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Effect Of Bacillus Subtilispriming On Growth And Pigment Composition Of Tomato Seedlings (Lycopersicum Esculantum Cv. Pusa Ruby) Under Different Levels Of Polyethylene Glycolstress Conditions

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Abstract

Objective: to explore the quantitative changes in photosynthetic pigments of *Lycopersicum esculantum* cv. Pusa Rubyseedlings to inoculation with *Bacillus subtilis*(ATCC No.: 11774) under different levels of polyethylene glycol 6000 (PEG 6000) stress using sustainable techniques such as priming with PGPB strain *Bacillus subtilis*.
Methods: This study was performed at laboratorycondition with *Solanum lycopersicum* L. cv. Pusa ruby seeds as factorial experimentunder Randomized Complete Design (CRD) with fourreplications. Effect ofdrought stress induced by different per cent level ofPEG 6000 treatments on drought tolerance in *Bacillus subtilis* primed tomato seedlings was studied. In this experiment, twenty*Bacillus subtilis* primed tomato (*Solanum lycopersicum* L. cv. Pusa ruby) seeds were placed in each per cent of PEG mediated drought stress treatment. One set without *Bacillus subtilis* primed tomato seeds were also treated with different level of PEG 6000 (1, 5, 10, 15, 20, 25, and 30%) mediated drought stress to observe the effect of *Bacillus subtilis* priming.
Results: Radicle protrusion (%), opening of cotyledonary leaves (%) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress at 0-25% and 0-15% respectively. Furthermore, the growth response parameters of *viz.* fresh weight (g) and dry weight (g) tomato were increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%). Chl *a* andChl *b* content was higher in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%). Furthermore, the carotenoid (μg g^{-1}) quantity was increased in tomato seeds primed with culture of *Bacillus*

subtilis as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-5%). Whereas, the quantity of anthocyanin (μg g^{-1}) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-15%).
Conclusion:*Bacillus subtilis* (ATCC No.: 11774) could be successfully used to enhance fruit production and fruit quality of tomato plants grown under controlled conditions.

Keywords:Tomato, Seed priming, Bacillus subtilis, Growth enhancement, Pigment composition

INTRODUCTION

A complex weather anomaly known as drought poses a major threat to the environment and to agriculture, forcing large swathes of arable land out of production [1]. Within the next forty years, it is predicted that the world's population will double, with the majority of that growth occurring in developing nations where hunger is already a reality [2]. One of the most common natural disasters in the world is a drought. By 2050, drought is predicted to seriously impair plant growth on over 50% of arable land [1]. Plant-water relationships are impacted by drought stress at the cellular and whole plant levels, which can result in both specific and nonspecific phenotypes as well as physiological reactions [3].

It has been well documented that several plant species, including barley [4], maize [5], rice [6], and wheat [7], experience reduced growth when under drought stress. Because they are sessile, plants must develop a variety of complex physiological, cellular, and molecular mechanisms to maintain homeostasis in order to adapt to and withstand any harsh environmental conditions [8,9]. Because of the imbalance in electron transport rates and the metabolic consumer activity of reductive power, drought stress is known to cause oxidative stress by raising the levels of reactive oxygen species [3]. Plants have evolved sophisticated antioxidant defense mechanisms, both enzymatic and non-enzymatic, to withstand oxidative stress [10].
Crop yield in the short term should not come at the expense of soil fertility. Maintaining soil fertility and organic matter levels depends in large part on recycling renewable resources. In addition, the crop that has been harvested needs to replenish the nutrients that have been taken out of the system. When there are organic nutrient sources nearby, new systems need to be created and developed to take advantage of them. In this situation, rehabilitating nutrient-depleted soils for food production may be aided by the microbiota. In order to make the soil ecosystem dynamic for nutrient turnover and sustainable for crop production, they are involved in a variety of biotic activities within it [11,12].Moreover, currently, the biologicalapproaches for improving crop production are gainingstrong status among agronomists and environmentalists followingintegrated plant nutrient management system.
Several strategies have been suggested for controlling the negative effects of drought stress in plants and breeding for tolerant varieties and genetic engineering are the most explored approaches [13]. However, the complexity of abiotic stress tolerance mechanisms makes the task of introducing new tolerant varieties very difficult and genetically modified plants are not accepted well in some regions [14]. An alternative strategy is to induce stress tolerance by using various chemical and biological agents in a process known as priming [15]. Plant growth promoting bacteria (PGPB) are bacteria that colonize the rhizosphere and enhance the growth of plants by direct and indirect mechanisms [16,17]. Several PGPB strains are also known toinduce abiotic stress tolerance in some plants such as salt and drought stress in wheat [18,19].
With these viewpoints, the present research investigation was conducted with the main aim to explore the quantitative changes in photosynthetic pigments of tomato (*Lycopersicum esculantum* cv. Pusa Ruby)seedlings to inoculation with *Bacillus subtilis* under different levels of polyethylene glycol 6000 (PEG 6000) stress using sustainable techniques such as priming with PGPB strain *Bacillus subtilis*.

