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RESEARCH ARTICLE

# EFFECT OF *BACILLUS SUBTILIS* QM3 ON MORPHOLOGY PARAMETERS DURING GERMINATION OF WHEAT SEEDS UNDER LEAD STRESS

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A R T I C L E I N F O

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A B S T R A C T

Lead (Pb) is the most common heavy metal contaminant in the environment. To investigate the alleviation of lead stress-induced inhibition of seed germination in wheat, wheat seeds (*Triticum aestivum* L.) of "*Linfeng* 3" were pre-soaked with $10^6$ and $10^7$ CFU/mL *Bacillus subtilis* QM3 for 12 h just before germination under hydroponic conditions and stressed with lead acetate, $Pb(CH_3COO)_2$, at five concentrations (50, 250, 500, 1000, 2000 mg/L). The changes of germination rate (GR), germination energy (GE), germination index (GI), vitality index (VI), root length (RL) and shoot length (SL) of wheat seeds had been studied. The results indicated that Pb is accumulated in a dose-dependent manner in wheat, which could exert harmfulness in the early development stage of wheat at inappropriate concentrations. The GR and GE of wheat seeds were reduced 35.0-80.3% and 1.50-58.56% respectively and GI and VI first increased to 103.96% and 120.83% respectively, and then reduced 21.68-58.79% and 8.68-86.52% respectively in wheat seeds after treatments with Pb from the second day till the end of the experiment. The RL and SL also first increased to 105.07% and 108.94% respectively, and then decreased to 2.25-54.79% and 10.35-63.88% respectively with Pb treatment. However, the *B. subtilis* QM3 influenced the variables evaluated. The GR and GE were found to be increased slightly ($p<0.05$), GI and VI were also found to be increased remarkably ($p<0.05$) in wheat seedlings by soaking with *B. subtilis* QM3 under Pb stress within 7 days after treatments compared with control. Pre-soaked with *B. subtilis* QM3 also could promote the growth of roots and shoots of wheat seeds under Pb stress. It was concluded that *B. subtilis* QM3 treatment on wheat seeds may be a good option to improve seed germination and crop establishment under leaded conditions.



## INTRODUCTION

As the amounts of industrial wastes increase and environmental disasters occur more and more often, the soil accumulates large quantities of environmentally hazardous substances and toxicants, including heavy metals [1]. Heavy metals are common non-biodegradable pollutants reported at elevated concentrations in many parts of the world. Due to their non-biodegradability and high toxicity, heavy metals are considered to be one of the most resistant pollutants affecting environments [2]. Most of heavy metals are known as growth inhibitors exerting various adverse effects on plants, which can lead to wider phytotoxic responses and decrease the yield and quality of agricultural crops, even cause health hazards to man and animals by entering the food chain [3]. For example, Pb, which emanates from several industrial activities, is one of the most toxic and common pollutant found in soils. Pb is not included in essential elements for plants, but they absorb it when it is present in their environment, especially in the rural areas where the soil is polluted by automotive exhausts and in fields contaminated with fertilizers containing heavy metal ingredients [4]. Plants absorb Pb from solution in the soil through their roots and, subsequently, the largest proportion of $Pb^{2+}$ is accumulated within roots in an insoluble form [5]. Toxic levels of Pb affect plant processes, as the metal reacts with important functional groups in macromolecules, and the activity of several enzymes is modified, some of which are important in photosynthesis, plant water status and mineral nutrition. The major processes affected are seed germination, seedlings growth, tolerance index, dry mass of roots and shoots [6].

Despite the importance of Pb contamination in the world, it remains unclear as to which economical species are able to resist Pb stress. Wheat is an important agricultural specie, it is grown on 17% of all crop areas and represents the staple food for 40% of the world's population, and is the primary food staple in the north of China, so it appeared to be of interest to research the alleviation of lead stress-induced inhibition on this specie [4]. Seed germination is the first step of the life of a plant and it is one of the most sensitive physiological process in plants, affected by hormonal interactions and environmental factors, both biotic and abiotic, and therefore to the presence of excess of metals. Germination responses of plant to environmental parameters determine their distribution in heavy metal environments [7]. The most widely used test for metal toxicity is the seed germination test. This method was developed to ascertain the toxicity of polluted liquid samples on seed germination and root elongation [8]. After permeation through the seed coat, the germination relies on the seed reserves for the supply of metabolites for respiration and metals can cause oxidative stress and disrupt the process, likewise interfere with the enzymes involved in the germination process.

