato plant branches was also measured at two-week intervals. At 8WAT, the leaf area per plant was determined using a leaf area meter machine (model LI -3100 Li-Cor Inc. Lincoln, Nebraska, USA). The dry weights of the plant were measured at 8WAT. Dry weights of the roots, shoots, and entire plant weight were measured using a weighing balance. Roots and shoots were separated by cutting off the stem at the ground level. Roots were cleaned off sand and other debris and left to air dry. After that, samples were dried for 72 hours at 70°C in a hot air oven to obtain root and shoot dry weights. After that, the total dry weight and the shoot: root ratio were calculated as follows:

$$\text{Shoot: Root} = \frac{Shoot\ dry\ weight(g)}{Root\ dry\ weight(g)}$$

Estimation of bacterial population in the soil rhizosphere

Ten grams of soil from the rhizosphere was added into a 250 mL Erlenmeyer flask containing 90 mL sterilized water. The flask was shaken on an orbital shaker for 10 minutes for ten minutes. One mL of the suspension was transferred into a test tube containing 9 mL of sterilized distilled water. A series of ten-fold dilutions of the soil suspension were made up to 10^9. Aliquots of 0.1 mL from prepared dilution were spread on respective TSA plates from the highest dilutions, and the plates underwent incubation for 24 hours at 28 ± 2°C, after which the number of colonies was determined as follows:

$$(\text{CFU g}^{-1}\text{ soil fresh weight}) = \frac{(Mean\ plate\ count)(Dilution\ factor)}{fresh\ weight\ of\ soil}$$

Physiological parameters

Chlorophyll content (SPAD Value)

Leaf greenness was measured using a portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan). Four measurements were made on each leaf per plant, two on either side of the midrib on all fully grown leaves (Khan *et al*., 2003). Chemical analyses were conducted using the same leaves (Alcantar *et al*., 2002). A portable photosynthesis system, model Li-6400 (Li-Cor Inc., Lincoln, Nebraska, USA), was used to measure the net photosynthetic rate (Pn) and transpiration rate (Tr) on a clear day at 8WAT. Reading was conducted before mid-day on the newest fully grown leaf, as stated by Huang *et al*. (2011).

N content of plant tissues

For nutrient analysis, samples of the roots and shoots (leaves and stems) were dried in an oven for 72 hours at 70°C. Dried samples were grounded, and 0.25 g weighed into a 100 mL digestion tube. Then, 5 mL of H_2SO_4 was added and allowed to stand for 2 hours. After that, 10 mL of 30% H_2O_2 was added, and the tubes were moved to the digesting block. The samples were heated at 450°C until the solution became clear. After cooling, the solution was filtered and sent to an autoanalyzer for N determination (LECO, 2008).

Nitrogen use efficiency (NUE %)

The whole tomato plants were harvested at 8WAT. The harvested plants were dried at 65°C for 72 hours and separated into shoots and roots. Total N content in plant parts was determined using the Kjeldahl method (Bremner, 1996). NUE was calculated using the formula below (IAEA, 2001):

$$NUE\ (\%) = \frac{Total\ N\ uptake\ -Total\ N\ uptake\ by\ control\ plant}{Amount\ of\ urea\ applied} \times 100$$

Statistical analysis

The data was statistically examined using ANOVA using Statistical analysis software (SAS) 9.4 (SAS, 2013) at a 5% level of confidence, and the treatment means were compared using Duncan's Multiple Range Test (DMRT). The principal component analysis (PCA) figure was plotted using JMP Version 14 (SAS Institute Inc., Cary, NC, USA, 2021).

RESULTS

Growth parameters

The application of bacteria showed better performance in the growing and developing tomatoes than fertilizer treatment, and UPMRB9N50 treatment shows significant superiority among other treatments at 8 WAT (**Table 2**). The plant responded according to the amount of N added

to the soil, but bacteria with 50% N application performed better than 100% N fertilizer application. At 8WAT, the highest plant height was recorded from UPMRB9N50, followed by UPMB10N50 and B0N100, while the control had the lowest. Relative to the control, UPMRB9N50 and UPMB10N50 treatments had 74.88% and 73.87% increase in plant height at 8WAT, respectively (**Table 2**). At 8WAT, the highest number of branches was observed in the UPMRB9N50 treatment (25), followed by UPMB10N50 (22.33) and B0N100 (19).