MATERIALS AND METHODS

Plant material and treatment

Tomato seeds (*Solanum lycopersicum* L. cv. Pusa ruby) were procured from National Bureau of Plant Genetic Resources (NBPGR), New Delhi and then seeds were multiplied and suitability trialsin different agro-climatic seasons at Defence Institute of Bio-Energy Research (DIBER) field station Pithoragarh, Uttarakhand.

Microbial culture *Bacillus subtilis* (ATCC No.: 11774) was procured from American Type Culture Collection (ATCC).

***Bacillus subtilis*priming**

Freshly stored *Bacillus subtilis*(ATCC No.: 11774) cultures were allowed to grow in nutrient agar media(Himedia) for overnight at 28°C, after which culture density was determined using the colony-forming unit (CFU) method. Priming was performed by soaking the tomato seeds in *Bacillus subtilis* solutions containing 10^7 bacteria ml^{-1} for overnight at 28°C with shaking at 150 rpm. Another set of tomato seeds was soaked in water to be used as a control. Twenty primed or non-primed tomato seeds were sown in disposable Petri plates and left to grow in controlled aseptic environment growth chambers (*LT-105* (*Percival Scientific Inc*., *Perry*, Iowa, USA)equipped with 22/16°C (day/night), 16/8-h photoperiods at 450 μmol m^{-2} s^{-1} and 70 ±2%. humidity. Plants were watered every other day for 1 month.

PEG treatment experimental design

This study was performed at laboratorycondition with *Solanum lycopersicum* L. cv. Pusa ruby seeds as factorial experimentunder Randomized Complete Design (CRD) with fourreplications. Effect ofdrought stress induced by different per cent level ofPEG 6000 treatments on drought tolerance in *Bacillus subtilis* primed tomato seedlings was studied. In this experiment, twenty*Bacillus subtilis* primed tomato (*Solanum lycopersicum* L. cv. Pusa ruby) seeds were placed in each per cent of PEG mediated drought stress treatment. One set without *Bacillus subtilis* primed tomato seeds were also treated with different level of PEG 6000 (1, 5, 10, 15, 20, 25, and 30%) mediated drought stress to observe the effect of *Bacillus subtilis* priming.

The tomato seeds were germinated inpetri dishes on two layers of filter paper in an aseptic environment growth chamber (*LT-105* (*Percival Scientific Inc*., *Perry*, Iowa, USA)equipped with 22/16°C (day/night), 16/8-h photoperiods at 450 μmol m^{-2} s^{-1} and 70 ±2%. humidity. Tomato seedlings were moistened with different per cent level solution of PEG 6000. One set of without *Bacillus subtilis* primed tomato seeds were moistened with deionized water maintained as control every other day for 1 month. After 3 days of seed sowing, the protrusion of the radicle was observed in control and other PEG 6000 treatments. Percentage of germination wasmeasured by ISTA (International Seed TestingAssociation) standard method. At end of the one month, the percentage of germination, germinationrate, the length of root and shoot of seedlings and dry matter weight of root and shoot were alsomeasured.

Estimation of chlorophyll (Chl) and carotenoids content

The Chl (*a*, *b* and total *a*+*b*) and total carotenoids contents were determined from fresh leaf samples. Leaf discs (100 mg) were placed in a test tube containing 10 mL of dimethylformamide (DMF) and stored for 24 h at 4 °C. The absorbance of the supernatant was read at 480, 647 and 666 nm in a monochromator base multimode detector (BioTek, Snergy 2, USA) with DMF as a blank. The contents of Chl *a*and*Chl b* were calculated according to Moran and Porath (1980) [20].

