



# Effects of *Bacillus subtilis* QM3 on Germination and Antioxidant Enzymes Activities of Wheat Seeds under Salt Stress

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## Abstract

Salt is an important environmental factor that affects wheat growth and development and restricts crop production. *Bacillus subtilis* QM3 is a strain of antagonistic strains which have biological control function. In this paper, the effects of wheat seed, and soaked treatment by *B. subtilis* QM3, on germination and antioxidant enzyme activities under salt stress were studied. The results showed that under salt stress, *B. subtilis* QM3 soaking could increase the germination percentage (GP), germination potential (GT), and germination index (GI) of wheat, and significantly increase the activity of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) ( $p < 0.05$ ). And high salt concentration had an adverse effect on wheat seed germination. When NaCl concentration were 100, 150, 200 mmol/L, GP, GT, GI decreased to 58.0%, 41%, 61 a/dy, and SOD, POD, CAT decreased to 133.12 g·min, 106.62 g·min, 6.68 g·min. However, the *B. subtilis* QM3 influenced the variables evaluated. After soaking with *B. subtilis* QM3, the morphological index GP, GT, GI was significantly increased by 12.99%, 41.18%, and 38.81% at NaCl concentration of 150 mmol/L. Under salt stress (50 mmol/L), *B. subtilis* QM3 treatment maximized the relative growth rate of CAT activity, which was 87.25%. Similarly, when the NaCl concentration was 150 mol/L, the relative growth rate of SOD activity and POD activity was the greatest, which were 35.81% and 36.57%. It was concluded that *B. subtilis* QM3 treatment on wheat seeds may be a good option to improve seed germination under salt stress.

## Subject Areas

Biochemistry, Bioengineering

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## Keywords

*B. subtilis* QM3, Seed Germination, Antioxidant Enzymes, Salt Stress

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## 1. Introduction

Salt stress is a widespread environmental problem, although great effort has been made on this problem [1]. It is well known that salinity affects almost every aspect of plant physiology and biochemistry. Salt stress decreases seed germination, retards plant development and reduces crop yield [2]. Excessive salt ions are absorbed by the plant, breaking the ion homeostasis of plant cells, causing osmotic stress, affecting the activity of the enzyme, and accumulating a large amount of toxic substances such as active oxygen in the cells. And several studies indicate that under salt stress, increased activity of antioxidant enzymes such as CAT, POD and SOD can significantly increase plant resistance.

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world. Seed germination is a crucial stage in the life history of wheat. Under salt stress, seeds are very sensitive to salinity during germination and seedling stage. The salinization of the soil and other abiotic factors severely restrict the enhancement of wheat production. Therefore, research breakthroughs in this area are of great significance for improving wheat yield and quality.

A few studies have focused on alleviation of salt stress of plant, while the studies on the mitigate effect of bacteria on plant growth under salt stress are limited. *B. subtilis* is a non-pathogenic bacterium that is widely found in nature and has strong resistance to stress. *B. subtilis* QM3 is an antagonistic strain with biological control functions. It is isolated from the feces of Qinghai yak [3]. Previous studies have shown that it can promote the germination of wheat seeds, but it has not been studied whether it can improve the salt tolerance of seed germination.

The aim of this study was to explore the influence of *B. subtilis* QM3 on germination (*GP*, *GT*, *GI*) and antioxidant enzymes activities (SOD, POD and CAT) of wheat growing under different concentrations of salinity stress. The research results will provide some theoretical and practical bases to increase crop production in salt lick.

## 2. Materials and Methods

### 2.1. Media

The commonly used media in this investigation were the beef extract peptone containing 1.0% peptone, 0.3% beef extract, 0.5% sodium chloride, 1.5% - 2.0% agar, pH 7.4 - 7.6, 1000 ml distilled water 121°C, 20 min sterilization.

The concentrations of salt were 0, 50, 100, 150, 200 mmol/L and were prepared freshly as NaCl.

## 2.2. Bacteria Materials

*B. subtilis* QM3 used in the present study come from the Microbiological Lab, the School of Life Science, Shanxi Normal University. A culture of *B. subtilis* QM3 was obtained by transferring a colony from the activated culture plate into a 250 mL flask containing 100 mL beef extract-peptone medium and shaking in an orbital shaker at 150 rpm at 37°C for 3 d [4]. Bacterial suspensions for the treatment of seeds were prepared as described: the bacteria are harvested by centrifugation at 3000× g for 10 min at 4°C. The supernatant is reserved, and the cells are washed by sterile water twice and resuspended in sterile water adjust to OD<sub>600</sub> = 0.872. There were 10<sup>8</sup> microbial cells per 1 ml solution. The concentration used in the experiment was 10<sup>6</sup> microbial cells per 1 ml solution.

