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Enhancing the NaCl Tolerance Potential of Wheat on Root Morphology and Osmoregulation Substance by Exogenous Application of *Bacillus subtilis* QM3

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Abstract

Bacillus subtilis QM3 is a strain of antagonistic strains which have biological control function. Salinity is the most important limiting plant factor for agriculture development and growth changes in wheat. The present study was carried out to test tolerance involved in salt stress alleviation in wheat (*Triticum aestivum* L.) through the root application of *B. subtilis* QM3. Experiment was performed after germinating for two days. Wheat root was first grown under *B. subtilis* QM3 with four levels (10^8 CFU/ml *B. sutilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4)), then poured with saline regime of NaCl of six concentrations (50, 100, 150, 200, 250, 300 mmol/L) compared along with control. The results of present study showed that *B. subtilis* QM3 clearly promoted the growth of the root morphology and the content of osmoregulation substance ($p < 0.05$), and 10^6 CFU/ml *B. subtilis* QM3 (M3) was found to be very effective in mitigation of NaCl stress by promoting the growth of the wheat root and adjusting the content of osmoregulation substance in wheat root.

Subject Areas

Microbiology

Keywords

B. subtilis QM3, Salinity, Osmoregulation Substance, Root Morphology, NaCl Stress

1. Introduction

Salinity is one of the major environmental challenges and it causes a substantial crop

revenue loss [1]. At present, soils of salt affected existed mostly under arid, semiarid climate because low humidity and high temperature covered approximately 800 million hectares of world [1]. According to incomplete statistics, 20% agriculture lands are affected by salinity at world level [2]. Growth of the crops is usually diminished due to high or fluctuating salt concentrations. Salt inclusion is one of the principal mechanisms which impair the osmotic adjustment due to accumulation of inorganic ions and particularly Na^+ and Cl^- to the toxic levels within plants through transpiration stream. The study showed that accumulation of Na^+ and Cl^- was increased and K^+ was decreased as concentration of NaCl was increased [3]. Generally, those plants have high affinity for K^+ uptake, while transporters will have low Na^+ uptake. Similar results had been observed in other reports [4] [5]. High concentration of NaCl in the soil poses osmotic stress and ionic stress, which reduce the ability of plants to absorb water and minerals [6]. Excessive accumulation of sodium (Na^+) ion in the cytosol damages cell membrane, which leads to leakage of electrolytes and affects metabolic activities in cytosol.

Seedling growth of several crops was affected by a detrimental factor, which created an osmotic potential in the rhizosphere of the plant. Na^+ and Cl^- compete with other nutrients including K^+, Ca^{2+}, and NO^{3-}, which may cause unbalance distribution and act negatively on biophysical and metabolic processes [7]. Roots and the whole crop inhibit the absorption of water or create toxic effect due to Na^+ and Cl^- [8]. A study conducted by Ahmad and Riffat [9] on peas showed that all these changes were associated with reduction in relative water contents and K^+ uptake. According to this study, proline and sugar contents were significantly increased while nitrate reductase activity and chlorophyll contents were decreased.

Wheat is the third largest crop products and occupies an important position in the globe. A world population growing in number will require more grain each year. However, the salinization of the soil and other abiotic factors severely restrict the enhancement of wheat production. So, it is necessary to find methods to improve the ability of wheat to tolerate salinity. It is well known that root is the most important part of cereal, which not only can support fixation plant, but also can absorb and store salinity in the soil. Therefore, the study of plant root system has important significance on effects of salinity in soil.

In the recent past, many researchers have focused on the performance of plant growth promoting rhizobacter. 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. *Bacillus subtilis*, a prokaryotic life form all around the world, is a big family that exhibits adaptation to include high salinity, drought, high and low temperatures and contaminated soils. *B. subtilis* QM3, a strain of antagonistic strains, has biological adjustment and control functions and is isolated from dung of Qinghai yak. Some studies have showed that *B. subtilis* QM3 improved germination rate of wheat seed and eased heavy metal stress of wheat seeding. So it is necessary to probe whether *B. subtilis* QM3 can mitigate inhibition of wheat root growth under salinity stress.

