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*Research Paper*

# Alleviation of drought stress of tomato plant regenerated via tissue culture inoculated with *Bacillus megaterium* (ATCC® 14581™)

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## ABSTRACT

Drought is one of the major limiting factors affecting crop productivity worldwide. Middle East in general is affected by water scarcity that mainly affects crop production. Tomato is one of the major stable vegetables consumed daily by Palestinian. This crop is drought sensitive; therefore, searching for reliable methods that enhances drought tolerance is highly favorable by farmers. One of the promising methods is utilizing a natural root colonizing bacteria to alleviate drought stress. In this work, a study was conducted to explore the efficiency of bacteria *Bacillus megaterium* (ATCC® 14581™) inoculum in enhancing the growth and physiological parameters of tomato (*Solanumlycopersicum*, L.) Var. Azmir plant derived via plant tissue cultured and normal seed derived plant when subjected to two levels of drought regimes. Under both drought-stress and non-stressed conditions, this bacteria successfully enhanced growth and physiological parameters. No significant differences were seen in all tested parameters between tissue culture and seed-derived plants. In general, high inoculum concentration inversely affects growth and physiological parameters regardless of the source of plant (treatment 4). The averages of shoots fresh weight, dryweight and plant height increased by 46.4%, 21.7% and 24.4% respectively than non-inoculated plant, while chlorophyll a, photosynthetic rate, and stomata conductance and respiration rate also increased by 54 %, 49 %, 39 % and 64%, respectively than non-inoculate plant( treatment 2). Exposure of inoculated plant to 8-week drought-stress (75 and 50% of total water amount required) enhanced the averages of shoot fresh weight, average dryweight and plant height by 20 and 23%, 24% and 25%, 34%, 30% respectively. Chlorophyll a increased by 51.9% and 46%. The photosynthetic rate increased by 39 and 45 %, stomata conductance increased by 32 and 50% and transpiration rate increased by 37.8and 25 % respectively (treatment 2).

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**Key words:** Tomato, Drought stress, *Bacillus megaterium* (ATCC® 14581™), endophyte, BAP, IAA.

## INTRODUCTION

Plants are subjected to various abiotic and biotic stresses during their life cycle. Drought negatively affects crop growth and productivity (Zhu, J.K. 2002). To cope with drought stress, plants have evolved different strategies at three levels: physiological, biochemical, and molecular levels (Araus et al., 2002; Chaves et al., 2003; Krasensky and Jonak, 2012). Drought affects cell water potential, photosynthesis, nitrate assimilation and various anabolic enzyme reactions (Zhang et al., 2010). In addition to Lipid peroxidation, enzyme inactivation, protein degradation, pigment bleaching and disruption of DNA strands (Pompelli et al., 2010). Recent reports have shown that endophytic bacteria which has direct interaction with host cell can help the plant to cope with abiotic stress more efficiently (Chauhan et al., 2015), these bacteria are called plant growth-promoting bacteria (PGB) which can colonize the internal plant tissue (Hardoim et al., 2008). The use of these bacteria is found to be very helpful in combating abiotic stress that limits the overall performance of plants under stress (Staudinger et al., 2016). Their action at physical and biochemical levels, PGB positively influence plant growth or reduce disease and abiotic stresses susceptibility (Dimkpa et al., 2009; Calvo et al, 2016). PGB are well-adapted to wide range of conditions and may have a strong potential to help their host-plant to cope with environmental stresses such as drought and enhance their growth under stress conditions (Vivas et al., 2003; Marulanda et al., 2009, 2010).

