

ALLEVIATION OF LITHIUM TOXICITY IN SORGHUM (*SORGHUM VULGARE* PERS.) BY INOCULATION WITH LITHIUM RESISTANT BACTERIA

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Abstract. The present study was carried out to assess the impact of lithium and the alleviation of its influence on the growth and physiological performance of *Sorghum vulgare* using lithium resistant bacteria. Various growth and physiological parameters were measured at 15 days intervals and plants were harvested after 150 days to assess the lithium up-take in both the above-and belowground parts. A significant reduction in the number of leaves, plant height, panicle number, and grain yield, was observed among the plants treated with lithium. While, these growth parameters exhibited a significant increase among plants inoculated with bacteria. There was not a significant difference on gas exchange rate in the lithium treated plants as compared to the plants treated with bacterial inoculation. A strong significant relationship was observed between lithium concentrations in roots and biomass accumulation factor. Furthermore, the concentration of lithium was significantly lower in above and below-ground parts of plants with bacteria as compared to those treated with lithium only. Our findings demonstrate that lithium can cause significant reduction in growth and yield of sorghum, although reduction can be compensated by applying lithium resistant bacteria. Such data can be useful for lithium remediation in agricultural crops.

Keywords: *plant growth promoting bacteria, vegetative, yield, physiology, pollution*

Introduction

Pollution, specifically that of soil and water has formulated the crisis for life on earth, wide efforts have been taken to explore solution for this disorder (Brown and Chaney, 2016). Lithium is extremely mobile element geochemically, so, the environmental and occupational health and safety risks are high due to its mobility (Aral and Vecchio-Sadus, 2008). Lithium is toxic to plants, and only a few plant species are also lithium tolerant. Lithium contents concentration may reach up to 1000 $\mu\text{g g}^{-1}$ of plant mass (Shahzad et al., 2016). In fact, it is not required for the growth and development of plant. Symptoms of lithium toxicity in plants are mostly chlorosis. Prime uses of lithium in today's daily life is for manufacturing of batteries (39%) ceramics and glass (30%) and rest in some other uses. Australia, Chile, China and Argentina are the largest lithium-producing countries in the world respectively (Jaskula, 2019). At present Lithium-ion batteries recycling is paying attention only on collection of economically viable elements like Cobalt and Nickel. Lithium is neglected even in sophisticated recycling systems because it is cheap and easily available for mining of virgin material. Currently lithium pollution is present in the countries where it is extracted and where finally the products of lithium are disposed off without any treatment (Ferreira et al., 2009; Dewulf et al., 2010; Wanger, 2011; Gu et al., 2017; Winslow et al., 2018).

Currently, there are not much studies about the phytotoxic effects of lithium, hence the metabolic role of lithium in plants is not fully documented (Hawrylak-Nowak et al., 2012; Franzaring et al., 2016). Being soluble in water it forms ions absorbed by plants (Aral and Vecchio-Sadus, 2008). Tolerance and uptake of lithium varies among plant families even in species according to their physiology for the ion up-take. Members of family leguminosae are most affected by lithium (Kabata-Pendias and Mukherjee, 2007). Plant and bacterial interaction has great importance, bacteria are helpful for plants to absorb nutrients and cope with different stresses faced by the plants. Bacteria synthesize plant hormones, which can cause direct effect on plant physiology. Bacteria also increase plant access to soil nutrients, and enhance plant growth. Such interaction result in the form of better growth and yield in plants (Hayat et al., 2010; Nadeem et al., 2014; Theka-Kutumela et al., 2020).

Sorghum (*Sorghum vulgare* Pers.) is an important crop cultivated in most of tropical, subtropical, and temperate regions. It is used worldwide for both cereal grain and fodder purposes. It belongs to C4 group of plants and have ability to face wide range of stresses (Gill et al., 2014; Zancheta et al., 2015; Jia et al., 2016). To the best of our knowledge the sorghum-bacterial interaction under lithium stress has not been much investigated previously. Major objectives of present study were to: (1) determine the effects of different concentrations of lithium and lithium resistant bacteria inoculation on the growth and yield of sorghum; (2) explore the role of different concentrations of lithium and inoculation of lithium resistant bacteria on the rate of photosynthesis, transpiration and stomatal conductance; (3) assess the uptake patterns (root, shoot and grains) of lithium in terms of bioaccumulation and translocation. For these objectives, we hypothesized that the significant reduction in plant growth and their physiology will be caused by the lithium, further the inoculation of lithium resistant bacteria will compensate the lithium effects. It was also hypothesized that there may be the relationship of lithium concentrations in plant parts and biomass accumulation factor. Findings of this study will add to understand tolerance of lithium toxicity in sorghum, and will provide eco-friendly approach to implement in the field.

Materials and Methods

Study site and experimentation

The pot experiment was conducted in the wire house of Botanic Garden GC University Lahore, Pakistan, containing *Sorghum vulgare* Pers. (sorghum) following the factorial experiment in a completely randomized design. Earthen pots of 35.56 cm diameter were used in this experiment, at start three seeds were sown in each pot, when plants were established, thinning was carried out and only one plant per pot was remained. There were six replicate pots per treatment and total of five treatments for lithium (0, 50, 100, 150 and 200 ppm and were labelled as T0, T1, T2, T3, T4) and five treatments for lithium and bacterial inoculations were applied (labelled as T0SB, T1SB, T2SB, T3SB, T4SB). Inoculum of lithium resistant bacteria (isolated from soil and identified as *Bacillus velezensis*) was prepared by dissolving eight gram of nutrient broth in some amount of distilled water and final volume was raised to one liter with the help of distilled water. This broth was added to flasks in which isolated lithium resistant bacterial colonies were added and kept at 37°C for 24 hours. Then 10 mL of liquid nutrient broth containing lithium resistant bacteria (10^7 CFU ml⁻¹) was mixed with 40 mL distilled water and was

poured into pots. The experiment was conducted for 150 days, and plants were destructively harvested.

Growth, yield and physiological assessment

During experiment biomass production was monitored through both physical and physiological parameters (Aslam et al., 2007). The parameters for growth included, number of leaves per plant, and total plant height of plant (cm), which were measured every 15 days. While the physiological parameters included the leaf area based maximum rate of photosynthesis, maximum stomatal conductance, rate of maximum transpiration using IRGA LCA4, in the ambient conditions in the morning time from 10:30 am to 11:30 am. These measurements were taken three times of study duration (first week of start of experiment, at the time of flowering and before the final harvest with mature seeds). The chlorophyll contents were measured using spectrophotometer (Spectroscan 80D). After final harvest, length of roots and shoots, fresh and dry mass of roots and shoots, number of panicle and grain yield were estimated in the Plant Eco-physiology Laboratory of GC University Lahore.

Determination of lithium

Plant material was placed in a porcelain crucible, which were placed in a muffle furnace for 5 hours at 550°C for the preparation of ash. Ash of plant material was taken and dissolved in 5 mL of 2N HCl solution and mixed with a stirrer. After fifteen minutes, the final volume was raised to 50 mL with the help of distilled water. The solution was mixed with the help of stirrer and then it was filtered by using Whatman No. 42 filter paper. The extract was collected in a flask and lithium was measured by flame photometer (S20 Spectrolab).

Translocation factor was calculated by following the method of Marchiol et al. (2004). Bioaccumulation factor (BAF) was computed according to Ruus et al. (2005).

Statistical analysis

The results obtained in experiment were expressed in terms of means and standard deviations. Data was statistically analyzed using one way ANOVA (*Duncan's Multiple Range test* (DMRT)) involving the comparison of the means of each treatment having the lithium application with the means of each treatment having the lithium application but also with the bacterial inoculation for each studied parameters. The statistical analyses, correlations between parameters and graphics were carried out using software Sigma Plot 12.5 version.

Results

Effect of lithium and bacterial inoculation on growth and yield of sorghum

A significant decreasing trend of number of leaves was observed from the T0 to T4 during the experiment duration of 150 days, but the significant increase in the number of leaves was observed in the treatment of T0SB as compared to T0 in all the observations (*Fig. 1*). There was also the significant increase in the number of leaves in T1SB as compared to T1 in 30 days observation and the similar significant difference was observed in 75 days, and 105 days duration. The significant increase in the number of leaves was also observed in T3SB as compared to T3 in 60, 90 135 and 150 days duration (*Fig. 1*).