Table 2. Plant height and number of branches of tomato as affected by the treatments at 8WAT

Treatment	Plant height (cm)	Number of branches
B0N0	47.83± 2.35 d	10.33±0.51 d
B0N50	53.33 ± 3.01 c	14.67±0.63 c
B0N100	62.67 ± 3.61 b	19.00±0.54 b
UPMB10N0	47.83 ± 2.60 d	11.00±0.12 d
UPMB10N50	67.63 ± 3.32 ab	22.33±0.17 ab
UPMB10N100	56.00±2.56 c	16.33±0.33 bc
UPMRB9N0	48.00±3.05 d	12.23±0.60 d
UPMRB9N50	70.33±2.78 a	25.00±0.75 a
UPMRB9N100	57.00±2.13 c	17.67±0.13 b

Note: A column sharing the same lower-case letter (s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Table 3 shows the effect of PGPB application with different N fertilizer levels on the total leaf area and plant dry weight. The application of PGPB and N fertilizer had a significant effect (P≤ 0.05) on the total leaf area of tomato plants under the prevailing conditions. Maximum leaf area was observed in the application of UPMRB9N50 (3256.67 cm2), followed by UPMB10N50 treatment, which was 66.02% and 9.42% higher than that produced by B0N0 and B0N100 treated plants, respectively. In addition, the result revealed a significant (P ≤0.05) difference in shoot, root, and total dry weight among the treatments. At the same time, there was no significant difference in the shoot/root ratio between control and treated plants. Additionally, a greater dry weight and shoot/root ratio were found in bacteria-treated plants with 50% N fertilizer.

Rhizosphere soil microbial population

In general, PGPB inoculations increased the bacterial population around the tomato root zones and surrounding soils compared to uninoculated soils (control). The results showed that a significantly (P≤ 0.05) higher bacterial population was observed in inoculated soil with 50% N compared with the other treatments (**Figure 1**). The lowest bacterial populations were recorded in the control soil (0% N, 0% N with inoculation).

Table 3. The total leaf area, dry weight and shoot: root ratio of tomato as affected by the treatments at 8WAT

Treatment	Total leaves area (cm^2)	Dry weight (g)			Shoot: Root
		Shoot	Root	Total	
B0N0	1106.67±34.880 h	14.3333± 0.667 g	2.3667±0.318 b	16.7±0.850 e	6.23±0.722 ab
B0N50	1453.33±14.530 f	19.6667± 0.333 e	2.8333±0.441 b	22.5±0.289 c	7.3667±1.364 a
B0N100	2950.00±17.321 c	23.3333± 0.333 c	4.16670±0.167 a	27.5±0.289 b	5.62±0.265 ab
UPMB10N0	1143.33±23.333 gh	15.3333±0.667 g	2.5333±0.338 b	17.8667±0.845 e	6.2367±0.760 ab
UPMB10N50	3166.67±12.019 b	26.3333±0.333 b	4.7667±0.145 a	31.1±0.379 a	5.5367±0.174 ab
UPMB10N100	1870.00±11.547 e	21.3333±0.333 d	4.5000±0.289 a	25.8333±0.441 b	4.78±0.308 b
UPMRB9N0	1170.00±11.547 g	17.3333±0.333 f	2.6333±0.296 b	19.9667±0.623 d	6.72±0.608 ab
UPMRB9N50	3256.67±14.530 a	27.6667±0.333 a	4.8667±0.067 a	32.5333±0.371 a	5.6867±0.072 ab
UPMRB9N100	2050.00±17.321 d	21.6667±0.333 d	4.6667±0.167 a	26.3333±0.441 b	4.6533±0.142 b