Anthocyanin content assay

Anthocyanin content was determined according to Mancinelli et al.(1975) [21]. Fresh leaf samples (100 mg) were washed with deionized water and cut into pieces (10 mm). Leaf pieces were transferred in to a sterile test tube containing 10 mL of methanol:water:concentrated HCl (80:20:1, v/v) and placed on a shaker in dark at 4°C. After 48 h, the sample extract was filtered through Whatman No. 1 paper and the absorbance was measured at 530 and 657 nm. Anthocyanin content was determined by using the following formula;

$$Anthocyanin\ content = A \times MW \times 10^4 / \varepsilon \times L$$

Where,

$A = A_{530}$ nm $- 0.3 \times A_{657}$ nm

MW (Molecular Weight) = 449.2 g/mol for cyanidin-3-glucoside

ε = 26900 mol L^{-1} cm^{-1}; L (path length) = 1 cm

10^4 is the factor for converting to μg g^{-1} FW

STATISTICAL ANALYSIS

Data based on replicates (at least six times) were subjected to an analysis of variance (ANOVA) test to determine the significance between the different treatments using CropStat for Windows (7.2.2007.2 module),

developed by the Biometrics unit, IRRI, Philippines. The treatment means were compared by Least Significant Difference (LSD) test at a significance level of p≤0.05.

RESULTS

The effect of *Bacillus subtilis* (ATCC No.: 11774) priming on seed germination in terms of radicle protrusion and opening of cotyledonary leaves and growth responses of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG-6000 mediated drought stress was represented in Table 1. Results depicted that radicle protrusion (%) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-25%). Similarly, percentage of opening of cotyledonary leaves was also increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-15%).

Furthermore, the growth response parameters of *viz.* fresh weight (g) and dry weight (g) tomato (*Solanum lycopersicum* L. cv. Pusa ruby) were increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%).

Table 1: Effect of *Bacillus subtilis*(ATCC No.: 11774) priming on seed germination in terms of radicle protrusion and opening of cotyledonary leaves and growth responses of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG-6000 mediated drought stress.

PEG Treatment (%)	Radicle Protrusion (%)		Opening of Cotyledonary		Fresh Weight (FW) (g)		Dry Weight (DW) (g)	
	P	NP	P	NP	P	NP	P	NP
0	81.25^{a}	77.38^{a}	73.88^{a}	60.75^{a}	8.28^{f}	6.02^{a}	0.79^{f}	0.54^{a}
1	79.50^{a}	65.88^{b}	57.63^{b}	30.13^{b}	10.94^{e}	3.93^{b}	1.06^{e}	0.35^{b}
5	60.00^{b}	55.88^{c}	50.38^{c}	0	13.76^{d}	2.2^{c}	1.36^{d}	0.20^{c}
10	43.75^{c}	40.13^{d}	39.13^{d}	0	16.69^{c}	0	1.61^{c}	0
15	21.87^{d}	9.63^{e}	1.75^{e}	0	19.52^{b}	0	1.88^{b}	0
20	4.63^{e}	4.25^{f}	0	0	23.44^{a}	0	2.31^{a}	0
25	2.00^{f}	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
LSD	5.02	5.21	4.31	4.63	1.10	0.63	0.20	0.07
SE	1.71	1.77	1.47	1.57	0.36	0.21	0.07	0.02

Different letters in each column indicate significant differences at p≤0.05, as per LSD test.
Standard error (SE) of each parameter are given in the last row.
P, Tomato seeds primed with culture of *Bacillus subtilis*; NP, Not-primed tomato seeds

The effect of *Bacillus subtilis* (ATCC No.: 11774) priming on alteration of photosynthetic pigments in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG-6000 mediated drought stress was represented in Table 2. Results delineated that Chl *a* andChl *b* content was higher in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%) (Figure 1 and Figure 2).

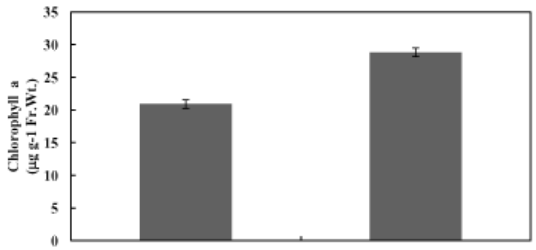

Fig. 1: Effect of *Bacillus subtilis* (ATCC No.: 11774) priming on Chl *a* content under PEG-6000 mediated drought stress