The significant increase in leaves number was observed in T3SB in 135 and 150 days duration. In the T4SB the significant increase in the number of leaves was only observed in 150 days duration (Fig. 1). Overall, among all the treatments, significant increase in the total number of leaves was observed, except, in the means of T2, T2SB during 30, 45 and 90 days duration. The similar non-significant difference was observed between the means of T1, T1SB in 45 and 120 days duration. The non-significant difference was also observed between T4, T4SB in 45, 60 and 90 days duration (Table S1).

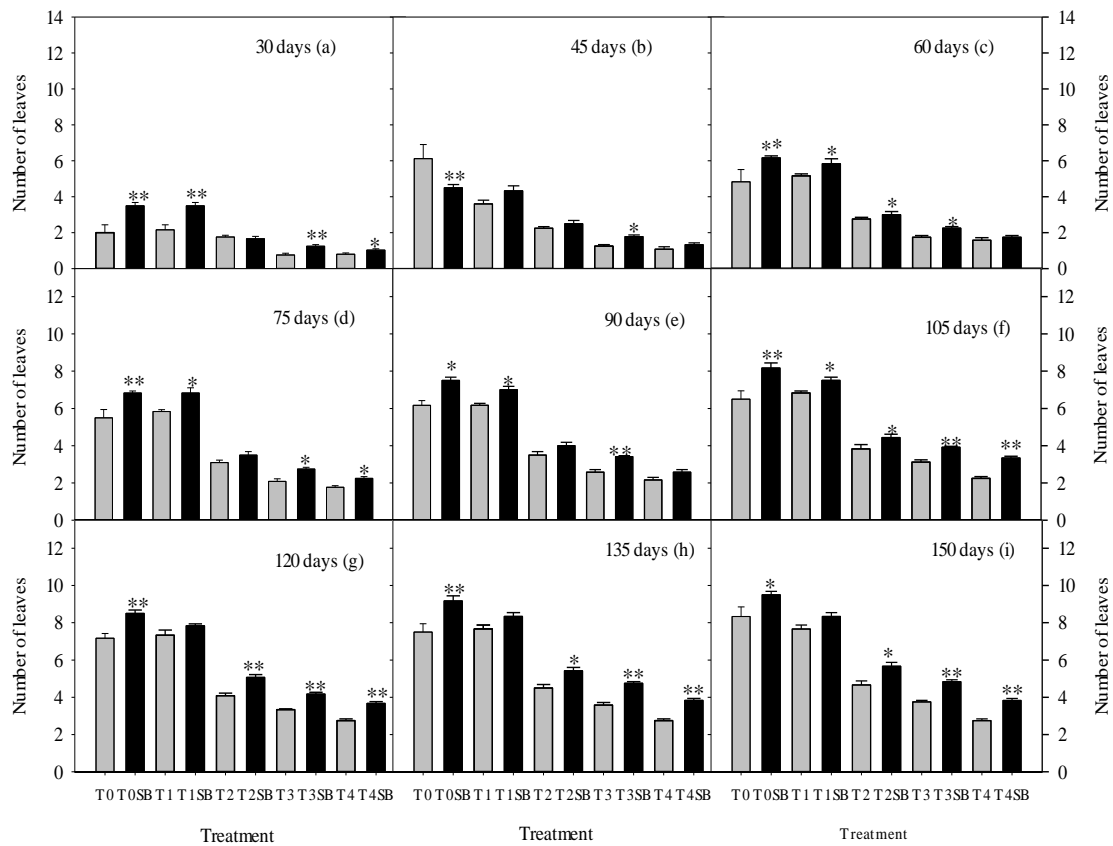


Figure 1. Mean of the total number of leaves per plant for each treatment with the number of days having 15 days intervals from 30-150 days. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment, while black bars indicate the soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

A significant decrease in the total height of sorghum plants was observed from T0 to T4 during the 150 days of experiment, but the significant increase in the plant height was observed in all the treatments having the soil with lithium but also inoculated with bacteria as compared to those having the lithium application only (Fig. 2). The significant increase in plant height was observed in T0SB as compared to T0 in 30 and 150 days duration only (Fig. 2). The similar significant increase in the plant height was also observed in T1SB, T2SB and T3SB as compared to T1, T2 and T3 in all the studied observations. While there was the consistent significant increase in plant height for T4SB as compared to T4 in all durations except the 30 days duration (Fig. 2). The non-significant increase in plant height was only observed between the mean of T0, T0SB at 60 days duration (Table S2).

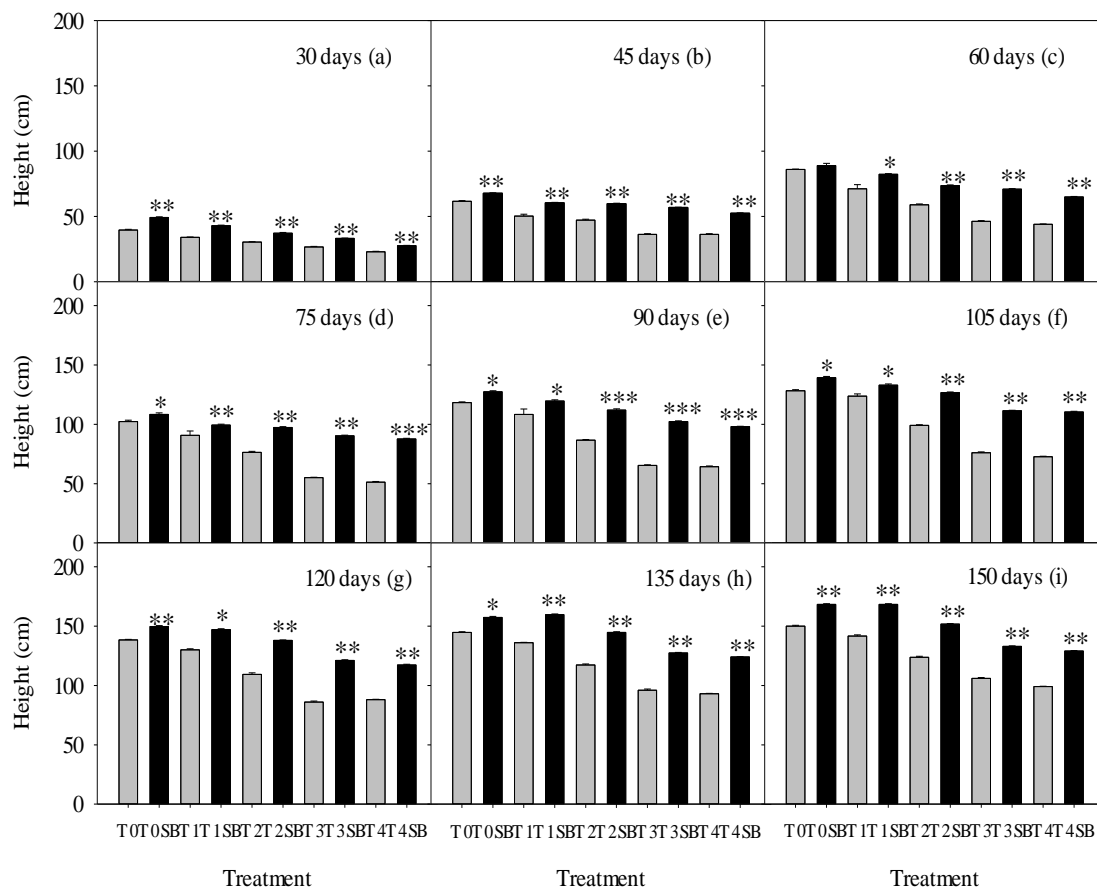


Figure 2. Mean total height of plants for each treatment with the number of days having 15 days intervals from 30-150 days. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment, while black bars indicate the soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

The consistent decrease in the panicle number per plant, panicle length, number of grains and grains yield was also observed across all treatments T0 to T4, while the significant increase in panicle number per plant, panicle length, number of grains and grains yield was observed in all treatments T0SB to T4SB (Fig. 3). While there was not the significant difference in the number of panicles for T1 and T1SB, T4 and T4SB, this non-significant difference was observed panicle length for T1 and T1SB (Table S3).