The present investigation intends to study the influence of *B. subtilis* QM3 on morphology and metabolites (total soluble sugar, soluble proteins, proline and malon dialdehyde) of wheat root 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. Plant Material and Bacterial Suspension Preparati

Seeds were purchased from the Research Institute of wheat in Shanxi Province of china *B.subtilis* strains QM3 were isolated from Shanxi normal university, college of life science microbiology laboratory. These strains were inoculated in a flask of 250 mL volume containing nutrient broth media (NB) and vigorous shaking (200 rpm) at 37˚C three to four days. These cultures were centrifuged 4000rpm for 10minutes at 20˚C each time, then refuse to go to clear liquid. This process was repeated use of sterile water three times. Finally these bacterial suspension liquid diluted to until the OD_{600nm} in 0.8 to 0.9 (10^8 CFU/ml *B. subtilis* QM3). Followed, 10^8 CFU/ml *B. subtilis* QM3 which bacterial suspension liquid was diluted 10 times (10^7 CFU/ml *B. subtilis* QM3), 100 times (10^6 CFU/ml *B. subtilis* QM3) and 1000 times (10^5 CFU/ml *B. subtilis* QM3) to reserve.

2.2. Wheat Germination, Bacterium Suspension Treatment and NaCl Stress Treatment

Seeds were selected to uniform, sterilized of 0.1% HgCl for 10 minutes then rinsed three times with sterile water and placed onto meter glass soaked in sterile water for 24 h. Then seeds were divided into five large groups germination of two days, each large group was placed in 7 petri dishes, five large groups respectively cultivated with sterile water (CK), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4) for 2 days. Then exogenous application of NaCl with seven doses (50, 100, 150, 200, 250 and 300 mmol/L) were applied along with control. After three days', root morphological indexes were measured and osmoregulation substance content were evaluated. In total, the seedlings were grown in a constant temperature light incubator (25˚C; 14 h/10 h light/dark period; and 55% relative humidity). There were three replications for each treatment.

2.3. Root Growth Analysis

Plants were harvested at day 7 and were divided into shoots and roots. Each gradient randomly selected three uniform roots to determine the root morphology index using the Win-RHIZO system [10]. There were three replications for each treatment.

2.4. Physiological Attributes

2.4.1. Soluble Total Sugar Content Assay

Total soluble carbohydrates were measured by anthrone colorimetry [11]. 0.1 g fresh

roots were cut up into 20 ml calibration tubes with 10 ml distilled water, which were liable to boil over about 30 min. The supernatant was filtrated to a volume of 20 ml calibration tube. There were three replications for extraction. Soluble total sugar were analyzed by reacting of 0.5 ml of sample extraction buffer with 1.5ml distilled water, 7.0 ml freshly prepared anthrone (1 g anthrone + 50 ml ethyl acetate + 98% H_2SO_4) which were incubated one minutes in boiling water bath. Then determination of the samples of natural cooled at 630 nm.

2.4.2. Soluble Total Protein Content Assay

Total protein concentration of the supernatant was determined according to the method described by [12] with bovine serum albumin as a standard. An amount of 2 gm of samples were grinded in mortar with 5 ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant of different samples were made equal by adding phosphate buffer solution. After extraction, 30 µl of different samples were mixed with 70 µl of distilled water separately. In all of these separate sample tubes 2.9 ml of Coomassie Brilliant Blue solution was then added and mixed thoroughly. The Total volume now was 3ml in each tube. All these tubes were incubated for 5 minutes at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (µg) of protein was calculated.

2.4.3. The Content of Proline

Proline was assayed according to sulfosalicylic acid colorimetry [13]. 0.25 g fresh roots were put into large test tubes with 10 ml 30% sulfosalicylic acid, which was liable to boil over about 10 min. Supernatants were filtrated to clean the tubes. Proline were measured that 2 ml of proline extraction buffer, 4.0 ml reaction liquid (2 ml of acid ninhydrin + 2 ml of glacial acetic acid) and which were stayed 30 minutes in boiling water bath. The reaction was stopped by placing test tubes in an ice water bath and then mixing vigorously with toluene. The chromophore containing toluene was separated and absorbance read at 520 nm using toluene as a blank.