Note: A column sharing the same lower-case letter (s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

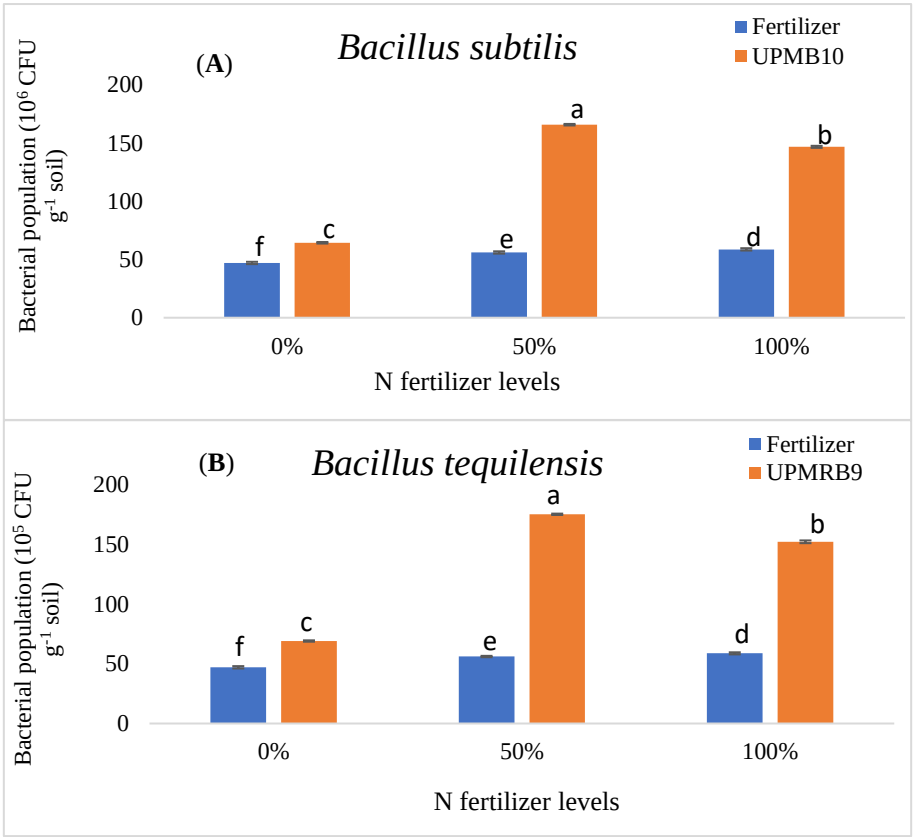

Figure 1. The total bacterial population in the root zone of tomato at 8WAT (**A**) *Bacillus subtilis* treated soil (**B**) *Bacillus tequilensis* treated soil.
Note: The standard error of the means is shown by the bars. A bar sharing the same lower-case letter (s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Physiological parameters

The mean chlorophyll content (SPAD value), photosynthetic rate (Pn), and transpiration rate (Tr) of the tomato plant at harvest (8 WAT) are shown in **Table 4**. Bacteria application enhanced the physiological parameters, particularly when supplemented with 50% N fertilizer. Maximum SPAD value was found in UPMRB9N50 (62.3) treatment, followed by UPMB10N50 (59.33) treatment. The UPMRB9N50 treated plants had 31.78% and 10.87%

higher SPAD values than B0N0 and B0N100 treated plants. Similarly, regarding the Pn, UPMRB9N50 had a significant ($P \leq 0.05$) higher value of 19.20 µmol CO2 $m^{-2}s^{-1}$, which differs significantly from the other treatments. UPMRB9N50 treated plants had 33.07 1.61% higher photosynthesis rates than the control and B0N100 treated plants. The significantly higher transpiration rate was also found in UPMRB9N50 (1.94 mmol H_2O $m^{-2}s^{-1}$), UPMB10N50 (1.91 mmol H_2O $m^{-2}s^{-1}$), and B0N100 (1.90 mmol H_2O $m^{-2}s^{-1}$) which differ significantly ($P\leq 0.05$) from the other treatments (Table 4). All control (inoculated or uninoculated) treatments were not significantly different ($P> 0.05$) from each other. The UPMRB9N50 treatment had a 25.26% higher transpiration rate than the control and 2.06% higher than the B0N100 treated plant.