Effect of lithium and bacterial inoculation on gas exchange, biomass and lithium contents in sorghum

A significant increase in the rate of maximum photosynthesis, stomatal conductance and rate of transpiration was observed from the initial week to the flowering time till the measurements taken during the seed maturation, the flowering time measurements were also significantly larger than the measurements of seed maturation time in the T0, T0SB to T2, T2SB (Fig. 4a-i). While there was a significant increase in the rate of maximum photosynthesis, stomatal conductance and rate of transpiration from the initial week to the flowering time till seed maturation (Fig. 4j-o, Table S4).

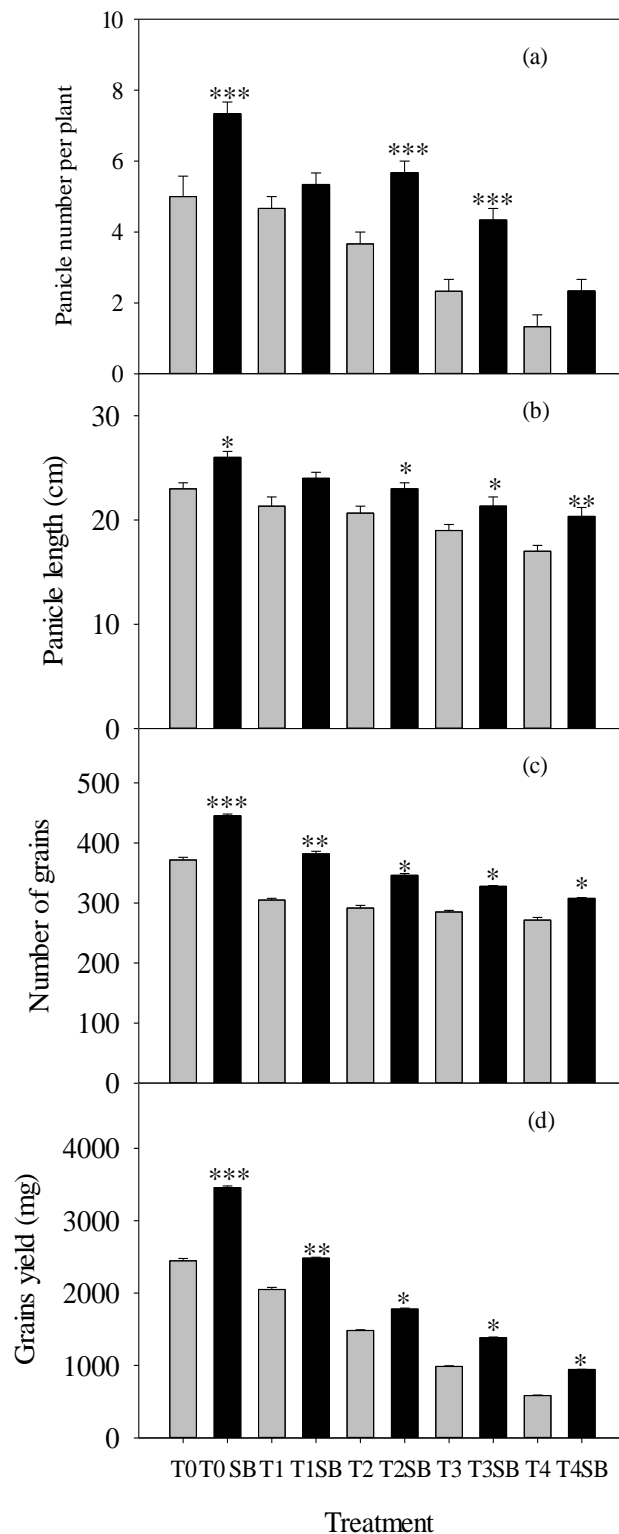


Figure 3. Mean panicle number per plant (a), panicle length (b), total number of grains per plant (c), and grain yield (d) for each treatment. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment, while black bars indicate the soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

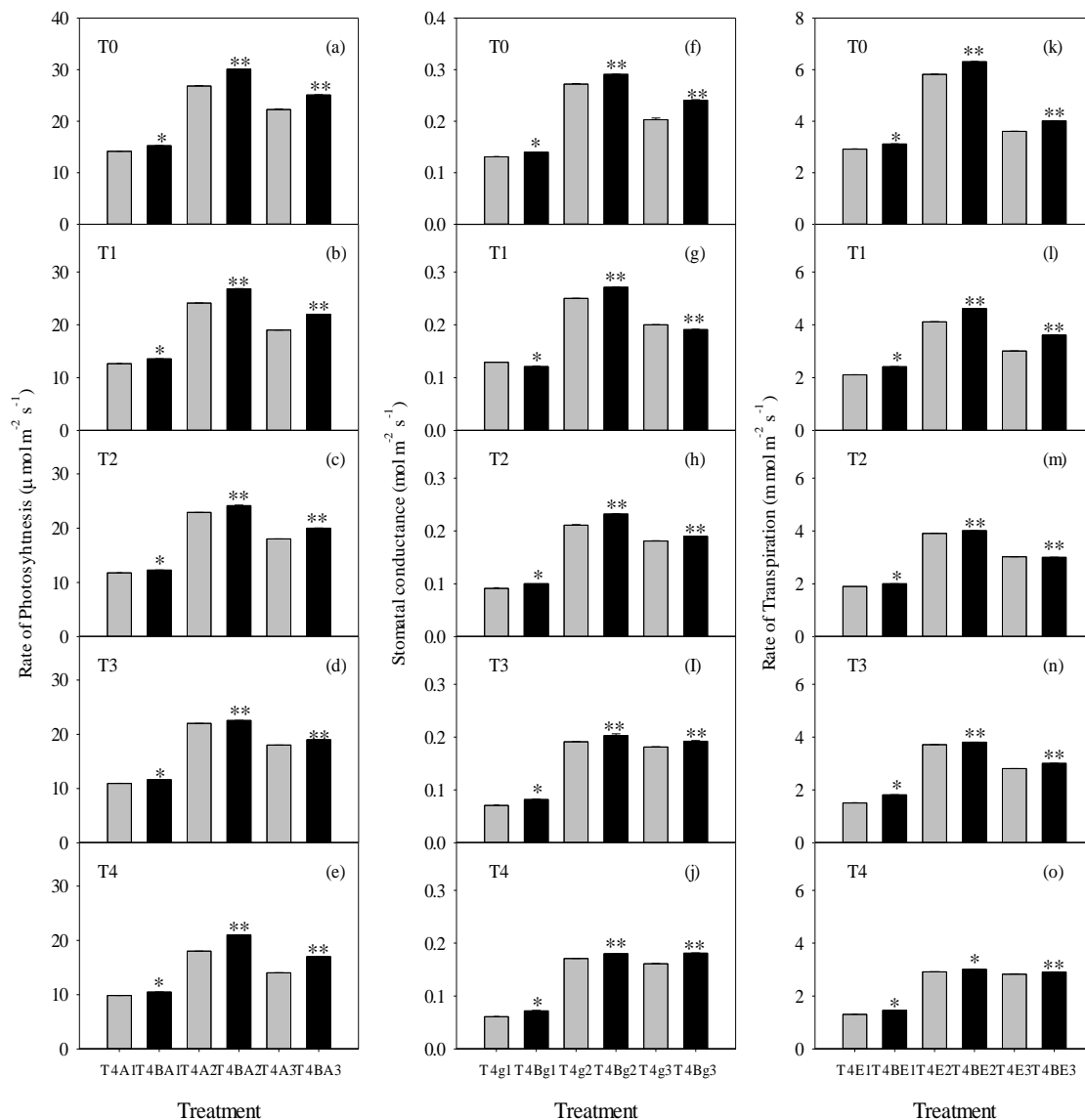


Figure 4. Mean of the maximum rate of photosynthesis, maximum stomatal conductance, rate of maximum transpiration at the first week of start of experiment, at the time of flowering and before the final harvest with mature seeds. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment, while black bars indicate the soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

There was not a significant increase in the shoot and root fresh weight, shoot and root dry weight, and root fresh and dry weight in T0 and T0SB and T1SB (Fig. 5). The non-significant difference in root fresh weight was also observed between T1 and T1SB, and T3 and T3SB, as well as between root dry weight of T3 and T3SB and T4 and T4SB. Specific root length and specific shoot length was observed across all the treatments from T0, T0SB to T4, T4SB (Fig. 5e,f). The comparison all the remaining treatments indicated the significant differences between the lithium treatments and the bacterial inoculations (Fig. 5, Table S5).