To find ways to reduce the adverse effect of heavy metal salts, one should study how they influence seed germination, development of seedlings (heterotrophic growth), and growth and development of plants. A promising approach is production and investigation of microorganism-based multifunctional biological preparations that can stimulate plant growth, protect plants from pathogenic organisms, and detoxify heavy metal salts in the soil [1]. Few studies on alleviation of heavy metal stress-induced inhibition of seed germination have been published. In relation to bacteria, the studies are limited to. To understand the biology of plants and microbial ecology, many studies performed with bacteria have focused on evaluating the colonization pattern of vegetative tissues, as well as the effects of bacteria on plant growth. These studies have been performed by inoculating the plants with bacteria, and comparing the inhibition of disease symptoms or germination [9].

*Bacillus subtilis* QM3 was the first isolation from Qinghai yak dung in China of a potential plant growth-promoting bacterium (PGPB) and a significant bio-control agent with a broad pathogen-inhibition spectrum against 11 phytopathogenic fungi, and it is a siderophore producer, investigated in this paper was illuminated to be a potential biocontrol agent [10]. The inoculation by PGPB represents an alternative as potential biofertilizer resources for heavy metal areas. Careful study is necessary to determine whether *B. subtilis* QM3 can alleviate inhibition of wheat seed germination which was induced by heavy metal stress.

The goal of this work was to investigate effect of *B. subtilis* QM3 on the germination of wheat seeds and on the growth and development of seedlings (heterotrophic growth) under impact of heavy metal (Pb) salts. Information from this study will be helpful to identify toxic critical values of Pb in soils based on wheat's response and these parameters; it will also provide a reference for eco-toxicity assessment of Pb in soils.

## MATERIALS AND METHODS

### *Bacteria Materials*

*Bacillus subtilis* QM3 used in the present study come from the Microbiological Lab, College of Life Science, Shanxi Normal University, China. A culture of *B. subtilis* QM3 was obtained by transferring acolony from the activated culture plate into a 250 mL flask containing 100 mL beef extract-peptone medium and shaking in an orbital shaker at 200 rpm at 30 °C for 4 d. Bacterial suspensions for the treatment of seeds were prepared as described: the bacteria are harvested by centrifugation at 8000×g for 10 min at 4 °C. The supernatant was reserved, and the cells were washed by sterile water twice and resuspended in sterile water. There were $10^8$ microbial cells per 1 mL solution. The concentrations used in the experiment were $10^7$ and $10^6$ microbial cells per 1 mL solution.

### *Wheat seeds*

The wheat seeds (*Triticum aestivum* L.) assayed were *Linfeng* 3, recommended by Wheat Research Institute, Shanxi, China. Prior to the germination test, all seeds were surface-sterilized with 5% (v/v) sodium hypochlorite solution for 10 min to prevent fungal growth, washed with distilled water for several times. Seeds were soaked with sterile water (W), $10^7$ CFU/mL (10*) and $10^6$ CFU/mL (100*) *Bacillus subtilis* QM3 for 12h.

### *Experimental conditions*

Seed germination test on filter paper was carried out in glass Petri dishes (90×90 mm) with one layer of filter paper and gauze on the bottom. The heavy metal (Pb) used in this study were in the form of acetate. Each dish contained 9 mL of metal solution (50, 250, 500, 1000, 2000 mg/L) or 9 mL of distilled water (control). Thirty seeds of wheat were then placed on a dish. The Petri dishes were then wrapped with Parafilm and placed in a germination chamber. Petri dishes were maintained for 12 h under dark conditions at 18°C and while maintained for 12 h under light conditions at 25°C.