Various PGB species have been tested for various crops such as wheat, maize, common beanand tomato; they found to enhance drought resistance in these crops (Hosseini et al., 2017; Garcia et al., 2017; Yanni et al., 2016; Calvo-Polanco et al., 2016) . Mechanisms that were used by PGR to enhance drought in plants are many (Yang et al., 2009). Some PGB are working at root levels, thus improving plant water absorption capacity, through the production of phytohormones such as indole acetic acid (IAA) (Gujral et al., 2013), Gibberellic acid (GA) and cytokinins (Boiero et al., 2007; Gagné-Bourque et al., 2015). Others reduce the production of ethylene through production of an enzyme such as deaminase enzyme that confers induced drought resistance (Saleem et al., 2015). Some PGB, work at gene level by modification of plant genes expression like early response to dehydration 15 or dehydration responsive element protein (Timmusk and Wagner, 1999; Gagné-Bourque et al., 2015). Accumulation of several organic solutes like soluble sugars, starch and amino acids was found in maize, inoculated with PGB (Vardharajula et al., 2011). *Bacillus* is one of the most common taxa of isolated endophytes used by researchers to increase yield and reduce pathogen infection (Lugtenberg and Kamilova, 2009). They promote plant growth promotion by increasing nutrient availability, production of volatiles and plant hormones synthesis (Ryu et al., 2003). In this research, we tested if application of *Bacillus megaterium* (ATCC® 14581™) on tomato plant derived via tissue culture or seed will enhance plants growth and physiological parameters.

## MATERIALS AND METHODS

### Inoculum preparation

*Bacillus megaterium* (ATCC® 14581™) was kindly provided by Dr Heba Al- Fares, Annajah National University. Bacteria was inoculated 12 hours before inoculum and accordingly diluted to have cellcount (2 x 10$^8$, 4 x 10$^8$ and8 x 10$^8$).

### Plant materials and growth conditions

The experiment was conducted under greenhouse conditions at the Palestine Technical University - Kadoorie. Plant were kept inpot with 5-litercapacitycontained (1:1 ratio) of vermiculite and peatmoss. Seeds of commercial tomato cultivar Azmir were surface sterilized using 20% of chlorox (sodium hypochlorite) for 15 minutes containing few drops of tween 20, and then were rinsed three times with sterile distilled water for three minutes each. Seeds were inoculated in Magenta boxes containing MS (Murashige and Skoog, 1962) media supplemented with 30 mg/l of sucrose and solidified with 8 mg/l of Agar. The pH was adjusted to 5.8 prior autoclaving at 121C° for 21 minutes. Cultured were transferred to growth room and kept for (10-14) days at 23C° under 16 h days light of 40 µmol m$^{-2s}$-1illumination and 8 h dark period. The cotyledons were excised from 14 days old seedlings and transferred in to MS medium supplemented with 1 mg /l zeatin and 0.1 mg/l IAA for shoot induction. The regenerated shoots were transferred into MS free hormone for shoot elongation. Well- rooted plants were hardened for 1 week before conducting drought tolerance experiments while one-week old seedlings were obtained from local nursery used along with tissue derived plants.

### Drought experiment

During the whole experiment, plants were kept under greenhouse conditions for 2 weeks before treated as follows: The overnight bacterial culture was centrifuged at 5000 rpm for 5 minutes, and diluted in sterile distilled water for a final of 2 x 10$^8$, 4 x 10$^8$ and 8 x 10$^8$ cells/ ml. The plants were kept for 2 days post inoculation. 1 ml of each concentration was applied to root zone every 4 weeks, while control plant received none. Well -watered (WW) plants were watered to field capacity three times per week based on pot weight. Pots of water-stressed plants (WS) received 50% or 75% of water field capacity. The data was recorded after 1 month of exposure to drought. All pots received a solution of N-P-K fertilizer 20-20-20 as needed.

### Growth and physiological parameters

#### *Plant biomass*

After 1-month post inoculation, the plant was harvested and fresh above shoots and its dry weight were recorded. For dry weight, the harvested shoots were dried at 110°C for 24 h.