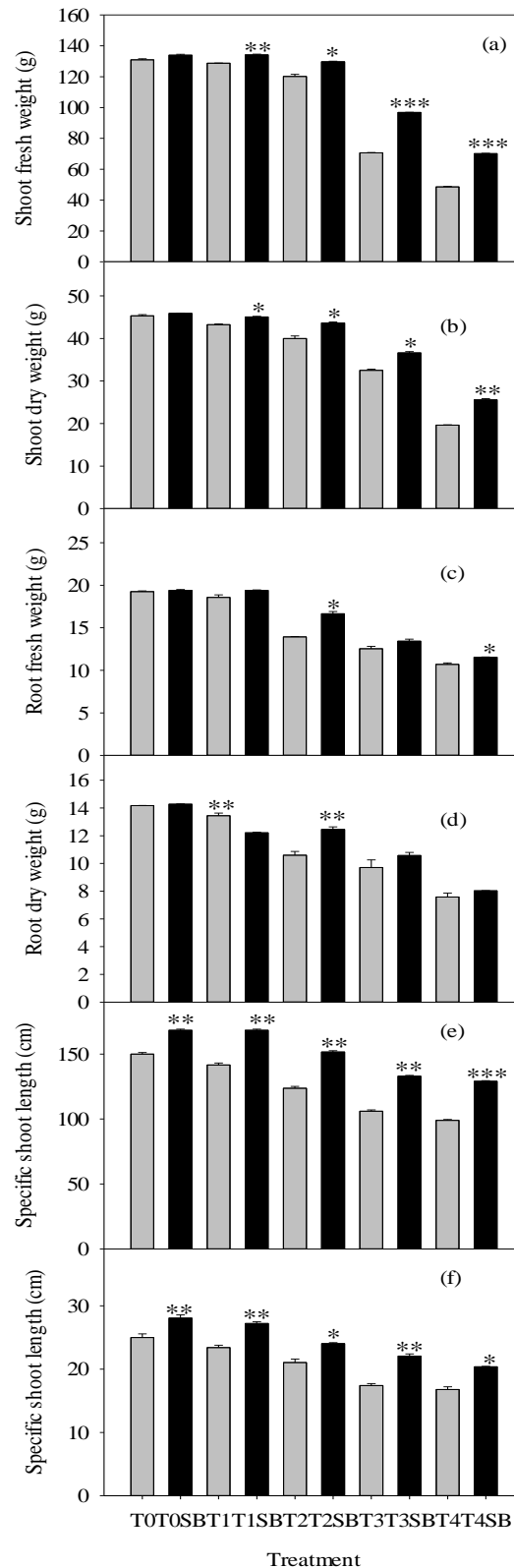


Figure 5. Mean fresh and dry weight of shoot (a,b) mean fresh and dry weight of root (c,d) mean specific shoot length and root length (e,f) for each treatment. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment and black bars indicate the soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

Similarly, the non-significant difference in chlorophyll a and chlorophyll b was observed between T1 and T1SB, and T0 and T0SB, respectively (Fig. 6a,c). For the rest of treatments there was a significant difference in the studied chlorophyll contents (Table S6).

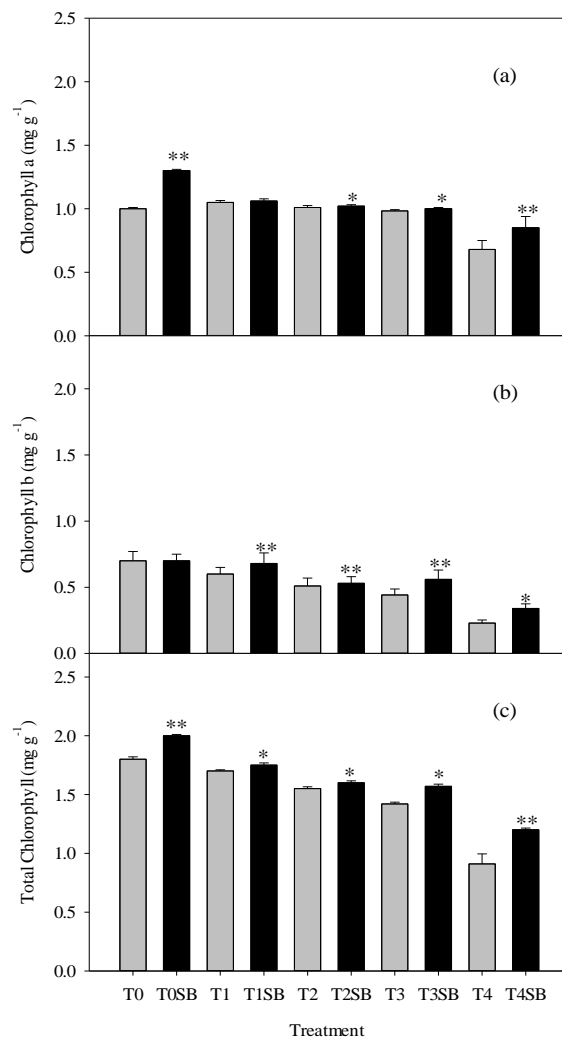


Figure 6. Mean chlorophyll a (a), chlorophyll b (b) and total chlorophyll (c) for each treatment. Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$. Grey bars indicate the soil with Lithium treatment, while black bars indicate soil with Lithium treatment and also application of bacteria. Error bars indicate SE (\pm)

A strong significant and positive relationship was observed between lithium concentration in roots with biomass accumulation factor (Fig. 7a). But, the slope of relationship was significantly different for the roots of plants with lithium concentration and application bacterial inoculation. Similarly, the lithium concentration in roots was also significantly and positively related to lithium concentration in grains (Fig. 7b). The translocation factor was significantly and positively associated with biomass accumulation factor across all the treatments of lithium, while translocation factor was significantly and negatively associated with biomass accumulation factor for the plants having lithium with bacterial inoculation (Fig. 8a,b).

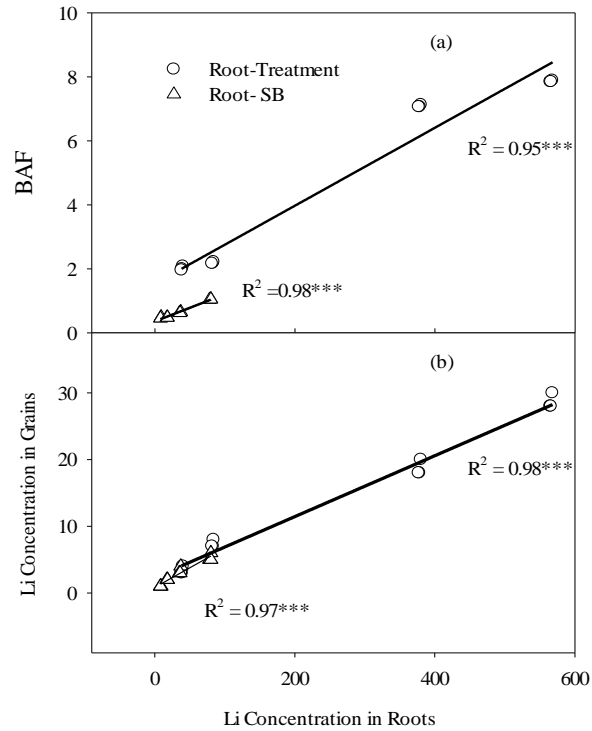


Figure 7. Relationship of Lithium concentration in roots with Biomass Accumulation Factor (a) and the Lithium concentration in grains (b). Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$