2.4.4. The Content of MDA

The content of MDA was measured by thiobarbituric acid colorimetry [14] [15].

2.5. Statistical Analysis

The obtained results were analyzed statistically by SPSS (version 17.0) statistical software. Data were analyzed by the analysis of variance (ANOVA) and treatment mean comparison by using least significance difference (LSD; $P = 0.05$). Values were expressed as means ± standard deviation (SD).

3. Results

3.1. *B. subtilis* QM3 Inoculation Effect on Wheat Root Morphology under NaCl Stress

The data collected on wheat root growth (Table 1), showed that *B. subtilis* QM3 (M1,

Table 1. Effects of *B. subtilis* QM3 on the growth of root under NaCl stress.

NaCl concentration(mmol/L)	Treatment	Len (cm)	SA (cm^2)	PA (cm^2)	Vol (cm^3)
0	CK1	10.16 ± 0.16 c	62.81 ± 0.78 d	19.44 ± 0.57 d	39.45 ± 0.78 d
	M1	10.60 ± 0.06 c	67.45 ± 0.49 c	21.44 ± 0.09 c	42.59 ± 1.87 c
	M2	15.39 ± 0.61 a	106.35 ± 1.03 a	33.86 ± 0.21 a	67.64 ± 0.84 a
	M3	13.54 ± 0.33 b	86.44 ± 1.9 b	26.36 ± 0.25 b	58.59 ± 0.94 b
	M4	11.94 ± 0.08 b	67.72 ±0.33 c	21.75 ± 0.09 c	43.30 ± 2.16 c
50	CK2	10.51 ± 0.38 d	66.66 ± 0.30 e	20.35 ± 0.27 e	42.38 ± 0.45 d
	M1	11.59 ± 0.11 c	71.62 ± 0.50 d	22.62 ± 0.26 d	44.54 ± 0.87 c
	M2	13.59 ± 0.32 a	90.36 ± 0.33 a	28.93 ± 0.17 a	57.55 ± 0.28 a
	M3	12.63 ± 0.25 b	75.71 ± 0.32 b	24.70 ± 0.19 b	48.39 ± 0.61 b
	M4	10.50 ± 0.14 c	74.61 ± 0.23 c	23.40 ± 0.13 c	45.44 ± 1.36 c
100	CK3	7.96 ± 0.08 c	52.73 ± 0.20 e	16.33 ± 0.20 c	31.42 ± 1.95 d
	M1	7.75 ± 0.12 c	60.54 ± 0.10 c	17.54 ± 0.66 bc	33.67 ± 0.31 c
	M2	8.56 ± 0.35 b	62.38 ± 0.26 b	18.48 ± 0.80 b	38.34 ± 1.56 b
	M3	9.94 ± 0.07 a	66.72 ± 0.16 a	20.21 ± 0.66 a	40.69 ± 0.33 a
	M4	7.94 ± 0.08 c	59.51 ± 0.22 d	17.09 ± 0.72 c	34.48 ± 1.03 c
150	CK4	6.51 ± 0.24 c	36.41 ± 0.32 e	11.05 ± 0.80 c	21.47 ± 0.69 d
	M1	6.53 ± 0.45 c	42.48 ± 0.17 c	13.98 ± 0.75 b	25.28 ± 0.52 c
	M2	7.52 ± 0.28 b	52.60 ± 0.23 b	15.40 ± 1.72 b	30.47 ± 0.34 b
	M3	8.74 ± 0.16 a	59.57 ± 0.25 a	17.74 ± 0.43 a	33.55 ± 0.84 a
	M4	6.72 ± 0.44 c	39.30 ± 0.16 d	11.77 ± 0.31 c	22.45 ± 0.38 d
200	CK5	5.02 ± 0.16 b	26.94 ± 0.15 d	8.79 ± 0.85 b	15.89 ± 0.19 d
	M1	5.64 ± 0.10 b	31.42 ± 0.33 c	9.72 ± 0.69 b	17.65 ± 0.76 c
	M2	6.35 ± 0.32 a	37.77 ± 0.16 b	11.81 ± 0.18 a	24.65 ± 0.32 b
	M3	6.90 ± 0.29 a	43.67 ± 0.40 a	13.41 ± 1.03 a	27.87 ± 0.14 a
	M4	5.47 ± 0.48 b	26.72 ± 0.18 d	8.86 ± 1.40 b	15.01 ± 0.59 e
250	CK6	3.82 ± 0.77 c	16.23 ± 0.40 b	5.11 ± 0.11 c	7.59 ± 0.31 c
	M1	3.75 ± 0.46 c	16.69 ± 0.39 b	5.53 ± 0.40 bc	7.60 ± 0.34 c
	M2	4.94 ± 0.07 b	17.47 ± 1.06 b	6.55 ± 0.72 b	9.56 ± 1.04 b
	M3	5.80 ± 0.16 a	19.42 ± 0.96 a	7.74 ± 1.11 a	11.41 ± 0.78 a
	M4	4.50 ± 0.09 bc	17.22 ± 0.37 b	5.73 ± 0.26 bc	6.61 ± 0.57 c
300	CK7	2.83 ± 0.16 b	11.21 ± 0.61 b	3.81 ± 0.88 ab	4.59 ± 0.31 ab
	M1	1.64 ± 0.37 c	9.60 ± 0.11 c	2.56 ± 0.76 b	2.61 ± 0.38 c
	M2	2.53 ± 0.41 b	10.70 ± 0.32 b	3.14 ± 0.30 ab	3.82 ± 0.29 b
	M3	2.97 ± 0.19 ab	10.72 ± 0.33 b	3.58 ± 0.41 ab	4.39 ± 0.59 ab
	M4	3.46 ± 0.30 a	12.36 ± 0.13 a	4.18 ± 0.95 a	5.18 ± 0.55 a