#### *Chlorophyll content*

Data on the concentrations of chlorophyll (Chl) A and B in tomato leaves were determined, using spectrophotometry as described by Arnon 1949. Briefly, Chl a and Chl b were extracted from 1 g tomato leaves in 80% acetone at 4°C for 24 h in the dark, and then the contents of Chl a and Chl b were determined by measuring the absorbance of the extracts at 663 and 645 nm,respectively. This experiment was repeated three times, with three replications per treatment.

#### *Photosynthetic rate, stomata conductance*

A portable photosynthesis unit (ADC Bio Scientific LCi-SD

**Table 1**: Effect of bacterium inoculum on average plant fresh weight (g), average dry weight and average plant height (cm) subjected to two drought regimes (75 and 50% of total supplied water).

| Treatment # | Bacterial CFU | Watering conditions (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | | | 75 | | | 50 | | |
| | | FW | DW | PH | FW | DW | PH | FW | DW | PH |
| 1 | 0 | TD $75.2^{b}$ | $20.7^{b}$ | $70.1^{b}$ | $60.3^{b}$ | $15.2^{ab}$ | $54.2^{b}$ | $54.4^{b}$ | $9.1^{b}$ | $48.6^{b}$ |
| | | SD $77.0^{b}$ | $23.3^{b}$ | $70.4^{b}$ | $61.1^{b}$ | $17.3^{a}$ | $55.3^{b}$ | $53.5^{b}$ | $8.7^{b}$ | $50.1^{b}$ |
| 2 | $2*10^{8}$ | $110.1^{a}$ | $25.2^{a}$ | $87.1^{a}$ | $72.2^{a}$ | $18.7^{a}$ | $67.2^{a}$ | $68.3^{a}$ | $12.2^{a}$ | $63.3^{a}$ |
| | | $115.5^{a}$ | $27.5^{a}$ | $87.2^{a}$ | $73.7^{a}$ | $18.5^{a}$ | $68.2^{a}$ | $70.1^{a}$ | $12.1^{a}$ | $66.5^{a}$ |
| 3 | $4*10^{8}$ | $107.1^{a}$ | $25.0^{a}$ | $84.5^{a}$ | $70.3^{a}$ | $16.7^{a}$ | $66.5^{a}$ | $67.2^{a}$ | $12.7^{a}$ | $62.3^{a}$ |
| | | $105.1^{a}$ | $27.3^{a}$ | $86.3^{a}$ | $69.3^{a}$ | $16.3^{a}$ | $67.2^{a}$ | $68.4^{a}$ | $11.6^{a}$ | $63.2^{a}$ |
| 4 | $8*10^{8}$ | $60.3^{c}$ | $15.2^{c}$ | $58.5^{c}$ | $52.2^{c}$ | $9.6^{c}$ | $52.2^{b}$ | $40.8^{c}$ | $8.5^{cb}$ | $45.0^{bc}$ |
| | | $61.2^{c}$ | $14.1^{c}$ | $56.3^{c}$ | $50.8^{c}$ | $8.3^{c}$ | $52.1^{b}$ | $42.5^{c}$ | $7.8^{b}$ | $51.0^{b}$ |

Means in a column with same letter(s) do not differ significantly according to DMRT at level of $p \leq 0.5$. SD: seed derived. TD: Tissue derived PH (Plant height).

System Serial No.34272) was used to monitor leaf net photosynthetic, transpiration rates and stomatal conductance. The measurements were taken in the morning between 9:00 and 11:00, when midday heat stress was absent. Mature leaves fully exposed to light (two leaves from each plant, three plants from each treatment) were selected.

## Statistical analysis

Statistical analysis data were statistically analyzed by Duncan's multiple range test (DMRT).

## RESULTS

The goal of this study was to characterize the response of tomato inoculated with the *Bacillus megaterium* (ATCC® 14581™) subjected to drought-stress and to determine if this bacteria could improve tomato plant drought tolerance.

### Growth parameters

The endophyte is well known for their capacity to enhance plant growth under stress conditions and among these are bacillus species. *Bacillus megaterium* (ATCC® 14581™) was found to be very effective in alleviating drought stress in this study.