Table 4. The SPAD value, photosynthetic rate and transpiration rate of tomato at 8WAT

Treatment	SPAD value	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-2} s^{-1})
B0N0	42.50± 2.35 i	12.85±0.22 i	1.45±0.01 d
B0N50	49.63 ± 0.21 f	15.72±0.14 f	1.62±0.03 c
B0N100	55.53 ± 1.01 c	18.88±0.2 c	1.90±0.04 a
UPMB10N0	43.66 ± 2.60 h	12.90±0.11 h	1.45±0.02 d
UPMB10N50	59.33 ± 1.31 b	19.10±0.09 b	1.91±0.01 a
UPMB10N100	51.63±0.61 e	17.77±0.12 e	1.67±0.02 b
UPMRB9N0	44.26±0.22 g	12.79±0.01 g	1.48±0.01 d
UPMRB9N50	62.30±0.99 a	19.20±0.03 a	1.94±0.03 a
UPMRB9N100	52.66±0.82 d	17.88±0.21 d	1.67±0.06 b

Note: A column sharing the same lower-case letter (s) indicates no significant difference at $P \leq 0.05$ based on Duncan's multiple range test means separation techniques

N content in plant tissue

The result of shoot, root, and total N content and nitrogen use efficiency (NUE) of the tomato is presented in **Table 5**. The shoot N content in UPMRB9N50 and UPMB10N50 treatments were not significantly different ($P> 0.05$), but they differed significantly ($P\leq 0.05$) from the other treatments. The lowest shoot N content was recorded in the control with 11.9333 mg N/g shoot DW, which did not differ significantly from UPMRB9N0 (11.9333 mg N/g shoot DW) and UPMB10N0 (11.8333 mg N/g shoot DW) treatments.

However, the root N content was about 50% of the shoot N content, with the highest in UPMRB9N50 and UPMB10N50 treatments, which did not differ significantly from one another ($P> 0.05$). The significantly lowest root N contents were recorded in B0N0 and UPMRB9N0 treatments. The NUE was found to differ significantly ($P\leq 0.05$) among the treatments, and the highest NUE was recorded in UPMRB9N50 (88.71%), followed by UPMB10N50 (81.94%) treatment. The NUE in UPMRB9N50 was 67.34 and 66.50% higher compared to B0N50 and B0N100, respectively. There was no significant difference ($P> 0.05$) between the B0N50 and UPMRB9N100 treatments regarding the NUE.

Table 5. The N content and NUE of tomato plant as affected by treatments at 8WAT

Treatment	N content in shoot (mg N/g shoot DW)	N content in root (mg N/g root DW)	Total N content (mg N/g plant DW)	Total N content (g/plant)	NUE (%)
B0N0	11.9333±0.067 e	5.8333 ± 0.033 f	17.7667 ± 0.088 e	0.29657 ± 0.014 g	-
B0N50	16.4±0.306 d	6.8667 ± 0.186 d	23.2667 ± 0.0.145 d	0.52343 ± 0.003 e	28.974 ± 1.446 cd
B0N100	19.6667±0.333 b	8.0333 ± 0.088 b	27.7 ± 0.252 b	0.76163 ± 0.003 c	29.717 ± 1.033 c
UPMB10N0	11.8333±0.441 e	6.4333 ± 0.233 e	18.2667 ± 0.674 e	0.32583 ± 0.15 fg	-
UPMB10N50	21.3333±0.333 a	8.8333 ± 0.088 a	30.1667 ± 0.418 a	0.93817 ± 0.017 b	81.941 ± 0.384 b
UPMB10N100	18.1667±0.167 c	7.6333 ± 0.088 c	25.8 ± 0.2517 c	0.66653 ± 0.013 d	23.64 ± 1.260 d
UPMRB9N0	11.9333±0.067 e	5.8333 ± 0.033 f	17.7667 ± 0.088 e	0.35477 ± 0.011 f	-
UPMRB9N50	21.5667±0.233 a	8.90 ± 0.058 a	30.4667 ± 0.176 a	0.99117 ± 0.013 a	88.71 ± 3.318 a
UPMRB9N100	18.7333±0.145 c	7.7667 ± 0.133 bc	26.5 ± 0.058 c	0.6978 ± 0.011 d	25.638 ± 0.992 cd