CK, M1, M2, M3 and M4 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4). Len, SA, PA and Vol in the table respectively represent the root length, the root surface area, the root projection area and the root volume. Values in the table are given as mean ± SD for 3 replicates. Different letters in the same column mean significant difference among treatments at 0.05 ($p < 0.05$).

M2, M3, M4) significantly increased wheat root growth as compared to control group (CK) at all NaCl (50, 100, 150, 200 and 250 mmol/L) levels, but at the NaCl concentration was 300 mmol/L, Compared with control group (CK7), M3 and M4 increased, M1 and M2 decreased wheat root growth. When without NaCl stress, even NaCl concentration was 0 mmol/L, M1, M2 ,M3 and M4 treatments compared with CK1, root length (Len), showed a obviously increase of 4.33%, 51.48%, 33.27% and 17.52%; surface area (SA), showed a respectively increase of 7.39%, 69.32%, 37.62% and 7.82%; projection area (PA), showed a respectively increase of 10.29%, 74.18% , 35.6% and 11.88%; volume (Vol), showed a respectively increase of 7.96%, 71.46%, 48.52%, and 9.76%. When without *B. subtilis* QM3 treatment, even, NaCl concentration were 50, 100, 150, 200, 250 and 300 mmol/L, wheat root growth were increased and then decreased. Under the NaCl concentration was 50 mmol/L, wheat root growth reached the maximum, CK2, M1, M2, M3 and M4 compared with CK1, root length (Len), showed a obviously increase of 3.44%, 14.07%, 33.76%, 24.31% and 3.35%; surface area (SA), showed a obviously increase of 6.13%, 14.03%, 45.32%, 21.76% and 19.99%; projection area (PA), showed a obviously increase of 4.68%, 16.36%, 48.82%, 27.06% and 20.37%; volume (Vol), showed a obviously increase of 2.39%, 12.90%, 45.88%, 22.66% and 15.18%. The above data shows, when NaCl concentration were 0 and 50 mmol/L, M1, M2, M3, and M4 was higher than that of CK, and M2 treatment was most significant than any other treatments. However, when NaCl concentration were 100, 150, 200 and 250 mmol/L, M1, M2, M3, and M4 were higher than that of CK, M3 treatment was most significant than any other treatments. So, When NaCl concentration were 50, 100, 150, 200, 250 and 300 mmol/L, not all concentrations of the *B. subtilis* QM3 bacteria liquid have a role in remission, and between different NaCl concentration stress , the effect of the same concentration of *B. subtilis* QM3 is different. In a certain degree, *B. subtilis* QM3 had an alleviation effect on the growth of the wheat root under NaCl stress.

