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Effects of Humic Acid Pretreatment on Germination of Tomato Seeds under NaCl Stress

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Abstract: The effects of different dilution ratios of Highly Active Humic Acid liquid on germination of tomato seeds under NaCl stress were studied by using "Jinpeng No.8" as the experimental material. The results showed that the NaCl stress had inhibitory effect on tomato seeds germination. Under the 150mmol/L NaCl stress, the appropriate concentration of Highly Active Humic Acid liquid could relieve NaCl stress to increase the germination energy, germination percentage, germination index, vigor index, radicle length and hypocotyl length of tomato seeds and decrease the content of malondialdehyde (MDA) in radicle. The optimal effects were observed when the Highly Active Humic Acid liquid was diluted 700 times.

Keywords: Humic acid; Tomato; Seed; NaCl stress

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Currently, research reports about the mitigation of salt (NaCl) stress in tomato seeds are abundant, and they propose multiple methods to mitigate the inhibitory effect of NaCl stress, including the inoculation of halotolerant bacteria, seed priming with gibberellin, seed priming with Ca^{2+}, 5-aminolevulinic acid pretreatment, pretreatment with different types of exogenous polyamines, etc.[1-8] Humic acid, which consists of diverse functional groups, exhibits favorable physical and biological activity. It can significantly improve soil quality, stimulate crop growth and development, and enhance crop resistance to stress[9], thereby representing a promising direction for the development of novel fertilizers in the 21st century.

The salt tolerance of seeds during the germination stage can be reflected from different perspectives by indicators such as germination energy, germination percentage, germination index and vigor index[5]. Malondialdehyde (MDA) is a product of membrane lipid peroxidation in the membrane system of plants under stressful conditions and serves as an indicator to measure the stress resistance of seeds under stress.

This experiment investigated the effects of Highly Active Humic Acid liquid pretreatment on different germination indicators of tomato seeds under NaCl stress. The findings aimed to offer insights into the application of Highly Active Humic Acid liquid for enhancing seed germination and mitigating NaCl stress.

1. Material and Method

1.1 Experimental Material

The experimental material was tomatoes (*Lycopersicon esculentum* Mill.), specifically the variety of "Jinpeng No.8".

The treatment liquid was Highly Active Humic Acid liquid (containing about 21.6g/L total humic acid), which was sourced from Xinyi Sumeng Fertilizer Co., Ltd. in Jiangsu Province.

1.2 Experimental Method

1.2.1 Germination experiment of tomato seeds under NaCl stress

Full and intact tomato seeds of relatively uniform size were chosen, sterilized with a 0.2% potassium permanganate solution for about 25min, and pretreated with distilled water for 6h. In a culture

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dish lined with double layers of filter paper, 50 seeds were placed on each layer, added separately with 10mL NaCl solution with concentrations of 50, 150, and 250mmol/L[10], and the distilled water served as the control (CK). After the filter paper fully absorbed the liquids, the culture dish was covered to prevent water evaporation. The same process was replicated three times for each treatment. The culture dish was then placed in a 27±1℃ thermostatic incubator for germination. The germinated tomato seeds were counted from Day 2, and the germination standard was defined as radicles of tomato seeds breaking the seed coats. This process was recorded for 7 consecutive days.

1.2.2 Germination experiment of tomato seeds treated with Highly Active Humic Acid liquid under NaCl stress

The tomato seeds underwent pretreatment with Highly Active Humic Acid liquid diluted 500, 600, 700, 800 and 900 times for 6h, and the distilled water served as the control treatment (CK). The same process was replicated three times for each treatment. Subsequently, 10mL NaCl solution with a concentration of 150mmol/L (which had been found in a previous experiment to have inhibitory effect on tomato seeds, but an excessive concentration would be of little guiding importance to the experiment) was added in each treatment. The germination experiment of tomato seeds was conducted using the experimental method as instructed in 1.2.1.

1.3 Measurement Indicators and Method

Germination Energy (GE) = (number of germinated seeds within 4 days/total number of experimental seeds) × 100%;

Germination Percentage (GP) = (number of germinated seeds within 7 days/total number of experimental seeds) × 100%;

Germination Index (GI) =$\sum Gt/Dt$ (Gt represents the number of germinated seeds, and Dt represents the number of days when the seeds are germinated);

Vigor Index (VI) = S×GI (S represents the fresh weight of seedlings on Day 7 of germination, and GI represents germination index).

Fifteen seedlings were chosen at random from each treatment. A ruler was used to measure radicle length and hypocotyl length, and a vernier caliper was used to measure radicle thickness. The thiobarbituric acid method[11] was used to determine the MDA content in the radicle, measured in μmol/(g·FW).

1.4 Data Analysis and Statistics

Excel 2013 and SAS software were used for statistics and analysis of the experimental data.

2. Result Analysis

2.1 Effects of NaCl Stress on the Germination of Tomato Seeds

The effects of NaCl stress on the germination of tomato seeds are shown in Table 1.