## 2.3. Seed Materials

Seeds of the Lin feng No.3 (*Triticum aestivum* L.) came from Shanxi academy of agricultural sciences. Healthy seeds of similar size and mass were selected about more than 4000 seeds. Prior to germination, seeds were surface sterilized with 0.1% mercuric chloride for 5 - 10 minute to avoid fungus attack, and subsequently rinsed with sterile water [1].

## 2.4. Germination Assay

Seeds were soaked with sterile water (Q), bacterium suspension (Y) and supernatant liquid (L) for 12h at 25°C. Immediately after soaking 50 seeds were sown in each petri dishes. A filterpaper was dipped into petri dishes (9 cm diameter) with 20 ml NaCl solution of 0 mg/L, 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L, respectively. The seeds were laid on filter papers uniformly with the embryo upturned and stored into the illumination plant box. The cultivated condition was set as follows: illumination lasting 12 h, light intensity is 1500 lux, temperature at 25°C (daytime) or 18°C (night), and moist condition [5]. To avoid changes in salinity, the original solution in each petri dish was removed daily and 10 ml of new solution was added and removed again as completely as possible [1]. All experiments described here were repeated three times. Seeds per petri dish were harvested 7 d after sowing.

## 2.5. Determination

### 2.5.1. Physiological Indexes

Radicle protrusion was used as the criterion of germination, and the numbers of germinated seeds on the day after initiation were measured germination percentage throughout the test period. Seeds showing a normal epicotyl were considered as being alive. In the germination experiment, follow the method described by Lina Zhou *et al.* [6]. The number of germinated seeds was recorded each day. *GP*, *GT* and *GI* were calculated following the equations:

$$\begin{cases} GP(\%) = (G_i/T) \times 100\% \\ GT(\%) = N^{3d}/T \times 100\% \\ GI = \sum(G_i/D_i) \end{cases} \quad (1)$$

where,  $G_t$  means number of germination at  $t^{\text{th}}$  day,  $T$  means selected number of seeds,  $N^{3^{\text{d}}}$  means number of germinated seeds in 3<sup>rd</sup> day,  $D_t$  means number of days up to  $t^{\text{th}}$  day.

### 2.5.2. Antioxidant Enzymes Assays

SOD was assayed by the photochemical method described by Giannopolitis and Ries [7]. POD activity was assayed following the method of Xu and Ye with minor modifications [8]. For this, 0.1 g of fresh seeds were homogenized in an ice bath in 5 mL 50 mmol/L borate buffer (pH 8.7) containing 5.0 mmol/L sodium hydrogen sulphite and 0.1 g PVP. The homogenate was centrifuged at  $10,000 \times g$  for 25 min at 4°C. The supernatant was used as enzyme extract. To 0.1 mL of the enzyme extract, a substrate mixture containing acetate buffer (0.1 mmol/L, pH 5.4), Ortho-Dianisidine (0.25% in ethyl alcohol) and 0.1 mL 0.75% H<sub>2</sub>O<sub>2</sub> was added. Also, CAT activity was assayed using the method described by Aebi [9].

### 2.6. Statistics Analysis

All data are the average of three repetitions. The obtained results were analyzed statistically by SPSS (version 11.0) statistical software. Data were analyzed by the analysis of variance and treatment mean comparison by using least significance difference (LSD;  $p = 0.05$ ).

## 3. Results

### 3.1. Effect on Wheat Seed Germination Soaked by *B. subtilis* QM3 under Salt Stress

Q, L and Y respectively represent the control group (treated with sterile water), supernatant liquid and bacterium suspension, GP, GT, and GI in the table respectively represent the germination percentage, the germination potential and the germination index. Data in the table is average  $\pm$  standard error. The uppercase letters in the same vertical column of the table represent significant differences ( $p < 0.05$ ) between sterile water treatments under different NaCl stress. Different lowercase letters represent a significant difference between the *B. subtilis* QM3 treatment and the Q under the same NaCl stress ( $p < 0.05$ ).