3.2. *B. subtilis* QM3 Inoculation Effect on Wheat Root Soluble Sugar Content under NaCl Stress

NaCl treatment caused soluble sugar content of M1, M2, M3 and M4 treatments for wheat root and the CK showed a tendency that soluble sugar content were gradually increased with the increase of NaCl concentration (Figure 1). Under the NaCl concentration were 50, 100, 150, 200 and 250 mmol/L, the content of soluble sugar of M1, M2, M3 and M4 treatment were higher than that of CK, M3 treatment was enhanced significantly as compared with any other treatments. However, when the NaCl concentration were 300 mmol/L, the content of MDA of wheat root of M2, M3 and M4 treatment were higher than that of CK, M1 treatment the content of soluble sugar was lowest. Among all the NaCl treatment, the higher the salt concentration, the better *B. subtilis* QM3 ease the results. At the NaCl concentration was 0 mmol/L, M1, M2, M3 and M4 treatment were higher than CK, M2 treatment was enhanced significantly as compared with any other treatments.

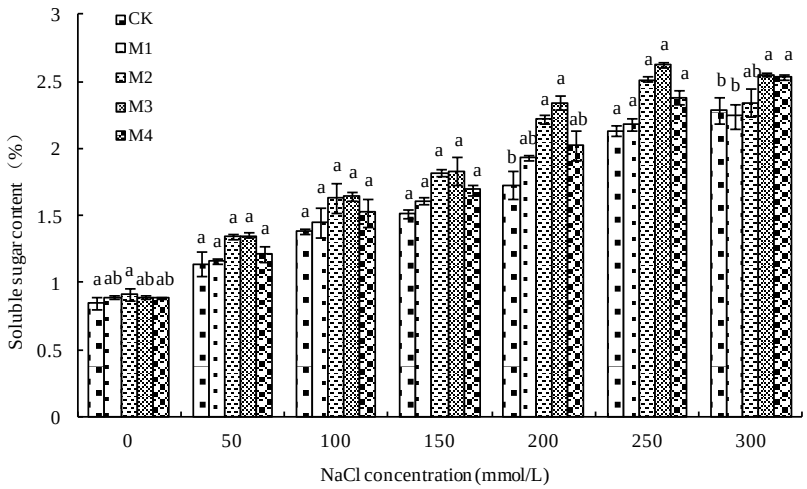

Figure 1. Effects of *B. subtilis* QM3 on content of soluble total sugar of wheat root under different NaCl stress. Values represent means of three independent replicates ± SD. CK, M1, M2, M3 and M4 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4). Different letters within a column mean statistically significant differences between the means ($p < 0.05$).

3.3. *B.subtilis* QM3 Inoculation Effect on Wheat Root Total Protein Content under NaCl Stress

With the increasing of NaCl concentration, the results showed that change of soluble protein content were increased after decreased in wheat root (**Figure 2**). Under the NaCl concentration was 150 mmol/L, wheat root soluble protein content reached the maximum. when without NaCl stress, *B. subtilis* QM3 had an obvious promotion to soluble protein content, compared with CK1, M1, M2 , M3 and M4 respectively increased by 3.98%, 10.45%, 4.13% and 6.10%. Under NaCl concentrations were 200 mmol/L, 250 mmol/ L, 300 mmol/L, soluble protein content of wheat root under the treatment of M1, M2, M3 and M4 were higher than that of CK, while under the NaCl concentrations were 50 mmol/L and 100 mmol/L, soluble protein content of wheat root under the treatment of M1, M2 and M3 were higher than that of CK and M4, while soluble protein content of M4 treatment was the lowest. When NaCl concentration was 150 mmol/L, there was no difference between CK, M1, M2 and M3, while soluble protein content of M4 treatment was the highest. In the NaCl solution concentrations were 0 mmol/L, 200 mmol/L, 250 mmol/L and 300 mmol/L, soluble protein content of *B. subtilis* QM3 treatment was enhanced obviously than CK. While the NaCl concentration were 50 mmol/L, 100 mmol/L, Some of the concentration of *B. subtilis* QM3 treatment was enhanced than CK, and 150 mmol/L among different treatments there was a slight change in the soluble protein content.