### *Germination assays*

Germination rate (GR) (%) was recorded every day for 7 days since wheat seeds were grown in distilled water and metal solution, and the seeds were considered to have germinated when a root longer than 1 mm. Other parameters of germination, including seed germination energy (GE) (%) and germination index (GI) (%) were also determined after 3 or 7 days of incubation, respectively. After 7 d growth, shoot length (SL) was measured from culms base to the tip of the longest leaf and root length (RL) was measured from the root-shoot junction to the tip of the longest root. Germination index (GI) and vitality index (VI) were calculated following the equations:

$$GI = \sum (Gt / Dt) \quad (1)$$

$$VI = \sum (Gt / Dt) * S \quad (2)$$

Where, Gt means germination rate at day t, Dt means day t, S means shoot length. Plants were harvested after 7 days.

### *Statistical analysis*

In all experiments, three replicates were performed for each sample, and each treatment was repeated three times. Data presented here are mean values and standard deviation (±SD). One-way analysis of variance (ANOVA) was conducted to determine the difference between different treatments (a significance level of 0.05 was used for all statistical tests).

## RESULTS

### Investigation of the effect of *B. subtilis* QM3 on germination of wheat seeds under the impact of Pb stress

The rate of germination may reflect the reaction rate of plant seeds to their living environment [11]. After soaked with *B. subtilis* QM3, wheat seeds were grown in different concentrations of Pb during the germination stage, results showed significant differences among treatments. Under our experiment conditions, Pb salt treatment had a clear inhibited effect on the GR of wheat seeds (Fig.1). The wheat seeds used in the present study had an average GR of 85.8 % after 7 d imbibition under control (no metal amendment) conditions. The GR was reduced to 80.3% and 72.3% when 250 and 500 mg/L of Pb were added to the incubation solution. When the concentration of Pb was higher, the GR was reduced to 68.0% and 35.0%, respectively. However, *B. subtilis* QM3 treatment had a clear stimulating effect on the germination of wheat seeds. All applied *B. subtilis* QM3 concentrations enhanced GR by 13.0-68.0%. The increased percentage of germination was significant differences at the Pb concentration of 0-2000 mg/L ($p$<0.05) and both the timing and final percentage of germination by day 7 were not affected by addition of 250 mg/L of Pb in the medium ($p$>0.05).

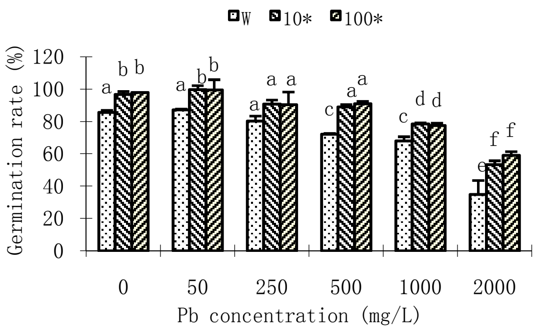


**Figure 1** Effects of *B. subtilis* QM3 on wheat seed germination rate (%) in the presence of Pb. Seed germination rates was codetermined after imbibition for 24 h under various treatments: control; treatment with 50, 250, 500, 1000, 2000 mg/L $Pb^{2+}$.

Data are mean ±SD for 3 replicates for calculation of seed germination. Different letters shown in the error bars mean significant differences among control and treatments ($p$<0.05).

Under our experiment conditions, GE of wheat seeds were decreased with increasing Pb stress (0 to 2000 mg/L) (Fig.2). GE of wheat seeds incubated in distilled water (Control)

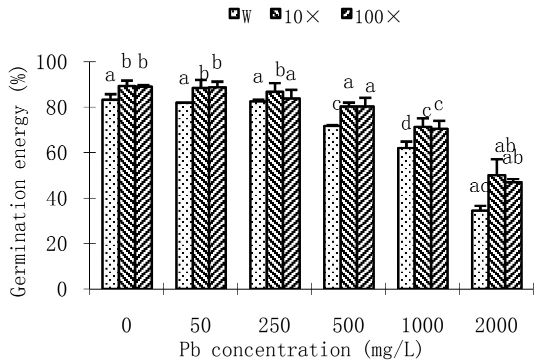


**Figure 2** Effects of *B. subtilis* QM3 on wheat seed germination energy (%) in the presence of Pb. Seed germination energy was codetermined after imbibition for 72 h under various treatments: control; treatment with 50, 250, 500, 1000, 2000 mg/L $Pb^{2+}$.