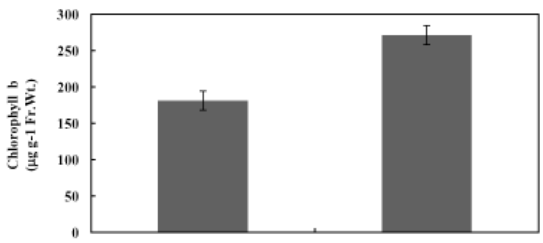

Fig. 2: Effect of *Bacillus subtilis* (ATCC No.: 11774) priming on Chl *b* content under PEG-6000 mediated drought stress

Table 2: Effect of *Bacillus subtilis*(ATCC No.: 11774) priming on alteration of photosynthetic pigments in tomato (*Solanum lycopersicum* L. cv. Pusa ruby) under PEG-6000 mediated drought stress.

PEG Treatment (%)	Chl *a* (µg g^{-1} FW)		Chl *b* (µg g^{-1} FW)		Carotenoid (µg g^{-1} FW)		Anthocyanin (µg g^{-1} FW)	
	P	NP	P	NP	P	NP	P	NP
0	25.83^{e}	20.92^{a}	271.23^{a}	181.36^{a}	0.57^{d}	0.54^{a}	1.80^{b}	1.21^{b}
1	31.03^{d}	16.04^{b}	251.01^{b}	146.55^{b}	0.63^{c}	0.30^{b}	2.24^{a}	1.34^{a}
5	42.34^{c}	4.84^{c}	233.73^{c}	134.41^{b}	0.65^{c}	0.29^{b}	2.30^{a}	0
10	49.96^{b}	0	222.05^{c}	0	0.91^{b}	0	1.60^{c}	0
15	77.78^{a}	0	201.10^{d}	0	1.04^{a}	0	0.70^{d}	0
20	75.18^{a}	0	120.60^{e}	0	1.01^{a}	0	0	0
25	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0
LSD	6.80	0.56	13.07	17.32	0.06	0.05	0.13	0.11
SE	2.31	0.19	4.44	5.90	0.02	0.02	0.04	0.03

Different letters in each column indicate significant differences at p≤0.05, as per LSD test.

Standard error (SE) of each parameter are given in the last row.
P, Tomato seeds primed with culture of *Bacillus subtilis*; NP, Not-primed tomato seeds
Furthermore, the carotenoid (µg g^{-1}) quantity was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-5%) (Figure 3). Whereas, the quantity of anthocyanin (µg g^{-1}) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-15%) (Figure 4).

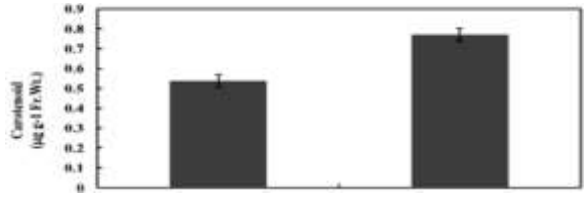

Fig. 3: Effect of *Bacillus subtilis* (ATCC No.: 11774) priming on Carotenoid content under PEG-6000 mediated drought stress

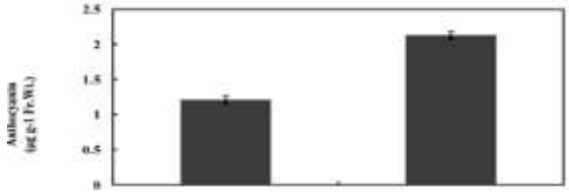

Fig. 4: Effect of *Bacillus subtilis* (ATCC No.: 11774) priming on Anthocyanin content under PEG-6000 mediated drought stress

DISCUSSION

Using the best agricultural technologies increases the productivity of producing vegetables. To boost crop productivity, a variety of growth regulators are employed, such as humic and bacterial preparations [22, 23]. Plant growth-promoting rhizobacteria (PGPR) are the active ingredients in bacterial preparations. They promote plant growth by a variety of pathways, including siderophore synthesis, plant hormone production, organic acid synthesis, and nitrogen fixation [24–28]. Furthermore, the use of PGPR is increasing in agriculture and may offer an attractive alternative to synthetic chemicals and fertilizers. Plant growth-promoting microorganisms are efficient microbial competitors that can promote plant growth by producing phytohormones and/or by increasing available nutrients through production of secondary metabolites or act as biocontrol agents to protect plants from infection by phytopathogens [29-33].With this scenario in the present study, we aimed to explore the quantitative changes in photosynthetic pigments of tomato (*Lycopersicum esculantum* cv. Pusa Ruby)seedlings to inoculation with *Bacillus subtilis* under different levels of polyethylene glycol 6000 (PEG 6000) stress using sustainable techniques such as priming with PGPB strain *Bacillus subtilis*.