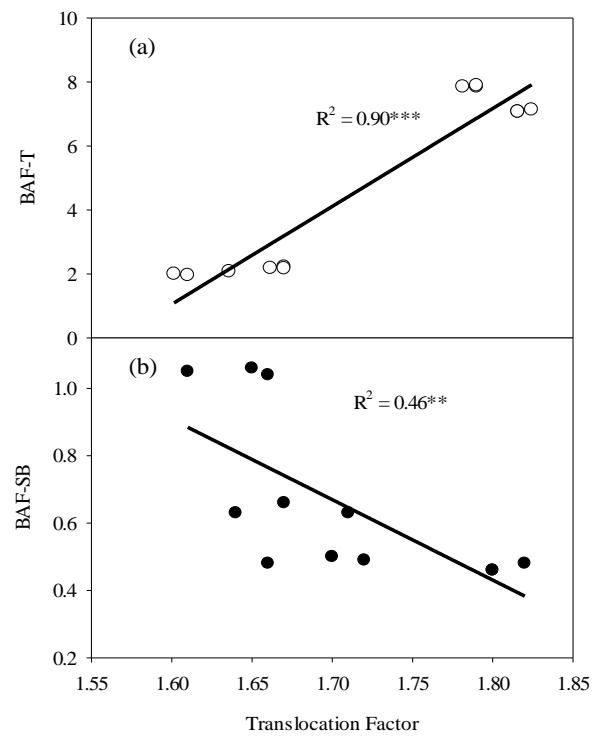


Figure 8. Relationship of translocation factor with Biomass Accumulation Factor having Lithium treatment (a) and with Biomass Accumulation Factor inoculated with Bacteria (b). Where *** indicate $P < 0.0001$, ** $P < 0.001$ and * $P < 0.05$

In all the plant parts, roots, shoots and grains the lithium was significantly lower in the plant parts which were inoculated with bacteria as compared to the plant parts having the application of various lithium concentrations (Fig. 9a-c). The lithium concentration was maximum in shoot, followed by the root and the minimum lithium was up-taken by the seeds (Table S7).

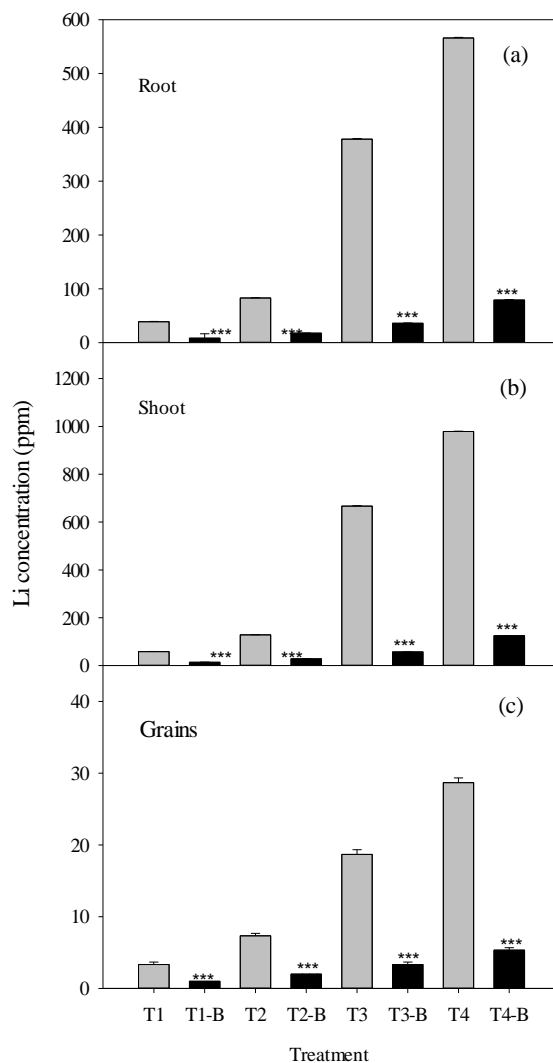


Figure 9. Lithium concentration in roots (a), shoots (b) and grains (c) for each treatment, where *** indicate $P < 0.0001$. Grey bars indicate the soil with Li treatment and black bars indicate the soil with Li treatment and also application of bacteria. Error bars indicate SE (\pm)

Discussion

Our study demonstrates that the lithium can impact the growth and yield of sorghum, but the application of bacteria can alleviate the impact. This supports our hypothesis and also highlights the importance of lithium resistant bacteria application in the remediating the lithium pollution for the studied species. Our results demonstrate that lithium significantly reduced the growth of plants, but the growth was compensated through the lithium resistant bacteria. As hypothesized, we found the strong relationship of lithium concentration roots and grains. In most of lithium treatments and the observations made

during the experiment, significant reduction in the number of leaves and plant height, panicle number, number of grains and grain yield indicating the overall reduction in plants growth and yield. This pattern was also reflected in the fresh and dry weight of above and below ground parts of plants. Such reductions indicate that the applied lithium concentration was toxic to sorghum. Our findings support the findings of Aral and Vecchio-Sadus (2008) who found the reduction in Citrus with lithium concentration. While, contradict with Schrauzer (2002), Kabata-Pendias and Mukherjee (2007) reporting the less impact on the growth of plants from Asteraceae, and Solanaceae when the lithium was applied in their soil.

We did not find the significant reduction in the area based rate of maximum photosynthesis, stomatal conductance, and transpiration which could be because of the reduction of number of leaves. The plant can have the adjustments in its gas exchange while exposed to some abiotic stress. The similar pattern was observed for most of treatment on chlorophyll contents, except for the T4, where all the chlorophyll a, b and total chlorophyll showed the significant increase when the bacterial inoculation was applied. Indicating the compensatory effect of bacterial inoculation.

We found that the significant and positive relationship between the lithium concentration in roots and biomass accumulation factor, but the inoculation of bacteria resulted in a significantly reduced slope for the relationship of BAF and lithium concentration in the roots. Indicating a mechanism in which bacteria would have work to reduce the effect of lithium. The similar pattern was observed for the lithium concentration in roots and lithium concentration in grains (Anjum et al., 2015). Such interaction between plant and microbes result in the formation of biofilm around the roots of plant. Successful colonization of microbes in the form of biofilm provides efficient shield to the plants against various stresses. There are different mechanisms involve to cope abiotic and biotic stresses via biofilm formation one of them is the production of exopolysaccharides. These exopolysaccharides are not only helpful against high ionic contents but also retain water and nutrients in these stressful conditions (Qurashi and Sabri, 2012; Kasim et al., 2016). This was supported by the results of our *Fig. 9*, where we observed highly significant reduction in lithium concentration in the plant parts, i.e., root, shoot, and grains. We found that the maximum concentration of lithium was found in shoot, followed by root and the minimum concentration was found in grains. This could be due to the plant mass accumulation to different organs. But in general, our results support the findings of (Jurkowska et al., 1998, 2003) in terms of lithium concentration to different parts of the plant.

Conclusion

Our study concludes that sorghum is susceptible to the lithium, when applied in the soil. As the significant reduction in the growth and yield were observed. Although, there was compensation for the reduction of growth by introducing the lithium resistant bacteria. The findings are useful for the cultivation of sorghum in particular and other cultivated crops in general, especially with reference to the lithium. Mechanisms of tolerance for Li are not yet clear and much work is underway to elucidate mechanisms of tolerance because of the consequences of Li is still poorly represented in many ecosystems. Li-pollution becoming a big environmental issue and having adverse implications for animals and plants like human update to these problems are crucial and require more detailed research.

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Conflict of interests. The authors declared that there is no conflict of interests regarding the publication of this paper.