### Effect of drought conditions on plant growth

The effect of *Bacillus megaterium* (ATCC® 14581™) application on the growth of tomato plants grown under normal and water stress conditions was evaluated. The tomato plant treated with each of the bacteria possessed greater in average plant height, average fresh weight and average dry weights than non-inoculated (control) plant, regardless of treatments (normal or water stressed; SD or TD (Table 1) except treatment 4 where high inoculum decreased the average of all growth parameters. There were no significant differences in plant height, fresh weight or dry weight between tomato plants obtained via tissue culture or seed in all treatments. Inoculating control plants with bacteria increase average fresh weight, average dry weight average plant height in all treatment except treatment 4, no significant difference was found in FW, DW or PH when inoculated with either 2 x $10^{8}$ per ml or 4 x $10^{8}$cellper ml. In addition to that, increasing bacterial concentration beyond this level negatively affect the growth parameters in all treatments. In general, average fresh weight decreased significantly when plant subjected to drought regime except treatment 4. Exposure of inoculated plant to 8-week drought-stress (75 and 50 % of total water amount required) enhanced the averages of shoot fresh weight, average dry weight and plant height by 20% and 23%, 24% and 25%, 34%, 30% respectively. It was well documented that plant growth promoting rhizo bacteria improved water stress tolerance in Plant. Banerjee et al. (2017) found that when rice was inoculated with bacteria, shoot and root biomass was significantly increased as well as he showed that they have a beneficial effect on crop and soil quality.

On other hand, Nand and Khan (2014) conducted an experiment on the effect of rhizobium inoculum on early plant growth on black and green gram and found that it caused subsequent improvement in root and shoot length and biomass. In our study this strain enhances biomass. This may be attributed to efficiency of plant to absorb water from soil by increasing their root system biomass. This strategy was one way that plant can avoid drought for long period and manage to delay the consequence of

**Table 2:** Effect of bacterium inoculum on chlorophyll content of drought subjected to two drought regimes (75% and 50 % of total supplied water).

| **Treatment #** | **Bacterial CFU** | **Cholorophyll contents (mg/g leaf tissue)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **100** | | **75** | | **50** | |
| | | **Chl a** | **Chl b** | **Chl a** | **Chl b** | **Chl a** | **Chl b** |
| 1 | 0 | TD 5.12$^{c}$ | 1.25$^{c}$ | 3.62$^{d}$ | 1.31$^{b}$ | 2.62$^{c}$ | 1.11$^{b}$ |
| | | SD 5.21$^{ac}$ | 1.31$^{b}$ | 3.87$^{d}$ | 1.1$^{b}$ | 2.87$^{c}$ | 1.3$^{b}$ |
| 2 | $2*10^8$ | 7.62$^{a}$ | 1.82$^{a}$ | 5.5$^{ba}$ | 1.94$^{a}$ | 3.84$^{a}$ | 1.64$^{a}$ |
| | | 1.20$^{a}$ | 1.8$^{a}$ | 5.89$^{a}$ | 1.55$^{a}$ | 3.89$^{a}$ | 1.65$^{a}$ |
| 3 | $4*10^8$ | 6.2$^{bc}$ | 1.6$^{a}$ | 4.9$^{bc}$ | 1.1$^{b}$ | 2.3$^{b}$ | 1.15$^{b}$ |
| | | 6.2$^{b}$ | 1.6$^{a}$ | 4.8$^{bc}$ | 1.2$^{b}$ | 2.8$^{b}$ | 1.2$^{b}$ |
| 4 | $8*10^8$ | 4.8$^{cd}$ | 1.2$^{b}$ | 3.8$^{c}$ | 0.98$^{bc}$ | 1.8$^{b}$ | 0.98$^{b}$ |
| | | 4.9$^{cd}$ | 1.3$^{b}$ | 4.0$^{c}$ | 1.0$^{bc}$ | 1.0$^{bc}$ | 1.0$^{b}$ |