Note: A column sharing the same lower-case letter(s) indicates no significant difference at P ≤0.05 based on Duncan's multiple range test means separation techniques

Principal Component Analysis (PCA)

To discover the existing relationships among some measured plant growth and physiological parameters of the tomato plant, a principal component analysis (PCA) was plotted (Figure 2). Each loading point represents the contribution of a variable (plant growth: branch number, total leaf area, total dry weight, shoot root ratio, plant physiology: chlorophyll content-SPAD value, photosynthetic rate, transpiration rate, and NUE) to the score. The first principal component (PC1) explains 85.90% of the experiment's variation, whereas the second principal component (PC2) explains 9.43%, so the first two principal components explain 95.33% of the total variation in the experiment. The result shows that UPMRB9N50 and UPMB10N50 explained the increase in SPAD value, photosynthesis rate, transpiration rate, branch number, total leaf area, plant dry weight, and plant nitrogen in the experiment. Furthermore, all the parameters were positively related to one another except S: R, which is negatively correlated with most parameters. Based on the PCA figure, the treatments were grouped into two- group 1 had BON100, UPMB10N50, UPMB10N100, UPMRB9N50, and UPMRB9N100, while group 2 had B0N0, BON50, UPMB10N0, and UPMRB9N0 as members. Group 1 explained the increase in SPAD value, transpiration rate, total leaf area, NUE, branch number, and total dry weight, particularly the UPMRB9N50 and UPMB10N50 treatments. However, the shoot: root ratio (S: R) was explained by the BON50 treatment.

DISCUSSION

The findings demonstrated that applying PGPB under different N levels on tomato (303 variety) enhanced the plant growth parameters, plant physiological parameters, N content in plant tissues, and potential use of N. Application of PGPB is a substitute sustainable farming method to increase yield, fruit quality, and soil fertility while preserving biodiversity (Tsegaye *et al*., 2021). These "plant growth-promoting microbes" (PGPM) promote plant growth through a variety of direct and indirect PGP mechanisms, including biological nitrogen fixation, the creation of numerous plant growth hormones, siderophores, HCN, various hydrolytic enzymes, and the solubilization of potassium, zinc, and phosphorus (Kour *et al*., 2020). Their co-inoculation with fertilizer containing 50% N enhanced the rhizosphere soil's fertility and made it easier for plants to absorb nutrients from the soil (Tsegaye *et al*., 2021). Our research consistently uses similar patterns as co-inoculating PGPB with chemical fertilizer to enhance soil fertility and boost plant growth and biomass.