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SUPPLEMENTARY INFORMATION

Table S1. One Way ANOVA showing the comparison of mean values number of leaves for each treatment, where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Total number of leaves in different durations					
Treatment	Duration (Days)	Mean (\pm SE)	MS	F	P
T0, T0SB	30	2.00 (0.18) - 3.5 (0.18)	6.75	33.75	<0.001
T1, T1SB	30	2.16 (0.27) - 3.5 (0.18)	5.33	16.0	0.003
T2, T2SB	30	1.76 (0.09) - 1.6 (0.31)	0.030	0.402	0.54
T3, T3SB	30	0.76 (0.09) - 1.25 (0.09)	0.701	13.92	0.004
T4, T4SB	30	0.80 (0.07) - 1.03 (0.07)	0.163	5.05	0.048
T0, T0SB	45	3.5 (0.18) - 4.5 (0.18)	3.00	15.0	0.003
T1, T1SB	45	3.66 (0.21) - 4.33 (0.27)	1.33	3.3	0.086
T2, T2SB	45	2.50 (0.09) - 2.50 (0.18)	0.18	1.5	0.24
T3, T3SB	45	1.25 (0.09) - 1.80 (0.09)	0.85	16.62	0.002
T4, T4SB	45	1.08 (0.13) - 1.33 (0.10)	0.188	2.04	0.183
T0, T0SB	60	4.83 (0.27) - 6.16 (0.10)	5.33	20.00	0.001
T1, T1SB	60	5.16 (0.10) - 5.83 (0.27)	1.33	5.00	0.049
T2, T2SB	60	2.76 (0.09) - 3.0 (0.18)	0.163	1.30	0.28
T3, T3SB	60	1.75 (0.09) - 2.25 (0.09)	0.75	15.00	0.003
T4, T4SB	60	1.58 (0.13) - 1.75 (0.09)	0.083	1.0	0.34
T0, T0SB	75	5.50 (0.18) - 6.83 (0.10)	5.33	40	< 0.001
T1, T1SB	75	5.83 (0.10) - 6.83 (0.27)	3.00	11.25	0.007
T2, T2SB	75	3.10 (0.13) - 3.50 (0.18)	0.48	3.15	0.11
T3, T3SB	75	2.08 (0.13) - 2.75 (0.09)	1.33	16.0	0.003
T4, T4SB	75	1.76 (0.09) - 2.75 (0.09)	2.90	57.63	< 0.001
T0, T0SB	90	6.16 (0.10) - 7.50 (0.18)	5.33	40.0	< 0.001
T1, T1SB	90	6.17 (0.15) - 7.0 (0.18)	2.08	15.62	0.003
T2, T2SB	90	3.5 (0.18) - 4.00 (0.18)	0.75	3.75	0.082
T3, T3SB	90	2.58 (0.13) - 3.41 (0.05)	2.08	31.25	< 0.001
T4, T4SB	90	2.16 (0.13) - 2.58 (0.13)	0.52	4.46	0.061
T0, T0SB	105	6.50 (0.18) - 8.16 (0.27)	8.33	25.0	< 0.001
T1, T1SB	105	6.83 (0.10) - 7.50 (0.18)	1.33	10.0	0.01
T2, T2SB	105	3.83 (0.23) - 4.43 (0.18)	1.08	4.09	0.071
T3, T3SB	105	3.17 (0.12) - 4.0 (0.05)	1.92	35.12	< 0.001
T4, T4SB	105	2.25 (0.09) - 3.33 (0.10)	3.52	60.35	< 0.001
T0, T0SB	120	7.16 (0.10) - 8.50 (0.18)	5.33	40.00	< 0.001
T1, T1SB	120	7.33 (0.27) - 7.83 (0.10)	0.75	2.81	0.12
T2, T2SB	120	4.08 (0.13) - 5.06 (0.14)	2.90	23.45	< 0.001
T3, T3SB	120	3.33 (0.05) - 4.12 (0.10)	2.08	50.00	< 0.001
T4, T4SB	120	7.66 (0.21) - 8.33 (0.21)	1.33	5.00	0.05
T0, T0SB	135	7.5 (0.18) - 9.16 (0.27)	8.33	25.00	< 0.001
T1, T1SB	135	7.66 (0.21) - 8.33 (0.21)	1.33	5.00	0.05
T2, T2SB	135	4.50 (0.18) - 5.42 (0.19)	2.52	12.10	0.006
T3, T3SB	135	3.58 (0.13) - 4.75 (0.09)	4.08	49.00	< 0.001
T4, T4SB	135	2.75 (0.09) - 3.83 (0.10)	3.52	60.35	< 0.001
T0, T0SB	150	8.33 (0.21) - 9.50 (0.18)	4.08	17.50	0.002
T1, T1SB	150	7.66 (0.21) - 8.33 (0.21)	1.33	5.00	0.05
T2, T2SB	150	4.66 (0.21) - 5.66 (0.21)	3.00	11.25	0.007
T3, T3SB	150	3.75 (0.09) - 4.83 (0.10)	3.52	60.35	< 0.001
T4, T4SB	150	2.75 (0.09) - 3.83 (0.10)	3.52	60.35	< 0.001

Table S2. One Way ANOVA showing the comparison of mean values for total plant height of each treatment, where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Total Plant height in different durations					
Treatment	Duration (Days)	Mean (\pm SE)	MS	F	P
T0 , T0SB	30	39.67 (0.55) , 49.33 (0.42)	280.33	191.1	< 0.001
T1 , T1SB	30	34.00 (0.36) , 43.00 (0.36)	243.0	303.75	< 0.001
T2 , T2SB	30	30.50 (0.18), 37.33 (0.42)	140.08	221.18	< 0.001
T3 , T3SB	30	26.66 (0.42), 3.33 (0.21)	133.33	200.00	< 0.001
T4 , T4SB	30	23.00 (0.3), 28.00 (0.21)	65.33	122.50	< 0.001
T0 , T0SB	45	61.66 (0.55), 67.66 (0.5)	108.78	57.68	< 0.001
T1 , T1SB	45	50.33 (1.52), 60.33 (0.55)	300.0	38.16	< 0.001
T2 , T2SB	45	47.33 (0.55), 60.0 (0.55)	45.3	244.64	< 0.001
T3 , T3SB	45	36.33 (0.55), 56.66 (0.42)	1240	845	< 0.0001
T4 , T4SB	45	36.43 (0.55), 52.11 (0.49)	768	411.6	< 0.001
T0 , T0SB	60	86.0 (0.36), 89.0 (1.67)	27.0	3.06	0.11
T1 , T1SB	60	71.33 (3.16), 82.3 (0.58)	363.0	12.1	0.006
T2 , T2SB	60	59.0 (0.73), 73.66 (0.55)	6455.3	254.7	< 0.001
T3 , T3SB	60	46.3 (0.55), 71.0 (0.36)	1825	1369	< 0.0001
T4 , T4SB	60	44.0 (0.36), 65.0 (0.36)	1323	1653	< 0.0001
T0 , T0SB	75	102.3 (1.11), 108.33 (1.28)	108.0	12.46	0.005
T1 , T1SB	75	90.66 (3.6), 99.3 (0.76)	225.3	5.28	0.04
T2 , T2SB	75	76.33 (0.91), 97.33 (0.76)	1323	310.07	< 0.0001
T3 , T3SB	75	55.0 (0.36), 90.33 (0.55)	3745	2809	< 0.0001
T4 , T4SB	75	51.33 (0.55), 87.66 (0.61)	3960	2121	< 0.0001
T0 , T0SB	90	118.33 (0.55), 127.3 (0.91)	243	70.1	< 0.01
T1 , T1SB	90	108.3 (4.5), 119.6 (0.91)	385.3	5.92	0.035
T2 , T2SB	90	86.66 (0.55), 112.0 (0.96)	1925	515.7	< 0.0001
T3 , T3SB	90	65.21 (0.35), 102.3 (0.85)	4107	2200	< 0.0001
T4 , T4SB	90	64.33 (0.41), 98.0 (0.36)	3400	2550	< 0.001
T0 , T0SB	105	128.3 (0.76), 139.3 (0.91)	363	85.0	< 0.001
T1 , T1SB	105	123.6 (2.0), 133.0 (0.96)	261.3	17.5	0.002
T2 , T2SB	105	99.0 (0.73), 126.7 (0.61)	2296	906	< 0.0001
T3 , T3SB	105	76.0 (0.73), 111.3 (0.55)	3745	1478	< 0.0001
T4 , T4SB	105	73.0 (0.42), 110.3 (0.51)	4256	2902	< 0.0001
T0 , T0SB	120	138.3 (0.58), 149.6 (0.76)	385.3	144.5	< 0.001
T1 , T1SB	120	130.0 (0.96), 147.0 (0.73)	867.0	195.0	< 0.001
T2 , T2SB	120	109.3 (1.2), 138.0 (0.36)	2465	462	< 0.0001
T3 , T3SB	120	86.0 (0.96), 121.0 (0.63)	3675	918	< 0.001
T4 , T4SB	120	88.0 (0.36), 117.3 (0.55)	2581	1936	< 0.001
T0 , T0SB	135	144.6 (0.55), 157.3 (0.91)	481.3	138.8	< 0.001
T1 , T1SB	135	136.0 (0.36), 159.6 (0.55)	1680	1260	< 0.001
T2 , T2SB	135	117.3 (0.76), 144.6 (0.55)	2241	840.5	< 0.001
T3 , T3SB	135	96.0 (1.0), 127.3 (0.49)	2945	788	< 0.001
T4 , T4SB	135	93.0 (0.36), 124.0 (0.40)	2883	3603	< 0.001
T0 , T0SB	150	150.0 (0.73), 168.3 (0.58)	1008	398	< 0.001
T1 , T1SB	150	141.6 (0.91), 168.3 (0.50)	2133	615	< 0.001
T2 , T2SB	150	123.6 (0.91), 151.6 (0.41)	2352	6778.4	< 0.001
T3 , T3SB	150	106.0 (0.63), 133.0 (0.36)	2187	1366	< 0.0001
T4 , T4SB	150	99.0 (0.36), 129.0 (0.29)	2700	3375	< 0.0001