Means in a column with same letter(s) do not differ significantly according to DMRT at level of p≤ 0.5

drought (Chaves et al., 2003; Meister et al., 2014) and was confirmed by study conducted by Kasim et al. (2013). These observations are in agreement with previous reports on the potential of endophytic bacteria in improving plant productivity and enhancement of drought tolerance (Creus et al., 2004; Mei and Flinn, 2010). Similar results was obtained by Kavamura et al. (2013) where *Zea mays* L. seedlings leaf area, stem and biomass shoot dry weight in a simulated water stress experiment when inoculated with bacillus spp. The bio inoculant *Bacillus megaterium* strain along with the use of fermented sugar beet as soil amendment in the presence of *arbuscularmycorrhizal* fungi promoted plant growth, biomass, nutritional content, and water level and reduced the conductance of stomata and the activities of enzymatic antioxidants in plants faced with drought stress. The amendment boosted the effectiveness of the bacteria toward supporting the growth and productivity of the stressed plant (Armada et al., 2014, Creus et al., 2004, Mei and Flinn, 2010). Bistgani et al. (2017) found the application of chitosan to a plant (thyme) undergoing drought reduced the effect of drought stress by 20% and increase in dry matter of the plant by 54% than non-inoculant.

## Effect of bacterium inoculum on physiological parameters

### *Chlorophyll content*

Inoculation plants with bacterium enhance chlorophyll contents. Inoculation with 1 ml of bacterium with 2 x $10^8$ increases chlorophyll a by 46% than control. Meanwhile, inoculation with bacteria with 8 x $10^8$cell / ml, adversely affect chlorophyll a content and decreased by 6%.When comparing, chlorophyll a and b content between seed derived or tissue derided plant, there was no significant differences (Table 2). Application of two drought regimes also affects both chlorophyll a and b in all treatment regardless of source of plant. No doubt that drought has a negative effect on chlorophyll content. It interferes with chlorophyll a more than b (Meher et al., 2018). The yellowing of the leaves is a result of the destruction of chlorophyll and lipid by oxygen radicals and hydrogen peroxide molecules (Meher et al., 2018). Similar results were found by Ahmadi et al. (2013), who obtained significant increases in two wheat genotypes inoculated with different strains of Azospirillum and Pseudomonas sp. Mutumba et al. (2018) showed that the inoculated plants showed significant increases in root length, stomata conductance and chlorophyll index. In our study, similar results were obtained were chlorophyll a and b significantly affected by drought regime and our bacteria strain enhanced chlorophyll a content by 46%.

### *Photosynthetic rate, stomata conductance and respiration rate*

The effect of different concentration of bacteria inoculum on physiological parameters is shown in Table 3. Inoculation plant with bacteria (2 x $10^8$ and 4 x $10^8$cells / ml) significantly enhances all physiological parameters regardless of drought conditions. Photosynthetic rate increased from 6.9 to 8.45 (22.4%). In our study, stomatal conductance increased by 32. % and transpiration rate by 63.0% when inoculated with bacteria (2 x $10^8$cell/ ml). No significant difference was found between tissue derived plant seed plant regardless of the treatment. In control plant, drought treatments reduce all physiological parameters. In tissue derived plant, photosynthetic rate decreased by 35% and 57.6%, stomata conductance by 40%, 65%, transpiration rate decreased 18% and 47% when subjected to 75% and 50% of water stressed plant, respectively. Reduction of stomata conductance is one of the first responses of plant subjected to drought stress. This

**Table 3**: Effect of different inoculum of bacteria on physiological parameters (photosynthetic rate, stomata conductance and transpiration subjected to two drought regimes (78% and 50 % of total water supplied).