Data are mean ±SD for 3 replicates for calculation of seed germination. Different letters shown in the error bars mean significant differences among control and treatments ($p$<0.05).

reached a maximum (83.25%) at 7 day, whereas imposition of 50 mg/L Pb led to a decrease of 1.50% in GE. With the increase of Pb concentration, GE was decrease by 13.81% and 25.53%. When the concentration of Pb was highest, GE had a decrease of 58.56%. However, a significant increase was observed in GE of wheat grown from seeds presoaked with *B. subtilis* QM3 compared to respective controls (no *B. subtilis* QM3-presoaking), and the increase was uniform across all concentrations of Pb applied (Fig.2). For example, soaked with $10^7$ CFU/mL *B. subtilis* QM3 led to 5.15 %-44.93 % increase compared with controls and soaked with $10^6$ CFU/mL *B. subtilis* QM3 led to 1.52 %-36.23 % increase compared with controls. It is particularly notable that the GE was no significant difference between soaked with $10^7$ CFU/mL and $10^6$ CFU/mL *B. subtilis* QM3. The results suggested that a certain concentration of *B. subtilis* QM3 could increase GE of wheat seed which grown in Pb salts.

Germination index (GI) and vitality index (VI) are two important parameters that reflect the seed quality. From Fig.3 and 4, it can be seen that GI and VI firstly increased, then decreased with the increase of Pb concentrations. When Pb

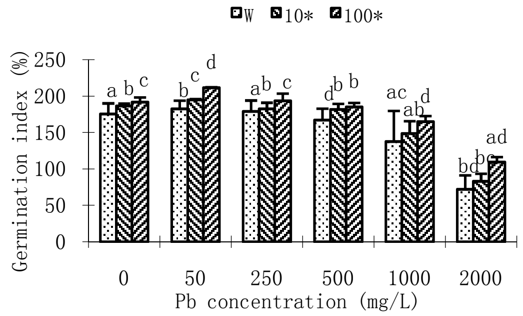


**Figure 3** Effects of *B. subtilis* QM3 on wheat seed germination index (GI) in the presence of Pb.

Data are mean ±SD for 3 replicates for calculation of seed germination. Different letters shown in the error bars mean significant differences among control and treatments ($p$<0.05).

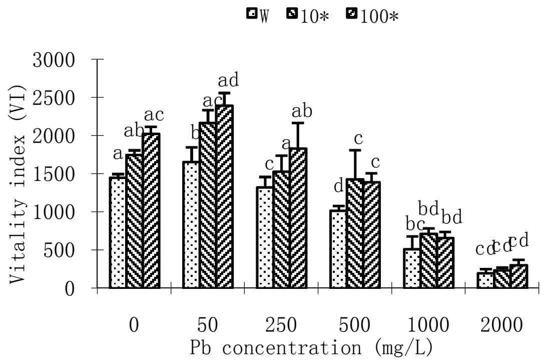


**Figure 4** Effects of *B. subtilis* QM3 on wheat seed vitality index (VI) in the presence of Pb.

Data are mean ±SD for 3 replicates for calculation of seed germination. Different letters shown in the error bars mean significant differences among control and treatments ($p$<0.05).

concentration was 50 mg/L, the GI and VI increased to 103.96% and 120.83%, respectively. They showed poor germination at high concentrations of Pb. GI increased by about 1.84 % at Pb concentration of 250 mg/L and then decreased by 21.68 % and 58.79 %, respectively when Pb concentration of 1000 and 2000 mg/L. VI decreased by 8.68 %, 30.01 %, 64.84 % and 86.52 %, respectively when Pb concentration of 250, 500, 1000 and 2000 mg/L. According to the statistical analysis, GI and VI at the highest Pb concentration had significant difference with those at other concentrations. However, the preparation of *B. subtilis* QM3 evidently protected the wheat seeds, reducing the effect of Pb. Soaked with $10^7$ CFU/mL *B. subtilis* QM3 led to 2.04 %-14.92 % increase in GI compared with controls (without *B. subtilis* QM3 soaking) and led to 15.49 %-40.79 % increase in VI compared with controls. Soaked with $10^6$ CFU/mL *B. subtilis* QM3 led to 8.20 %-51.32 % increase in GI compared with controls (without *B. subtilis* QM3 soaking) and led to 29.09 %-54.56 % increase in VI compared with controls. The results showed that presoaked with *B. subtilis* QM3 could increase GI and VI of wheat seed including those grown in Pd salts.