Table S3. One Way ANOVA showing the comparison of mean values of number of Panicles, Panicle length, number of grains per Panicle and total number of grains per plant. Where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Number of Panicles				
Treatment	Mean (\pm SE)	MS	F	P
T1 , T1SB	4.66 (0.33), 5.33 (0.33)	0.67	2.00	0.23
T2 , T2SB	3.66 (0.33), 5.66 (0.33)	6.00	188.00	0.013
T3 , T3SB	2.33 (0.33), 4.33 (0.33)	6.00	18.00	0.013
T4 , T4SB	1.33 (0.33), 2.33 (0.33)	1.50	4.50	0.10
Panicle Length				
T0 , T0SB	23.0 (0.5), 26.0 (0.57)	13.50	13.5	0.021
T1 , T1SB	21.3 (0.88), 24.0 (0.57)	10.66	6.40	0.065
T2 , T2SB	20.66 (0.66), 23.00 (0.58)	8.16	7.00	0.057
T3 , T3SB	19.00 (0.6), 21.33 (0.88)	8.16	4.90	0.091
T4 , T4SB	17.0 (0.51), 20.33 (0.90)	16.7	10.00	0.034
Number of grains per Panicle				
T0 , T0SB	371.66 (4.41), 445.0 (2.88)	8066	193.6	< 0.001
T1 , T1SB	305.00 (2.88), 381.66 (4.41)	8816	211	< 0.001
T2 , T2SB	291.66 (4.41), 346.0 (3.05)	4428	102.5	< 0.001
T3 , T3SB	285.0 (2.88), 327.6 (1.45)	2730	174.2	< 0.001
T4 , T4SB	271.6 (4.41), 307.6 (1.46)	1944	60.12	0.001
Grains per plant				
T0 , T0SB	2446.6 (31.79), 3458.3 (22.04)	135204	683.57	< 0.001
T1 , T1SB	2050 (28.68), 2483.33 (12.09)	281666	192.04	< 0.001
T2 , T2SB	1483.3 (8.81), 1780.0 (11.54)	132016	416.89	< 0.001
T3 , T3SB	986.67 (8.82), 1383 (8.89)	236016	1011	< 0.001
T4 , T4SB	583.33 (8.81), 945.0 (2.88)	196204	1519	< 0.001

Table S4. One Way ANOVA showing the comparison of mean values for Photosynthetic rate, transpiration, and stomatal conductance for each treatment in three different durations. Where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Photosynthetic rate					
Treatment	Duration (Days)	Mean (\pm SE)	MS	F	P
T0 , T0SB	Initial Week	14.73 (0.01), 15.23 (0.01)	1.8	2317	< 0.001
T1 , T1SB	Initial Week	12.65 (0.02), 13.57 (0.02)	1.25	987	< 0.001
T2 , T2SB	Initial Week	11.7 (0.01), 12.28 (0.001)	0.385	888	< 0.001
T3 , T3SB	Initial Week	10.93 (0.03), 11.67 (0.01)	0.700	362.28	< 0.001
T4 , T4SB	Initial Week	9.83 (0.01), 10.43 (0.001)	0.634	1086	< 0.001

Photosynthetic rate					
Treatment	Duration (Days)	Mean (\pm SE)	MS	F	P
T0 , T0SB	Flowering time	26.83 (0.08), 30.10 (0.01)	16.07	1369	< 0.001
T1 , T1SB	Flowering time	24.12 (0.01), 26.83 (0.01)	10.98	19392	< 0.001
T2 , T2SB	Flowering time	22.90 (0.01), 24.16 (0.01)	2.35	199.96	< 0.001
T3 , T3SB	Flowering time	22.01 (0.01), 22.57 (0.01)	0.48	810.47	< 0.001
T4 , T4SB	Flowering time	18.01 (0.01), 21.02 (0.01)	13.56	31292	< 0.001
T0 , T0SB	Seed formation	22.26 (0.14), 25.10 (0.06)	12.42	328.49	< 0.001
T1 , T1SB	Seed formation	19.0 (0.01), 22.0 (0.01)	13.37	50512	< 0.001
T2 , T2SB	Seed formation	18.0 (0.01), 20.0 (0.00)	5.94	23760	< 0.001
T3 , T3SB	Seed formation	18.0 (0.00), 19.01 (0.01)	1.54	11552	< 0.001
T4 , T4SB	Seed formation	14.0 (0.01), 17.02 (0.01)	13.44	26013	< 0.001
Transpiration					
T0 , T0SB	Initial Week	2.92 (0.01), 3.11 (0.00)	0.058	183.21	< 0.001
T1 , T1SB	Initial Week	2.11 (0.00), 2.41 (0.01)	0.138	828	< 0.001
T2 , T2SB	Initial Week	1.90 (0.00), 2.00 (0.00)	0.016	961.0	< 0.001
T3 , T3SB	Initial Week	1.50 (0.01), 1.81 (0.01)	0.138	637.0	< 0.001
T4 , T4SB	Initial Week	1.31 (0.00), 1.45 (0.01)	0.032	276.57	< 0.001
T0 , T0SB	Flowering time	5.81 (0.01), 6.31 (0.01)	0.365	1152	< 0.001
T1 , T1SB	Flowering time	4.11 (0.01), 4.61 (0.01)	0.3775	2045	< 0.001
T2 , T2SB	Flowering time	3.91 (0.00), 4.01 (0.00)	0.015	150.0	< 0.001
T3 , T3SB	Flowering time	3.71 (0.00), 3.80 (0.00)	0.0131	196.0	< 0.001
T4 , T4SB	Flowering time	2.91 (0.01), 3.01 (0.00)	0.014	84.10	< 0.001
T0 , T0SB	Seed formation	3.60 (0.01), 4.01 (0.01)	0.248	13353	< 0.001
T1 , T1SB	Seed formation	3.01 (0.00), 3.61 (0.00)	0.540	5400.0	< 0.001
T2 , T2SB	Seed formation	3.023 (0.00), 3.00 (0.00)	0.00	18.0	0.013
T3 , T3SB	Seed formation	2.80 (0.00), 3.00 (0.00)	0.06	1800	< 0.001
T4 , T4SB	Seed formation	2.82 (0.01), 2.91 (0.00)	0.0104	28.40	0.006
Stomatal Conductance					
T0 , T0SB	Initial Week	0.131 (0.00), 0.140 (0.00)	0.00	168.2	< 0.001
T1 , T1SB	Initial Week	0.130 (0.00), 0.121 (0.00)	0.00	81.0	< 0.001
T2 , T2SB	Initial Week	0.091 (0.00), 0.10 (0.00)	0.00	42.5	0.003
T3 , T3SB	Initial Week	0.07 (0.00), 0.08 (0.00)	0.00	64.05	0.001
T4 , T4SB	Initial Week	0.061 (0.00), 0.072 (0.00)	0.00	90.75	< 0.001
T0 , T0SB	Flowering time	0.272 (0.001), 0.291 (0.001)	0.000	196.0	< 0.001
T1 , T1SB	Flowering time	0.250 (0.00), 0.271 (0.00)	0.000	496.12	< 0.001
T2 , T2SB	Flowering time	0.211 (0.00), 0.232 (0.00)	0.00	240.25	< 0.001
T3 , T3SB	Flowering time	0.91 (0.00), 0.203 (0.00)	0.00	13.29	0.022
T4 , T4SB	Flowering time	0.171 (0.00), 0.180 (0.00)	0.00	420.50	< 0.001
T0 , T0SB	Seed formation	0.20 (0.00), 0.241 (0.00)	0.00	121.45	< 0.001
T1 , T1SB	Seed formation	0.200 (0.00), 0.191 (0.00)	0.00	168.20	< 0.001
T2 , T2SB	Seed formation	0.181 (0.00), 0.190 (0.00)	0.00	196.0	< 0.001
T3 , T3SB	Seed formation	0.181 (0.00), 0.192 (0.00)	0.00	72.25	0.001
T4 , T4SB	Seed formation	0.161 (0.00), 0.181 (0.00)	0.00	531.57	< 0.001