| **Treatment #** | **Bacterial CFU** | **Photosynthetic rate(µmol m-2 s-1)** | | | **Stomata conductance (mol m-2 s-1)** | | | **Transpiration rate (mmol m-2 s-)** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **100** | **75** | **50** | **100** | **75** | **50** | **100** | **75** | **50** |
| 1 | 0 | TD 5.67$^{c}$ | 3.66$^{b}$ | 2.40$^{b}$ | 0.57$^{b}$ | 0.34 b | 0.20$^{b}$ | 16.10$^{b}$ | 13.20$^{bc}$ | 8.40$^{b}$ |
| | | SD 5.0 $^{c}$ | 3.91 $^{b}$ | 2.0$^{b}$ | 0.51$^{b}$ | 0.37b | 0.21$^{b}$ | 17.10$^{b}$ | 15.10$^{b}$ | 8.8 0$^{b}$ |
| 2 | 2 x 10$^{8}$ | 8.45$^{a}$ | 5.10$^{a}$ | 3.50$^{a}$ | 0.80$^{a}$ | 0.45a | 0.30$^{a}$ | 26.50a | 18.20$^{a}$ | 10.5$^{a}$ |
| | | 8.20$^{a}$ | 5.90$^{a}$ | 3.10$^{a}$ | 0.84$^{a}$ | 0.45$^{a}$ | 0.31$^{a}$ | 27.20$^{a}$ | 19.10$^{a}$ | 11.1$^{a}$ |
| 3 | 4 x 10$^{8}$ | 6.50$^{b}$ | 4.30$^{a}$ | 3.50$^{a}$ | 0.78$^{a}$ | 0.44$^{a}$ | 0.32$^{a}$ | 18.5$^{b}$ | 12.30$^{b}$ | 9.10$^{ab}$ |
| | | 6.90$^{b}$ | 4.10$^{a}$ | 3.10$^{a}$ | 0.80$^{a}$ | 0.41$^{a}$ | 0.31$^{a}$ | 19.1$^{b}$ | 15.20$^{b}$ | 8.80$^{ab}$ |
| 4 | 8 x 10$^{8}$ | 4.45$^{c}$ | 3.10$^{c}$ | 1.10$^{c}$ | 0.42$^{b}$ | 0.21$^{c}$ | 0.09$^{c}$ | 17.2$^{b}$ | 12.2 0$^{bc}$ | 7.80$^{b}$ |
| | | 4.80$^{c}$ | 3.55$^{c}$ | 1.30$^{c}$ | 0.48$^{b}$ | 0.35$^{b}$ | 0.11$^{c}$ | 18.1$^{b}$ | 11.2 0$^{bc}$ | 6.90$^{c}$ |

Means in a column with same letter(s) do not differ significantly according to DMRT at level of p≤ 0.5

will decrease water loss by transpiration (Flexas and Medrano, 2002). Photosynthetic rate will subsequently being affected by stomata closure as CO2 will be limited. To achieve better growth under drought, fine balance between photosynthesis and water transpiration must met. Drought stress reduce photosynthetic rate in plant. In a legume plant (*Aspalathuslinearis*) when was subjected to drought condition via with holding of water supply to the plant, photosynthetic rate of the plant by 40% reduction as well as 61% reduction in stomatal conductance, as a consequences of continuous closure of stomata to maintain intracellular water content and cut down on water loss from the leaves. In our study, also in control plant, photosynthetic rate stromal conductance and respiration rate are affected by drought conditions. An inoculation with the bacteria inoculum of 2 x 10$^{8}$ CFU improved photosynthetic rate, stomatal conductance and respiration rate. It is an accordance with a study by Durán et al. (2016) who showed an improved rate of photosynthesis and, consequently, increase leaf water potential, which plays an important role in the tolerance of plants to water stress.

## CONCLUSION

These results indicate that *B.megaterium* (ATCC® 14581™) improves tomato growth under drought stress condition. These results hopefully will contribute to the development of a microbial agent to improve the yield of tomato commercial varieties exposed to environmental stresses.

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