As shown in the table, different concentrations of NaCl treatment had different effects on the germination of tomato seeds. As the concentration of NaCl solution increased, the germination energy, germination percentage, germination index and vigor index of tomato seeds decreased. Specifically, high concentrations (150 and 250mmol/L) of NaCl stress had significant inhibitory effects on the germination energy, germination percentage, germination index and vigor index of tomato seeds, which was basically consistent with the previous research results on cucumber [8], wheat [12, 13], corn[14] and other crops.

Table 1 Effects of NaCl Stress on the Germination of Tomato Seeds

NaCl Concentration (mmol/L)	Germination Percentage (%)	Germination Energy (%)	Germination Index	Vigor Index
CK	93.95a	96.22a	20.89a	0.77a
50	90.85a	93.03a	16.86b	0.71a
150	62.55b	74.40b	10.15b	0.43b
250	49.17c	55.46c	7.56c	0.35b

Note: Different lowercase letters in the same column of data indicate significant difference ($P<0.05$), the same below.

2.2 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Germination Characteristics of Tomato Seeds under NaCl Stress

The effects of different dilution ratios of Highly Active Humic Acid liquid on the germination of tomato seeds under 150mmol/L NaCl stress are shown in Table 2.

As shown in the table, except for the pretreatment with Highly Active Humic Acid liquid diluted 500 and 900 times, other tomato seeds undergoing Highly Active Humic Acid liquid pretreatment demonstrated germination indicators all superior than those of CK. On the whole, all of the four germination indicators exhibited an initial increase followed by a decrease as the dilution ratio of Highly Active Humic Acid liquid increased. This trend suggested that the appropriate concentration of Highly Active Humic Acid liquid could, to some extent, mitigate the inhibitory effects of NaCl on the germination of tomato seeds. However, an excessively low dilution ratio of Highly Active Humic Acid liquid may result in penetration-induced water loss in tomato seeds, thereby affecting their germination. The Highly Active Humic Acid liquid diluted 700 times was most effective in mitigating NaCl stress, with the germination energy, germination percentage, germination index and vigor index of tomato seeds increasing by 17.03%, 16.17%, 66.11% and 65.12% when compared to those of CK, respectively.

Table 2 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Germination of Tomato Seeds under 150mmol/L NaCl Stress

Dilution Ratio of Highly Active Humic Acid liquid	Germination Energy (%)	Germination Percentage (%)	Germination Index	Vigor Index
CK	62.55cd	74.40cd	10.15c	0.43bc
500	51.99d	69.406d	9.87c	0.37c
600	68.40ab	79.14bc	12.60bc	0.45bc
700	73.20a	86.43a	16.86a	0.71a
800	67.15b	82.17ab	14.97ab	0.49b
900	58.23c	72.43d	11.38c	0.44bc

2.3 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Radicle and Hypocotyl Growth of Tomato Seeds under NaCl Stress

The effects of different dilution ratios of Highly Active Humic Acid liquid on the radicle and hypocotyl lengths of tomato seeds under 150mmol/L NaCl stress are shown in Figure 1 and Figure 2, respectively.

As shown in the figures, the pretreatment with 5 different concentrations of Highly Active Humic Acid liquid was all found to facilitate the radicle and hypocotyl growth of tomato seedlings under 150mmol/L NaCl stress, and such facilitation effect appeared to first increase and then decrease as the dilution ratio of the Highly Active Humic Acid liquid increased. However, it facilitated radicle growth more than hypocotyl growth. The most significant effects were observed when the Highly Active Humic Acid liquid was diluted 700 times, leading to a 129.33% increase in radicle length and a 34.93% increase in hypocotyl length of tomato seedlings. These experimental results showed that Highly Active Humic Acid liquid can mitigate the inhibitory effect of NaCl stress on the growth of tomato seedlings and that the optimal mitigation effects are achieved by the Highly Active Humic Acid liquid diluted 700 times.

2.4 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Radicle Thickness of Tomato Seedlings under NaCl Stress

The effects of different dilution ratios of Highly Active Humic Acid liquid on the radicle thickness of tomato seedlings under 150mmol/L NaCl stress are shown in Figure 3.

As shown in the figure, the thickness of seedling radicle showed a significant increase in comparison to the CK (except for the Highly Active Humic Acid liquid diluted 500 times) following the addition of 5 different dilution ratios of Highly Active Humic Acid liquid under 150mmol/L NaCl stress. The seedling radicle undergoing pretreatment with Highly Active Humic Acid liquid diluted 700 times exhibited the greatest thickness, showing a 55% increase compared to the CK. This substantially mitigated the inhibitory effect of NaCl stress on radicle thickness, suggesting that Highly Active Humic Acid liquid has the potential to mitigate the inhibitory effect of NaCl stress on the growth of seedling radicle to some extent and that the optimal mitigation effects are achieved by Highly Active Humic Acid liquid with an appropriate concentration (diluted 700 times).

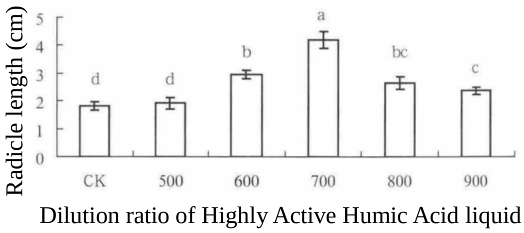

Figure 1 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Radicle Length of Tomato Seedlings under 150mmol/L NaCl Stress

Note: Different lowercase letters in the figure indicate significant differences (P<0.05), the same below.