In this experiment, seeds germinated for 3 days were selected to determine their germination indexes. The results are shown in **Table 1**. Compared with the control group (Q), *B. subtilis* QM3 (L, Y) significantly increased the level of wheat seed germination (50, 100, 150, 200 mmol/L). When without NaCl stress, L and Y treatments compared with Q, the GP was significantly increased by 4.44% and 5.22%; the GT increased by 6.58% and 15.79%; the GI increased by 6.30% and 22.05%. This result indicated that *B. subtilis* QM3 had an obvious promotion effect on GP, GT, GI. When without *B. subtilis* QM3 treatment, Treatment of wheat seeds with 100 mmol/L and higher concentrations of the NaCl caused inhibition of morphogenesis of wheat seedlings germination. When NaCl concentration were 100 mmol/L, L and Y treatments compared with Q, the GP was significantly increased by 11.11% and 11.6%; the GT increased by 23.33%

**Table 1.** Effect on wheat seed germination soaked by *B. subtilis* QM3 under salt stress.

NaCl Concentration (mmol/L)	GP (%)			GT (%)			GI (a/dy)		
	Q	L	Y	Q	L	Y	Q	L	Y
0	95.0 ± 0.11Aa	96.0 ± 0.14a	97.0 ± 0.05a	82.0 ± 0.61Ab	87.0 ± 0.47a	98.0 ± 0.31a	137.0 ± 0.95Ab	153.0 ± 0.78a	155.0 ± 1.02a
50	94.7.0 ± 0.16Aa	96.5 ± 0.11a	97.0 ± 0.12a	81.4 ± 0.83Ab	88.0 ± 0.54a	88.6 ± 0.35a	136.0 ± 0.24Ac	145.0 ± 0.65b	150.0 ± 0.81a
100	81.0 ± 0.17Bc	90.0 ± 0.25b	90.4 ± 0.07a	60.0 ± 0.57Bc	72.0 ± 0.63b	75.0 ± 0.27a	80.0 ± 0.87Bb	102.0 ± 0.72a	101.0 ± 0.87a
150	77.0 ± 0.09Cc	82.0 ± 0.15b	86.7 ± 0.10a	51.0 ± 0.33Cc	68.0 ± 0.37b	72.0 ± 0.52a	67.0 ± 0.74Cc	90.0 ± 01.05b	93.0 ± 0.98a
200	58.0 ± 0.28Db	62.0 ± 0.24a	63.0 ± 0.03a	41.0 ± 0.65Db	48.0 ± 0.43a	50.0 ± 0.38a	61.0 ± 0.86Db	67.0 ± 0.58a	66.0 ± 0.86a

and 25%; the *GI* increased by 27.5% and 26.25%; when NaCl concentration were 150 mmol/L, L and Y treatments compared with Q, the *GP* was significantly increased by 12.99% and 12.6%; the *GT* increased by 33.33% and 41.18%; the *GI* increased by 37.31% and 38.81%; when NaCl concentration were 200 mmol/L, L and Y treatments compared with Q, the *GP* was significantly increased by 10.34% and 12.07%; the *GT* increased by 17.07% and 21.95%; the *GI* increased by 9.83% and 8.2%. The above data shows, when NaCl concentration was 100, 150 and 200 mmol/L, Y and L was higher than that of Q. When NaCl concentration were 150 mmol/L, *B. subtilis* QM3 had the best treatment effect. In a certain degree, *B. subtilis* QM3 had an alleviation effect on the germination of the wheat seeds under NaCl stress.

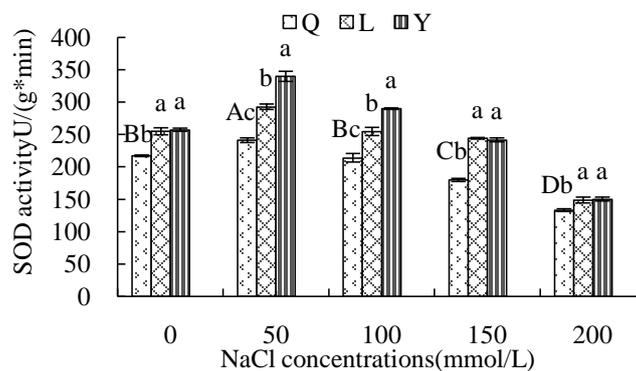
### 3.2. *B. subtilis* QM3 Soaking Effect on Activities of Antioxidant Enzymes under Salt Stress