**Investigation of the effect of *B. subtilis* QM3 on growth and development of wheat seedlings under lead stress**

To examine whether *B. subtilis* QM3 is involved in Pb-induced inhibition of root and shoot growth, the effect of *B. subtilis* QM3 on root and shoot growth in the absence and presence of Pb was investigated. As shown in Table 1, even a low level of Pb did have effect on root growth of wheat seeds. However, following seed germination, stress symptoms in response to exposure to a high level of Pb, including shorter and thickened roots, which changed from creamy color to brown, reduced root hair formation, wilting, and browning at the edge of the seeds were all less evident in 7-day-old wheat seedlings grown from seeds growth with Pb (results not shown). Pb markedly inhibited root length (RL) of wheat seedlings (Table.1). Table.1 shows the RL first increased, then decreased with Pb treatment. The wheat seedlings used in the present study had an average RL of 7.10 cm after 7 d imbibition under control conditions (no metal amendment). When the concentration of Pb was 50 mg/L, it increased slightly to 105.07%. The RL was reduced to 96.20%, 54.79%, 6.06% and 2.25% when 250, 500, 1000 and 2000 mg/L of Pb were added to the incubation solution. These results indicate that the wheat seedling was sensitive to Pb, and the growth of root was all inhibited by high concentration of Pb (500-2000 mg/L). However, soaked with $10^7$ CFU/mL *B. subtilis* QM3 led to 9.52%-48.84% increase in RL compared with controls (without *B. subtilis* QM3 soaking) and soaked with $10^6$ CFU/mL *B. subtilis* QM3 led to 13.00%-62.50% increase in RL compared with controls. These implied a partial protection from the effects of Pb (Table. 1). When grown in Pb medium wheat seedlings from seeds that were pretreated with *B. subtilis* QM3 had significantly longer roots than untreated seedlings ($p$<0.05), and in the absence of Pb, pretreatment with *B. subtilis* QM3 did also affect RL compared to the control ($p$<0.05).

Other vegetative response endpoints, such as shoot growth, were examined to investigate metal toxicity. Pb also markedly inhibited SL of wheat seedlings (Table. 2). Table.2 shows the SL first increased, then decreased with Pb treatment. When the concentration of Pb was 50 mg/L, it increased to 108.94%. After then it decreased. The SL reduced by 10.35%, 26.12%, 51.76% or 63.88%, respectively, at the Pb concentrations of 250, 500, 1000 and 2000 mg/L. According to the statistic analysis (Table 2), Pb had a significant adverse effect on SL ($p$<0.05) of wheat seedlings. Results suggest that root growth was more sensitive to Pb than shoot growth. There were significant differences in SL among wheat seedlings pretreated singly with $10^7$ and $10^6$ CFU/mL *B. subtilis* QM3, *subtilis* QM3 evidently protected the shoot system, reducing the effect of Pb. Soaked with $10^7$ CFU/mL *B. subtilis* QM3 led to 11.5%-28.5% increase in SL compared with controls (without *B. subtilis* QM3 soaking) and SL was not affected at the highest Pb concentration (2000 mg/L). Soaked with $10^6$ CFU/mL *B. subtilis* QM3 led to 19.3-24.6% increase in SL compared with controls and SL was also not affected by addition of 2000 mg/L of Pb in the medium. The results suggest that the level of SL was elevated in Pb-exposed seedlings grown from seeds pretreated with *B. subtilis* QM3, whose value was significantly different from that of the *B. subtilis* QM3-free controls.

**Table 1** Effects of *B. subtilis* QM3 on length of root (cm) in wheat in the presence of Pb.

| | **Pb concentration (mg/L)** | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **50** | **250** | **500** | **1000** | **2000** |
| W | 7.10±2.33 a | 7.46±2.00 d | 6.83±0.00 d | 3.89±0.03 ac | 0.43±0.08 bc | 0.16±0.04 cd |
| 10* | 7.98±2.14 b | 8.57±1.54 c | 7.48±0.65 a | 4.59±0.12 ab | 0.64±0.03 ad | 0.19±0.06 cd |
| 100* | 8.38±1.17 c | 8.43±0.21 c | 7.76±0.13 b | 4.58±0.22 ab | 0.51±0.02bd | 0.26±0.05 ba |

Data are mean ±SD for 3 replicates for calculation of length of wheat roots. Different letters within a column mean significant differences among control and treatments ($p$<0.05).