Our study results revealed that radicle protrusion (%) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-25%). Similarly, percentage of opening of cotyledonary leaves was also increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-15%). Furthermore, the growth response parameters of *viz.* fresh weight (g) and dry weight (g) tomato were increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG

6000 mediated drought stress (0-20%). Chl *a* andChl *b* content was higher in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%). Moreover, the carotenoid (μg g^{-1}) quantity was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-5%). Whereas, the quantity of anthocyanin (μg g^{-1}) was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-15%). The results of our study findings are comparable with literature findings reported by various other research investigators.

Characterization of the carotenoids, mainly β-carotene and lycopene during storage and various ripening stages, shows drastic developments in sustainable yield and quality parameters of tomato [34]. Regulation of carotenoid biosynthesis and high-accumulation lycopene during tomato fruit development is widely studied [35-37]. Lycopene possesses the highest antioxidant potential among the carotenoids and several other antioxidants found in fruits and vegetables [38]. In our study the carotenoid (μg g^{-1}) quantity was increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-5%). Thus, the addition of PGPR enhances lycopene content in tomato fruits and can potentially contribute to antioxidant levels of diets. This potent antioxidant activity of lycopene protects from a variety of reactive oxygen species and reactive nitrogen species, thus helping in preventing chronic diseases in humans [35,39].

In our study the growth response parameters of *viz.* fresh weight (g) and dry weight (g) of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) were increased in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%).These findings were in accordance with various others studies reported in the literature wherein authors have demonstrated the positive effect of inoculation of tomato plants with strains of *Bacillus*[40,41].

Lucy et al., stated that in agriculture, benefits due to the application of PGPR include increased germination rate, root growth, increased shoot and root weights, increased leaf area, higher chlorophyll content, greater nitrogen content, higher protein content, enhanced tolerance to drought, delayed leaf senescence, and improved crop yield [16]. In our study Chl *a* andChl *b* content was higher in tomato seeds primed with culture of *Bacillus subtilis* as compared to not-primed tomato seeds under PEG 6000 mediated drought stress (0-20%). These findings are in concurrence with the findings of Kachigan and Garcia et al [42,43].

Plants are the primary source of food, shelter, and various remedial approaches [44]. Tomato (*Solanum lycopersicum*L. cv. Pusa ruby) is regarded as the second most vegetable crop next to potato in the agricultural implications of human consumption. According to agricultural statistics, tomatoes along with sweet corn and snap beans constitute 93% of crop production and processing strategies. The positive benefits of tomato consumption have been rigorously proved against a variety of diseases like chronic degenerative diseases, owing to the escalated content of significant phytochemicals with potent health benefits like the carotenoids (β-carotene and lycopene), the glycoalkaloids (dehydrotomatine and α-tomatine), ascorbic acid, tocopherols, and many phenolic and flavonoid compounds [45,46].

CONCLUSIONS

The results of our study clearly demonstrated that *Bacillus subtilis* (ATCC No.: 11774) promotes the growth of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) mainly through enhancement of seed germination potential and growth response parameters *viz.* fresh weight and dry weights of tomato. Furthermore, *Bacillus subtilis* (ATCC No.: 11774) promotes the growth of tomato (*Solanum lycopersicum* L. cv. Pusa ruby) through augmentation of chlorophyll, carotenoids and anthocyanin contents. These findings could be accredited to the production of indole compounds of Indole Acetic Acid (IAA) type and solubilization of phosphate by the strain. However, other mechanisms could be inducing the beneficial effects obtained *in-vivo,* and hence further *in-vivo* studies are recommended. Our study findings delineated that, *Bacillus subtilis* (ATCC No.: 11774) could be successfully used to enhance fruit production and fruit quality of tomato plants grown under controlled conditions.

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AUTHORS CONTRIBUTION

Kamal Kant Patra was involved in the execution of all the laboratory work and data analysis; Keshamma E was associated provided the research guidance and manuscript editing. All authors have read and approved the final manuscript before its submission.

CONFLICTS OF INTERESTS

None to declare.

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