Table S5. One Way ANOVA showing the comparison of mean values of shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, specific shoot length, and specific root length across different treatments. Where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Shoot Fresh weight				
Treatment	Mean (\pm SE)	MS	F	P
T1 , T1SB	128.7 (0.17), 134.20 (0.41)	44.82	146.2	< 0.001
T2 , T2SB	120.21 (1.31), 129.6 (0.40)	133.95	47.13	0.002
T3 , T3SB	70.66 (0.20), 96.90 (0.057)	1032.0	14770	< 0.001
T4 , T4SB	48.56 (0.29), 70.13 (0.24)	697.68	3195.48	< 0.001
Root Fresh weight				
T0 , T0SB	19.25 (0.081), 19.40 (0.098)	0.0353	1.44	0.296
T1 , T1SB	18.57 (0.29), 19.39 (0.058)	1.009	7.52	0.052
T2 , T2SB	13.92 (0.03), 16.64 (0.26)	11.12	107.40	< 0.001
T3 , T3SB	12.53 (0.29), 13.43 (0.23)	1.21	5.83	0.073
T4 , T4SB	10.73 (0.17), 11.54 (0.02)	1.067	23.82	0.008
Shoot Dry weight				
T0 , T0SB	45.33 (0.26), 45.87 (0.057)	0.443	3.96	0.11
T1 , T1SB	43.25 (0.14), 45.06 (0.12)	4.95	93.55	< 0.001
T2 , T2SB	40.00 (0.57), 43.63 (0.27)	19.83	32.57	0.005
T3 , T3SB	32.50 (0.26), 36.60 (0.306)	25.21	102.91	< 0.001
T4 , T4SB	19.65 (0.038), 25.56 (0.29)	52.45	391.76	< 0.001
Root Dry weight				
T0 , T0SB	14.17 (0.00), 14.27 (0.024)	0.015	15.25	0.017
T1 , T1SB	13.44 (0.19), 12.20 (0.05)	2.29	37.0	0.004
T2 , T2SB	10.59 (0.26), 12.45 (0.18)	5.17	32.36	0.005
T3 , T3SB	9.70 (0.55), 10.57 (0.22)	1.127	2.11	0.22
T4 , T4SB	7.58 (0.28), 8.03 (0.01)	0.299	2.415	0.195
Specific Shoot length				
T0 , T0SB	150.00 (1.15), 168.3 (0.88)	504.16	159.21	< 0.001
T1 , T1SB	141.66 (1.45), 168.33 (0.88)	1066	246	< 0.001
T2 , T2SB	123.66 (1.45), 151.66 (0.88)	1176	271.38	< 0.001
T3 , T3SB	106.00 (1.00), 133.0 (0.57)	1093	546.75	< 0.001
T4 , T4SB	99.0 (0.57), 129.0 (0.57)	1350	1350	< 0.001
Specific Root length				
T0 , T0SB	25.0 (0.57), 28.11 (0.61)	14.10	15.55	0.02
T1 , T1SB	23.40 (0.37), 27.23 (0.29)	21.88	6.70	0.001
T2 , T2SB	21.05 (0.53), 24.40 (0.15)	13.38	29.17	0.006
T3 , T3SB	17.42 (0.29), 22.08 (0.32)	32.48	111.336	< 0.001
T4 , T4SB	16.80 (0.41), 20.37 (0.06)	19.11	71.69	0.001

Table S6. One Way ANOVA showing the comparison of mean values of chlorophyll a, chlorophyll b, and total chlorophyll across different treatments. Where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Chlorophyll a				
Treatment	Mean (\pm SE)	MS	F	P
T1 , T1SB	1.20 (0.15), 1.06 (0.001)	0.028	0.855	0.408
T2 , T2SB	1.01 (0.001), 1.02 (0.001)	0.000	45.0	0.003
T3 , T3SB	0.98 (0.00), 1.009 (0.004)	0.001	30.48	0.005
T4 , T4SB	0.68 (0.00), 0.85 (0.00)	0.043	26112	< 0.001
Chlorophyll b				
T0 , T0SB	0.703 (0.00), 0.703 (0.00)			1.00
T1 , T1SB	0.60 (0.001), 0.682 (0.001)	0.0096	1873	< 0.001
T2 , T2SB	0.512 (0.00), 0.531 (0.00)	0.000	336	< 0.001
T3 , T3SB	0.44 (0.00), 0.56 (0.00)	0.021	9257	< 0.001
T4 , T4SB	0.23 (0.00), 0.34 (0.00)	0.0181	18150	< 0.001
Total Chlorophyll				
T0 , T0SB	1.80 (0.003), 2.03 (0.03)	0.079	47.13	0.002
T1 , T1SB	1.71 (0.005), 1.75 (0.00)	0.002	51.53	0.002
T2 , T2SB	1.55 (0.00), 1.61 (0.001)	0.0057	84.09	< 0.001
T3 , T3SB	1.41 (0.005), 1.57 (0.002)	0.039	705.37	< 0.001
T4 , T4SB	0.91 (0.00), 1.22 (0.01)	0.143	714.322	< 0.001

Table S7. One Way ANOVA showing the comparison of mean values of lithium concentration in shoot, lithium concentration in root, and lithium concentration in grains. Where T0, indicates the soil without lithium and bacterial inoculation, T1, T2, T3, T4 indicate the application of different concentration of lithium and T0SB, indicates the soil without lithium application but with the inoculation of bacteria, T1SB, T2SB, T3SB and T4SB indicate the different concentrations of lithium with the application of bacterial inoculations

Lithium Concentration (Shoot)				
Treatment	Mean (\pm SE)	MS	F	P
T1 , T1SB	1288.68 (0.70), 28.70 (0.43)	15000	18000	< 0.0001
T2 , T2SB	667.0 (1.52), 57.00 (0.61)	55815	139537	< 0.0001
T3 , T3SB	978.66 (1.2), 125.0 (0.57)	1093120	409920	< 0.0001
Lithium Concentration (Root)				
T0 , T0SB	39.0 (0.6), 8.33 (0.33)	1410	2116	< 0.0001
T1 , T1SB	83.0 (0.61), 17.66 (0.355)	6402	9604	< 0.001
T2 , T2SB	378.33 (0.88), 36.0 (0.60)	17755788	105472	< 0.0001
T3 , T3SB	566.35 (0.88), 79.35 (0.67)	355753	194047	< 0.0001
Lithium Concentration (Grains)				
T0 , T0SB	3.33 (0.25), 1.00 (0.00)	8.16	49.0	0.002
T1 , T1SB	7.33 (0.31), 2.00 (0.00)	42.66	256.0	< 0.001
T2 , T2SB	18.66 (0.71), 3.33 (0.21)	352.66	423.20	< 0.001
T3 , T3SB	28.66 (0.66), 5.33 (0.33)	816.67	980.00	< 0.001