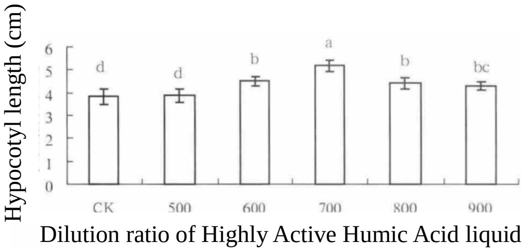

Figure 2 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Hypocotyl Length of Tomato Seedlings under 150mmol/L NaCl Stress

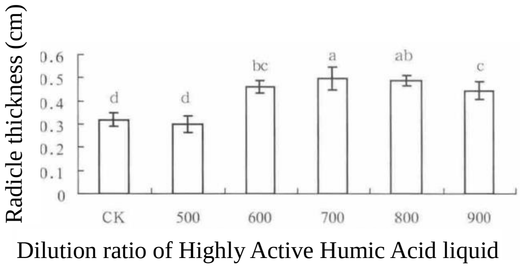

Figure 3 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the Radicle Thickness of Tomato Seedlings under 150mmol/L NaCl Stress

2.5 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the MDA Contents in Tomato Seedling Radicle under NaCl Stress

The effects of different dilution ratios of Highly Active Humic Acid liquid on the MDA contents in tomato seedling radicle under 150mmol/L NaCl stress are shown in Figure 4.

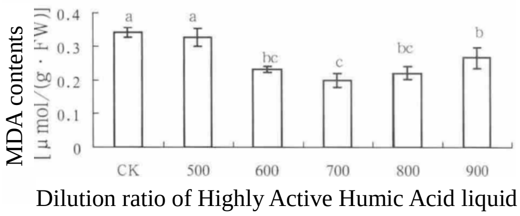

Figure 4 Effects of Different Dilution Ratios of Highly Active Humic Acid liquid on the MDA Contents in Tomato Seedling Radicle under 150mmol/L NaCl Stress

As shown in the figure, the seedlings experienced elevated membrane lipid peroxidation under NaCl stress, which meant the cell membrane system of the seedlings was severely damaged to the extent that it had inhibited the growth of the seedlings. Upon the application of Highly Active Humic Acid liquid, the MDA contents in the seedling radicle first decreased and then increased as the dilution ratio of the applied liquid increased. The lowest MDA contents were observed when the seedlings underwent pretreatment with Highly Active Humic Acid liquid diluted 700 times, exhibiting a 45.33% decrease compared to the CK. These results showed that Highly Active Humic Acid liquid has the potential to protect the cell membrane system to some extent and mitigate the detrimental effect of NaCl stress on germinating seedlings. The optimal mitigation effects are achieved by the Highly Active Humic Acid liquid diluted 700 times.

3. Conclusion and Discussion

Hu Xiaohui et al. contended that exposure to NaCl stress led to a significant decrease in the germination energy, germination percentage, germination index, radicle length and hypocotyl length of tomato seeds while the MDA contents showed a significant increase[4]. Wang Naiqiang et al. contended that NaCl stress could disrupt the integrity of cell plasma membrane, resulting in reduced cell selective permeability and intracellular ion imbalance, which could further inhibit the water absorption by the seed embryo and the germination of tomato seeds due to insufficient intracellular water[6]. In this experiment, as the NaCl concentration increased, the germination energy, germination percentage, germination index and vigor index of tomato seeds were gradually decreased. These findings were basically consistent with the conclusions of previous research.

Guo Wei et al. found that humic acid pretreatment could lead to increased glutathione content, reduced reactive oxygen, and lower membrane lipid peroxidation, thereby decreasing the MDA content[15]. In this experiment, Highly Active Humic Acid liquid with different concentrations enhanced the salt tolerance of tomato seeds, with the optimal effects observed when the Highly Active Humic Acid liquid was diluted 700 times.

In conclusion, the appropriate concentration of Highly Active Humic Acid liquid can increase the germination energy, germination percentage, germination index and vigor index of tomato seeds, promote their radicle and hypocotyl growth, and reduce the MDA content under 150mmol/L NaCl stress, thereby promoting the normal germination and growth of tomato seeds. Consequently, applying Highly Active Humic Acid liquid pretreatment with an appropriate concentration is a viable approach to enhancing the salt tolerance of tomato seeds. Nonetheless, further research is required to elucidate the internal mechanisms through which Highly Active Humic Acid liquid promotes the germination of tomato seeds.

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In this test, due to the lack of analysis on the quantity of viable bacteria in bacillus megaterium, bacillus mucilaginosus and azotobacter chroococcum found in the post-fermentation medicine residue, which means that the number of viable bacteria in the medicine residue remains ultimately unclear, it remains uncertain if these three bacteria remain viable after the fermentation process. Therefore, additional testing and analysis are needed.

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