3.4. *B.subtilis* QM3 Inoculation Effect on Wheat Root Proline Content under NaCl Stress

Without *B. subtilis* QM3 treatment, the results exhibit that the content of proline were

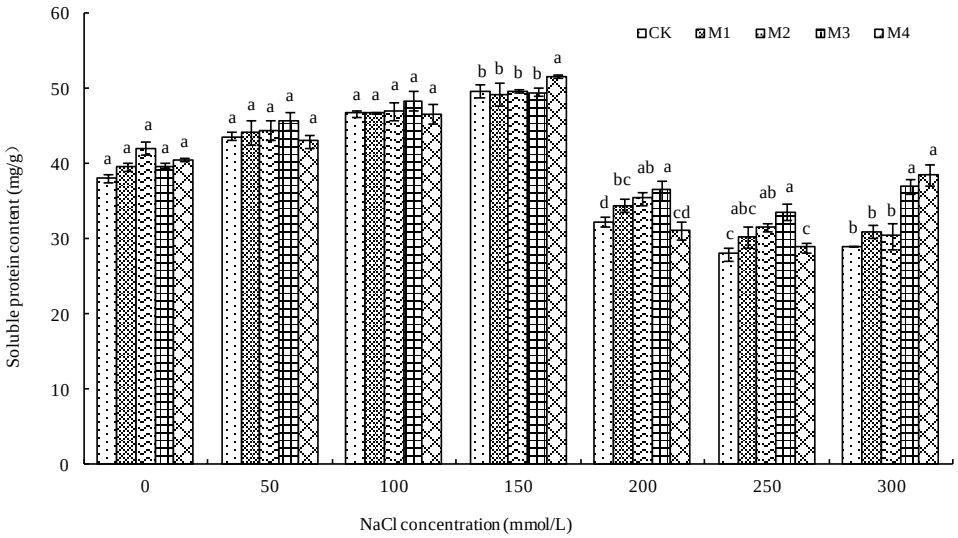

Figure 2. Effects of *B. subtilis* QM3 on content of total protein of wheat root under different NaCl stress. CK, M1, M2, M3 and M4 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4). Values in the chart are given as mean ± SD for 3 replicates. Different letters in the same NaCl concentration column mean significant difference among treatments ($p < 0.05$).

increased under the increasing of NaCl concentration, under 300mmol/L NaCl concentration which reached the maximum (Figure 3). Under the different concentrations of NaCl stress, a certain concentration of *B. subtilis* QM3 treatment significantly decreased the content of proline. On the whole, the content of proline of wheat root under the NaCl treatment of M1, M2 and M3 were lower than that of CK and M4, However, when NaCl concentrations were 0 mmol/l and 50 mol/L, M1 treatment was reduced obviously than any other treatment, and when NaCl concentrations were 100 mmol/l, 150 mmol/L, 200 mmol/L, 250 mmol/L and 300 mmol/L, M3 treatment was the lowest at all. In all treatment groups, compared with CK, the M4 treatment almost didn't have any relief effect.

3.5. *B.subtilis* QM3 Inoculation Effect on Wheat Root MDA Content under NaCl Stress

With the increasing of NaCl concentration, the content of MDA of wheat root of M1, M2, M3 and M4 treatments for wheat root and CK showed a tendency that the content of MDA were increased in NaCl treatment levels(Figure 4). Under the NaCl concentration were 50, 100, 150, 200, 250 and 300 mmol/L, the content of MDA of M1, M2, M3 and M4 treatment were lower than that of CK, M3 treatment was significantly as compared with any other treatments. M1, M2, M3 and M4 respectively increased by 11.37%, 28.68% 42.36% and 8.94% when the NaCl concentration was 50 mmol/L. Under the NaCl concentration was 0 mmol/L, the content of MDA of wheat root, compared with CK1, M1, M2 and M4 no obvious different, M3 was the lowest.