**Table 2** Effects of *B. subtilis* QM3 on length of shoot (cm) in wheat in the presence of Pb.

| | **Pb concentration (mg/L)** | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **50** | **250** | **500** | **1000** | **2000** |
| W | 8.50±0.09 a | 9.26±0.79 b | 7.62±0.44 ab | 6.28±0.68 ac | 4.10±0.67 ad | 3.07±1.29 bd |
| 10* | 9.48±0.17 b | 11.17±0.93 d | 8.64±0.77 a | 8.07±1.80 a | 5.23±0.13 bc | 3.14±0.89 bd |
| 100* | 10.59±1.15 c | 11.31±0.87 d | 9.42±0.77 b | 7.49±0.21 ab | 4.99±0.10 bc | 2.82±0.99 cd |

Data are mean ±SD for 3 replicates for calculation of length of wheat shoots. Different letters within a column mean significant differences among control and treatments ($p$<0.05).

## DISCUSSION

In the present study, we reported that applications of *B. subtilis* QM3 bacterial suspension alleviated the inhibition of wheat seed germination process involved in the germinative morphology parameter and seedling growth of wheat in the presence of different concentrations of Pb (Tables 1 and 2; Figs. 1- 4), further providing evidence for the beneficial effect of *B. subtilis* QM3 on plant seeds in Pb condition.

Pb is one of the most abundant and ubiquitously distributed toxic elements [12]. It exerts adverse effects on morphology [13], growth [4] and photosynthetic processes [14] of plants and causes inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition. The most common response of plants to stress conditions, including excess concentrations of heavy metals, is restriction of growth [15]. Plant's tolerance to heavy metals is usually estimated on the basis of the degree of their root or shoot growth inhibition by the metal present in a nutrient solution [16].

In 2010, Yingli Yang et al. reported that high concentrations of Pb significantly inhibited seed germination and the growth of roots and shoots [3]. Mostafa Lamhamdi et al. also reported that Pb accumulation in seedlings was positively correlated with the external concentrations, and negatively correlated with morphological parameters of plant growth [5], revealing the toxic action of Pb in plant growth and development. However, some researches [4, 17, 18] found that low concentrations of Pb in the nutrient solution stimulated seed germination, while high concentrations resulted in the inhibitory effect, suggesting the dual role of Pb in plants. These dual effects of Pb on seed germination were partially confirmed by our experiment with treatments of various Pb solutions (Tables 1 and 2; Figs 3 and 4). The results indicated the decrease in GR and GE (Figs 1 and 2) of the studied seeds along with the increase of Pb concentrations. That is due to that Pb could possibly be attributed to the interference with metabolic and biochemical processes associated with normal growth and development of the plants [19]. The phenotypic were statistically significant differences with the increase of Pb concentrations. High concentrations of Pb (1000 and 2000 mg/L) played much influence on the restraining the development of seedlings than low concentrations of Pb (50 mg/L). Therefore, exploring various ways to improve crop productivity and/or alleviate Pb stress effects is one of the major areas of concern.

Nowadays, there were many ways to alleviation of heavy metals stress-induced inhibition of seed germination and growth of plants, most of them were physical and chemistry method. For example, the results of ZongBo Qiu et al. showed that pretreated with He-Ne laser had a positive physiological effect on the growth of cadmium stressed seedlings [20]. Meng Wang et al. studied six types of nanoparticles (NPs) (Kaolin, montmorillonite, hydroxyapatite, $Fe_3O_4$, α-$Fe_2O_3$ and γ-$Fe_2O_3$) to alleviated cadmium-induced root growth inhibition in crop seedlings using standard toxicity test, results showed that NPs had a short-term effect of alleviation [21]. NO functions as cell signaling molecule in plants and also play important role in the regulation of plant responses to both abiotic and biotic stress conditions [22-24], it often as heavy metals stress modulator in crop plants. Treatments with exogenous indole-3-acetic acid and salicylic acid [25], glutathione [26], hydrogen sulfide [27], phosphate compounds [28] and $H_2O_2$ [29] were reported also could alleviate the inhibition in plants under abiotic stress conditions.