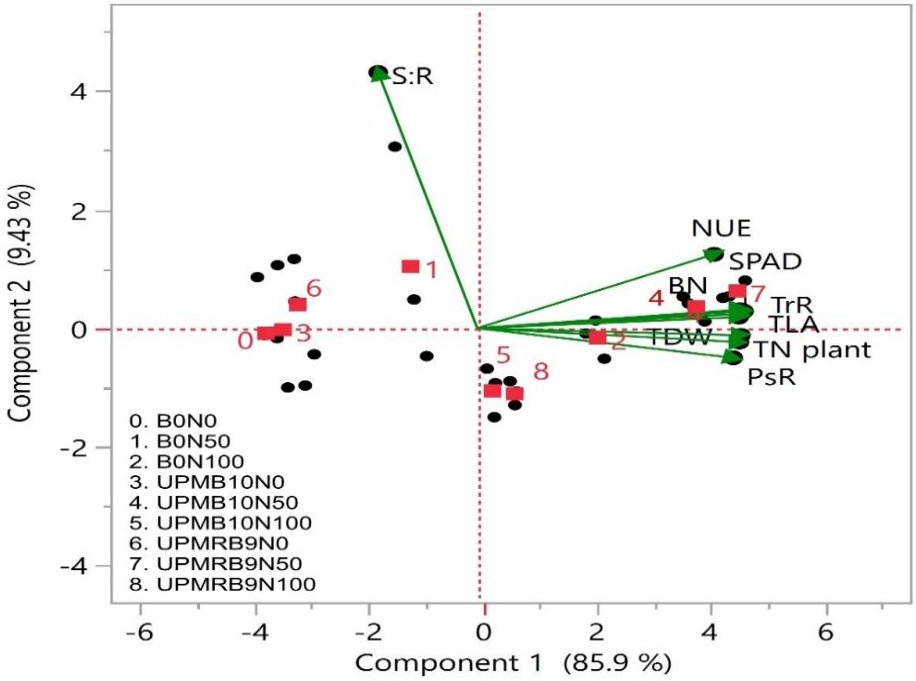

Figure 2. Principal component analysis biplot of the effects of treatments on tomato plants. PsR-photosynthetic rate, TrR-transpiration rate, BN-branch number, TN-plant total nitrogen, TLA-total leaf area, TDW-total plant dry weight, NUE-nitrogen use efficiency, S: R-shoot root ratio

The number of tomato plant branches at 8WAT followed the same increasing trends as plant height (**Table 2**). Our findings supported earlier research that indicated the most significant plants in height were seen when 70% of the appropriate N rate was applied to the soil (Marini *et al*., 2015). The amount of nutrients in the soil, soil temperature, the length of the day, and other environmental conditions all impact plant height in addition to those mentioned above. Under the study's glasshouse circumstances, the soil's moisture content and temperature were favorable for plant growth; therefore, regardless of N levels, all plants grew to their typical height. On the other hand, using PGPB inoculants with 50% of the prescribed amount of chemical fertilizer could help to reduce the adverse effects of chemical fertilizers on the health of the soil. This study applied PGPB with different N levels; thus, it is an environmentally friendly technology that can minimize soil pollution and maximize crop returns. *Bacillus subtilis* (UPMB10) and *Bacillus tequilensis* (UPMRB9) have been researched the most and have a variety of plant growth-promoting qualities, including the ability to solubilize inorganic phosphate, nitrogen-fixing biologically, solubilize potassium, produce IAA, and produce iron siderophore (Shultana *et al*., 2020). Present study findings revealed that most tomato plant development, fruit production, production-related variables, and N content were improved by applying PGPB alone or combined with N levels.

The application of PGPB significantly ($P \leq 0.05$) influenced plant leaf area under N levels (**Table 3**). Accordingly, several studies revealed that applying PGPB along with N fertilizer boosted crops such as corn (Lin *et al*., 2019) and tomato (Garcia *et al*. (2004) leaf area. The highest root dry weight observed in UPMB9N50 treated plants agrees with Tsegaye*et al*. (2021), who found higher dry weight in bacteria-inoculated plants. This could result from increased phosphate solubilization, increased atmospheric N_2 fixation by applying PGPB, or a combination of PGPB strains and chemical fertilizers. Masood *et al*. (2020) conducted two pot experiments in a related study to examine the impact of *Bacillus pumilus*on tomato plant growth when paired with N fertilizer. As a result of the inoculation, the shoot dry weight and root dry weight of this PGPB significantly increased, mainly when additional N fertilization was used, according to their findings. This study revealed that optimum N fertilizer (50% N)

with bacteria inoculation increased microbial population in soil (**Figure 1**). To avoid direct competition for scarce resources and available space, free-living soil bacteria could shift chemotactically and colonize new locations, according to Compant *et al*. (2010). This has resulted in the dynamic of the PGPB population in the rhizosphere.