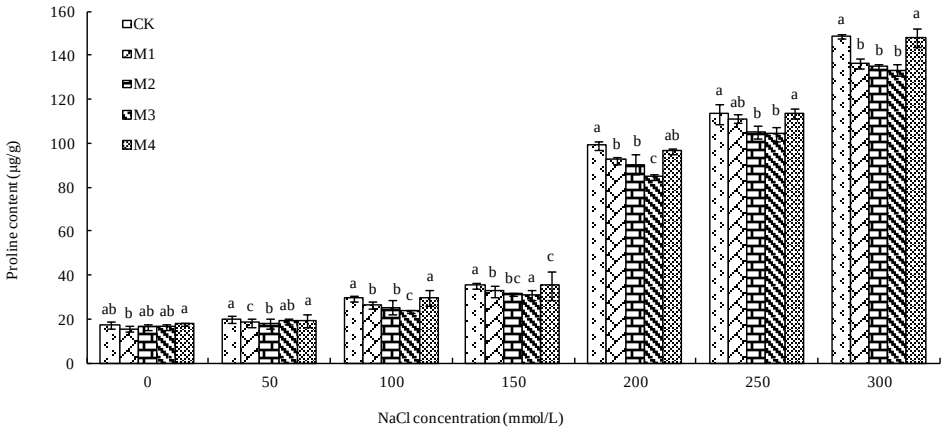

Figure 3. Effects of *B. subtilis* QM3 on content of proline of wheat root under different NaCl stress. CK, M1, M2, M3 and M4 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4). Values in the chart are given as mean ± SD for 3 replicates. Different letters in the same NaCl concentration column mean significant difference among treatments ($p < 0.05$).

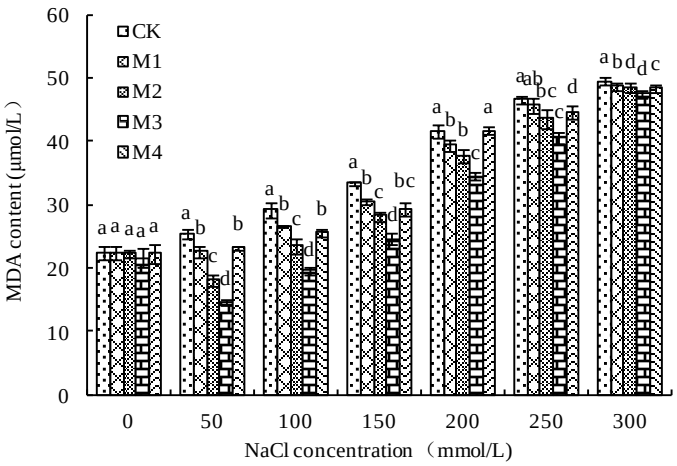

Figure 4. Effects of *B. subtilis* QM3 on content of MDA of wheat root under different NaCl stress. Values represent means of three independent replicates ± SD. CK, M1, M2, M3 and M4 respectively represent the control group (treated with sterile water), 10^8 CFU/ml *B. subtilis* QM3 (M1), 10^7 CFU/ml *B. subtilis* QM3 (M2), 10^6 CFU/ml *B. subtilis* QM3 (M3) and 10^5 CFU/ml *B. subtilis* QM3 (M4). Different letters within a column mean statistically significant differences between the means ($p < 0.05$).

4. Discussion

Higher NaCl concentration, the dominant salt in the soil, has a direct effect on plant growth, poses osmotic stress and ionic stress, which reduces the ability of plants to absorb water and minerals [6] [16].

NaCl stresses affect negatively first seed germination and then root length. Plant early growth stage is more sensitive as compared to later stages so inoculation with bacteria having ability to mitigate salinity stress can be helpful for plants to overcome from

stress [17] [18] [19].

Different researchers have also reported that PGPR inoculation also improved seed genrmination under salinity stress [20] [21] [22]. Na^+ and Cl^- are necessary to plant growth, then a number of reports have showed that high concentration of NaCl stress inhibit plant root and proliferation [23] [24] [25] [26]. Our result was carried out to evaluate exogenous application of *Bacillus subtilis* QM3 enhancing NaCl tolerant potential of wheat through assessing on root morphology and osmoregulation substance attributes, which showed that NaCl stress caused a significant inhibition on wheat root growth, including the root length, root surface area, root volume and root projection. However, under the NaCl concentration of 50 mmol/L, the wheat root growth was promoted, it may due to the dual role of NaCl in plant. From other NaCl concentrations stress, in a certain degree, *B. subtilis* QM3 had an enhanced the NaCl tolerance potential on the growth of the wheat root.