However, microbe-based removal is now considered to be an effective alternative method to the conventional processes and is receiving greater levels of interest for potential uses in bioremediation [30]. Plant Growth Promoting Rhizobacteria (PGPR), whose role is still underestimated, plays an important (or perhaps essential) role in improving plant growth. The comprehensive understanding of bacterial plant growth promoting mechanism helps to get sustainable agriculture production under biotic and abiotic stresses [31]. The inoculation by plant growth promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) represents an alternative as potential bio fertilizer resources for salty areas was observed by Edgar Omar Rueda-Puente et al. [32]. The establishment of Arbuscular mycorrhizal (AM) symbiosis involves major changes in the physiology of the host plant and a modulation of plant responses to biotic and abiotic stresses [33, 34]. In particular, AM symbioses have received much attention for their ability to alleviate heavy metal stress in plants [35]. *Pseudomonas putida* Rs-198 strains could protect against salt stress and promote cotton seedling growth, it also exhibited the ability to increase the cotton's germination rate and healthy stand [36]. Strains of *Bacillus subtilis* have earlier been shown to synthesize plant growth-promoting substances, such as gibberellins and indole acetic acid, ACC deaminase, extracellular phytase, chitinase, antifungal peptides and increased yields in various crop plants [37, 38].The present study demonstrated how inoculation of *B. subtilis* QM3 ameliorated inhibition heavy metals induced stress in wheat and improve its growth under hydroponics conditions (Figs 1-4 and Tables 1 and 2). Stimulation of root growth and effective root area for enhanced water and nutrient uptake is the most important stress management tool because a healthy, strong and proliferated root system plays major role in helping the plant to maintain optimal growth and development under stress conditions [39]. An overall increase in the wheat plant seed germination, growth and profuse rooting observed in the *B. subtilis* QM3 inoculated plants compared with the uninoculated controls is in accordance with earlier studies.

## CONCLUSIONS

Some studies have shown that heavy metals as cadmium [40, 41, 17], lead [3, 5, 42] and copper [16, 43] produce the strongest effect on a plant's phenotype and genotype. Model experiments showed that lead salt inhibited wheat seed germination. Treatment of wheat seeds with 500 mg/L and higher concentrations of the Pb salt caused inhibition of root morphogenesis of wheat seedlings at early stages of their development. However, low Pb concentrations stimulated seed germination and root morphogenesis.

This study revealed the way the *B. subtilis* QM3' influences on seed germination under lead stress. It was shown that at the concentrations of $10^7$, $10^6$ CFU/mL *B. subtilis* QM3 of the preparation could reduce the adverse affect of the lead salt on wheat seed germination. It was particularly important to estimate the feasibility of reducing the inhibitory effect of the lead salt on growth and development of wheat seedlings. In the experiments, *B. subtilis* QM3 reduced inhibitory effect of the lead salt on root development and stimulated root morphogenesis. At 50-500 mg/L of the lead salt, the *B. subtilis* QM3' preparation reduced the inhibitory effect of lead.

Our experimental datas and evidences suggest significance of *B. subtilis* QM3 treatment to wheat under hydroponic conditions in ameliorating the lead stress possibly through increased the percentage of germination and root elongation utilizing microbes in the wheat rhizosphere along with upregulation or repression of set of stress responsive response in plants. To our knowledge, this study is the first analysis of plant growth promotion in wheat plants responding to lead stress for 7 days in the presence of PGPB. The results suggested that *B. subtilis* QM3 exerts a significant beneficial effect on the growth and development of wheat subjected to Pb stress. Despite not explored at the overall level, our results strongly point to the suggestion that the *B. subtilis* QM3 play an important role in the growth of wheat to moderate Pb stress. The results presented here constitute an initial step toward the characterization and understanding the networking of abiotic and biotic stress signals in wheat to respond the adverse growth conditions. Development of a plant as a whole depends on an early phases of plant seed germination. Results based on the beneficial plant-microbe interactions indicate that it is possible to develop *B. subtilis* QM3 as bio-inoculant or buffer and mitigate adverse effects of heavy metals at early phases of plant seed germination.

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