The result of the SPAD measurement showed that N supply enhanced the Chlorophyll content of tomatoes with or without inoculation (**Table 4**). Throughout the growth stages, SPAD readings rose with rising N rates, and more excellent chlorophyll content was seen when high N fertilizer rates were used (Lin *et al*., 2019). A similar result was found by Masood *et al*. (2020), who found an 8% increase in chlorophyll content in *Bacillus pumilus* inoculated plants. The improvement in chlorophyll content in UPMRB9N50 might be related to some protection provided by TPC, which is also supported by the PCA (**Figure 2**). Our findings agree with Akram *et al*. (2019), who reported that *the Bacillus* megaterium strain increased the levels of Chlorophyll in tomato plants. This study's photosynthetic rate (Pn) decreased with increasing N levels. The lowest Pn was presented in the B0N0 treatment (**Table 4**). The increase in photosynthesis increases the accumulation of aboveground biomass, which influences tomato yield (Du *et al*., 2017). The better growth of plants depends on the rate of photosynthesis, as the assimilation of CO_2 accounts for around 90% of plant biomass (Long *et al*., 2006). According to Mia and Shamsuddin (2010), rice plants' overall photosynthetic rate significantly increased when applied with specific Rhizobia strains. In our present findings, the photosynthetic rate increased with an increase in fertilizer, while a higher plant transpiration rate was obtained in bacteria supplemented with 50% N fertilizer levels. Masood *et al*. (2019) found that non-inoculated treatments significantly increased leaf transpiration and photosynthetic rates in rapeseed (*Brassica napus L.*) compared to the control treatment. The PCA analysis of our result (**Figure 2**) indicated that UPMRB9N50 and UPMB10N50 treatments had correlated strongly with photosynthesis rate. Our findings agree with Costa-Santos *et al*. (2021), who found that inoculation of *Bacillus zhangzhouensis* positively enhances photosynthesis, thus supporting a positive correlation by PCA analysis.

Nitrogen content in the plant is directly related to biomass production and N concentration. In this study, concentrations of N in plant tissues varied substantially between N treatments. Whether PGPB inoculants were applied or not, N concentrations increased with the increase in N rate (**Table 5**). Compared to the unfertilized control, plants that received 50% and 100% of the recommended N rate had significantly higher shoot and root N concentrations. Other researchers also found the same findings from their experiment in corn (Lin *et al*., 2019). Urea fertilizer improves NUE by decreasing N losses, which are dispersed throughout the rhizosphere area, and reduces the risk of N loss, increasing crop availability and uptake potential (Yao *et al*., 2018). In UPMRB9N50 treatment, NUE was highest (88.71%), which led to the highest plant yield of 12.0467 t/ha. This result indicated that PGPB inoculation improves NUE in tomato plant production under optimum N fertilization. The plant N content and N uptake depend on the demand or availability of N within the soil. The N demand in tomatoes depends on their growing stages, and N availability depends on their soil adsorption and loss minimization processes. The fast mineralization of N reduces N losses and rapidly supplies N to plants, increasing NUE and reducing the risk of environmental contamination.

CONCLUSION

A glasshouse study has shown that selected PGPB strains inoculated with 50% of the optimum N level boosted tomato growth and plant physiology and produced tomato biomass, increased bacterial population, and N content of tissues comparable to or superior to the 100% N fertilization level. The extensive N fertilization could have obscured the impact of the PGPB inoculations. Because of this, PGPB applications should be viewed as instruments that will supplement N usage efficiency methods by improving N absorption efficiency, hence

minimizing N losses and lowering the quantity of applied N. However, more research is required to identify the approach of nitrogen fertilization reduction that may be attained when applying PGPB to various crops and with various nitrogen fertilizers, as well as to evaluate the best field management techniques for enhancing the efficacy of PGPB in actual field circumstances.

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