#### 3.2.1. SOD Activity

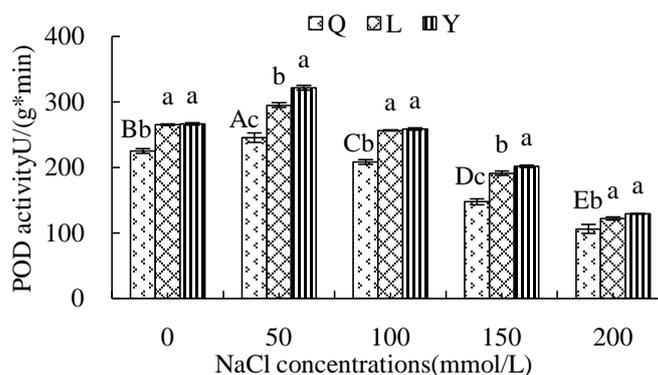
*B. subtilis* QM3 soaking effect on SOD activity under salt stress (Figure 1). With the increase of salt concentration, the SOD activity of wheat seeds increased and then decreased. Basically, under the salt concentration of 50 mmol/L, the SOD activity reached a maximum level, compared with Q, L and Y respectively increased by 21.35% and 40.84%; under the NaCl concentration were 50, 100, 150 and 200 mmol/L, the SOD activity of L and Y treatment was higher than control treatment (Q). When the salt concentration was 150 mmol/L, the increase was the largest compared with Q, and L and Y increased by 35.81% and 34.42%, respectively. At a salt concentration of 200 mmol/L, L and Y increased by 11.9% and 12.91%, respectively, compared to Q. Under the salt stress (50, 100, 150, 200 mg/L), *B. subtilis* QM3 could improve SOD activity.

#### 3.2.2. POD Activity

NaCl treatment caused POD activity of L and Y treatments for wheat seed and the Q showed a tendency that POD activity were increased and then decreased with the increase of NaCl concentration (Figure 2). Under the salt concentration of 50 mmol/L, the POD activity reached a maximum level. Compared with Q, L and Y respectively increased by 20.27% and 31.17%, under the NaCl concentration were 50, 100, 150 and 200 mmol/L, the POD activity of L and Y treatment



**Figure 1.** *B. subtilis* QM3 soaking effect on SOD activity under salt stress. Q, L, and Y respectively represent the control group (treated with sterile water), supernatant liquid and bacterium suspension. The uppercase letters in the figure represent significant differences ( $p < 0.05$ ) between sterile water treatments under different NaCl stress. Different lowercase letters represent a significant difference between the *B. subtilis* QM3 treatment and the Q under the same NaCl stress ( $p < 0.05$ ).

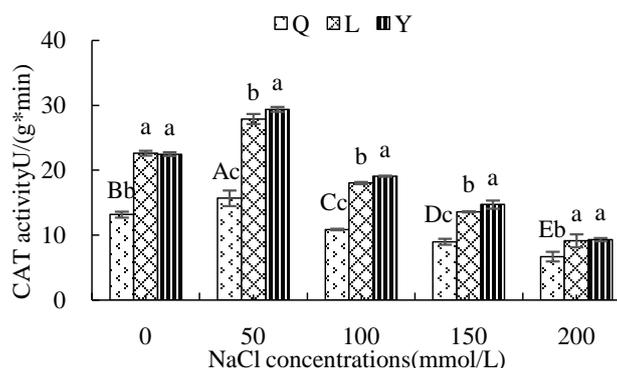


**Figure 2.** *B. subtilis* QM3 soaking effect on POD activity under salt stress. Q, L, and Y respectively represent the control group (treated with sterile water), supernatant liquid and bacterium suspension. The uppercase letters represent significant differences ( $p < 0.05$ ) between sterile water treatments under different NaCl stress. Different lowercase letters represent a significant difference between the *B. subtilis* QM3 treatment and the Q under the same NaCl stress ( $p < 0.05$ ).

were higher than that of control treatment (Q). When the salt concentration was 150 mmol/L, the increase was the largest compared with Q, and L and Y increased by 29.45% and 36.57%, respectively. Under the salt stress (50, 100, 150, 200 mg/L), *B. subtilis* QM3 could improve POD activity.

### 3.2.3. CAT Activity

With the increase of salt concentration, the CAT activity of wheat seeds increased and then decreased (Figure 3). On the whole, under the NaCl concentration were 50, 100, 150 and 200 mmol/L, the CAT activity of L and Y treatment were higher than that of control treatment (Q). However, when NaCl concentrations were 50 mol/L, *B. subtilis* QM3 treatment was increased obviously than any other treatment, and L and Y increased by 77.68% and 87.25%, respectively. And when NaCl concentrations were 200 mmol/L, *B. subtilis* QM3 treatment



**Figure 3.** *B. subtilis* QM3 soaking effect on CAT activity under salt stress. Q, L, and Y respectively represent the control group (treated with sterile water), supernatant liquid and bacterium suspension. The uppercase letters in the figure represent significant differences ( $p < 0.05$ ) between sterile water treatments under different NaCl stress. Different lowercase letters represent a significant difference between the *B. subtilis* QM3 treatment and the Q under the same NaCl stress ( $p < 0.05$ ).

was the lowest at all. Under the salt stress (50, 100, 150, 200 mg/L), *B. subtilis* QM3 could improve CAT activity.