As plant osmoregulation substances are concerned in regulating a series of physiological and biochemical processes, changes in physiological conditions like proline, protein and carbohydrate [27], biosynthesis the analysis of the role of new plant growth regulators in crop abiotic stress tolerance is being a great deal of focus these days [28]. It has been commonly reported that salt stress is one of the major causes of oxidative damage to plant tissues [29].

Sugars perform important regulatory functions in plants including photosynthesis [30] and carb ohydrate partitioning [31] [32]. Soluble sugar to osmotic adjustment, the increase of its concentration could increase the concentration of cell protoplasm, to maintain normal membrane function.

Excessive salt accumulation is the obvious effects on protein metabolism, which inhibit synthesis and promote decomposition. The inhibit protein synthesis directly causes may be the synthesis of amino acids.

The accumulation of amino acids was involved in osmotic adjustment, free radical scavenging, maintenance of protein and membrane integrity [33]. A number of reports have showed the amount of the content of proline increased significantly and gradually with increasing NaCl concentration. Proline serves as an important compatible osmolyte, and its accumulation is believed to reduce cellular water potential and avoid deleterious toxicity of high ionic strength, has also been proposed to serve as reactive oxygen species scavenger [34] and its accumulation can stabilize the structure of membranes and proteins to minimize the damage of cells under salt stress.

It was reported that MDA content on different plant species increased significantly with the processing salt level rising [35] [36]. These increases may be attributed to that salinity could modify the membrane structure and stimulate O_2 production, which facilitates lipid peroxidation [37].

Study found that the content of soluble sugar, proline and MDA increased in NaCl concentration level. However, the content of soluble protein increased in low NaCl level (50, 100, 150 and 200 mmol/L), decreased in high NaCl concetration level (200, 250 and 300 mmol/L).Under the same NaCl concentration level, Basically, M1, M2, M3 and M4

treatment compared with control group, under the different level can be increased or decreased osmoregulation substance to increase the osmotic regulation ability of wheat root, enhancing the NaCl tolerance potential on wheat root, and M3 treatment is most obvious effect than any other treatments.

On the whole, plant self-protection ability is limited, a certain of NaCl concentration stresses in wheat root, compared with the control group, the treatment of *B. subtilis* QM3 contributed to osmotic regulation ability of the wheat root improved to a certain degree, so as to restrain the excessive accumulation of Na^+ and Cl^- caused permeability damage, reduce lipid peroxidation of cell membrane and maintain the relative integrity and orderliness, at last, enhanced the NaCl tolerance potential on the growth of the wheat root. By comparison, 10^6 CFU/ml *B. subtilis* QM3 bacteria liquid treatment was enhanced obviously than any other treatments. 10^8 CFU/ml *B. subtilis* QM3 *and* 10^7 CFU/ml *B. subtilis* QM3 has a higher concentration, and 10^6 CFU/ml *B. subtilis* QM3 has a lower concentration, these two reasons may lead to their alleviate effect are not as good as 10^6 CFU/ml *B. subtilis* QM3 solution.

5. Conclusion

When the plants suffer NaCl stress, bacterium induces the concentration of osmoregulation content to improve the penetration ability by combining with wheat roots, resulting in increased plant tolerance to NaCl. In this study, the wheat root inoculated with 10^6 CFU/ml *B. subtilis* QM3 conformed better effect in terms of root morphology, soluble sugar content, total protein content, MDA content and proline content as compared to 10^8 CFU/ml *B. subtilis* QM3, 10^7 CFU/ml *B. subtilis* QM3 and 10^5 CFU/ml *B. subtilis* QM3. Such studies are necessary to select a suitable bacterium and an optimal concentration of a suitable bacterium to be used as bioinoculant for sustainable wheat production under saline areas.

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