#### 4. Discussion

Excess salt stress can reduce seed germination, delay plant development, break the ion homeostasis of plant cells, affect the activity of enzymes, and accumulate a large amount of toxic substances such as active oxygen in cells. Salt stress can reduce seed germination, delay plant development, break the ion homeostasis of plant cells, affect the activity of enzymes, and accumulate a large amount of toxic substances such as active oxygen in cells.

Compared with the late stage, the early growth stage of the plant is more sensitive to salt, so it is particularly important to inoculate bacteria with reduced salt stress to make it more salt-tolerant [10]. Our result was carried out to evaluate exogenous application of *B. subtilis* QM3 enhancing NaCl tolerant of wheat through assessing on seed germination and antioxidant enzyme activity. The results show that excess salinity adversely (100, 150, 200 mmol/L) affected the germination of wheat, regardless of the *B. subtilis* QM3 treatment and the salt-stress level. And with increasing salt concentration, germination was progressively inhibited in the absence of bacteria. However, when the seeds were inoculated with *B. subtilis* QM3 the extent of seeds suppression was decreased and these treated seeds had greater *GP*, *GT* and *GI* than untreated seeds. On the other hand, *GP*, *GT* and *GI* significantly decreased with an increase in NaCl concentration from 100 to 200 mmol/L. When NaCl concentration were 150 mmol/L, *B. subtilis* QM3 had the best treatment effect. This clearly demonstrates that the selected bacterium can alleviate some of the debilitating effects of salt stress, and bacterial suspension has the best treatment effect.

Under the condition of salt treatment, crops accumulate excessive reactive oxygen species (ROS), which cause peroxidation damage to crops. Plant cells

have armed with a distinct antioxidant defense system that keeps ROS at minimal level by employing various enzymes like SOD, POD and CAT [11]. These enzymes resistance is positively correlated with enzyme activity.

SOD modulates relative amounts of  $O_2^{\cdot-}$  and decreases the risk of  $^{\cdot}OH$  radical formation.

CAT is the main scavenger of strong oxidant  $H_2O_2$  in peroxisomes and it converts  $H_2O_2$  to water and molecular oxygen. Generally, the increases of CAT activity are a strategy for improving the salt tolerance [12].

Like CAT, POD plays also a vital role in plant defense against oxidative stress by scavenging  $H_2O_2$  in chloroplast, cytosol, mitochondria and peroxisome of plant cells [13].

In our study, with the increase of salt concentration, SOD activity POD activity and CAT activity of all treatments for wheat seeds showed a tendency that SOD activity and POD activity were increased and then decreased. Under the NaCl concentration of 50 mmol/L, the wheat antioxidant enzyme activity was promoted, it may due to the dual role of NaCl in plant. When with *B. subtilis* QM3 treatment, enzyme activity of wheat seeds under the treatment of L and Y were higher than that of Q. When the NaCl concentration was 50 mol/L, CAT activity was the greatest. Under salt stress (150 mmol/L), *B. subtilis* QM3 treatment maximized the relative growth rate of SOD activity and POD activity. And Y treatment was enhanced obviously than any other treatments. Based on these findings, *B. subtilis* QM3, was effective with respect to alleviating the negative effect of salinity on the growth of Wheat.

Overall, our results implied that salt stress caused severe damage on morphological indicators and antioxidant enzyme activity during the germination period of wheat seeds. And *B. subtilis* QM3 was effective with respect to alleviating the negative effect of salinity on the growth of Wheat.

## 5. Conclusion

To conclude, inoculating seeds with *B. subtilis* QM3 increased the germination percentages, GT and GI of wheat exposed to salt stress. When the plants exposed to NaCl, the bacterium depressed NaCl induced oxidative stress by improving antioxidant enzymes (SOD, POD, CAT), and promoting relative plant growth, resulting in increased plant tolerance to NaCl. The results of this study demonstrate that excess salinity is detrimental to wheat growth and *B. subtilis* QM3 inoculated have a positive effect on growth of wheat under salt stress. Selection of suitable inoculation methods is more conducive to promote wheat production, thus increasing crop yield. This phenomenon may be related to the combination of bacteria and wheat seeds, but the deeper reasons need to be further studied.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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