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Physiological Responses of Tomato Seedlings (*Lycopersicon Esculentum)* to Salt Stress

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Abstract

Tomato seedling was treated under different concentration of NaCl ranged from 0 to 300 mM. Effects of salt stress on the content of growth and osmotic adjustment substance, the superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities and generation rate of $O_2^{\cdot-}$ of tomato seedling were studied. The result showed that the content of fresh weight (FW), dry weight(DW), K^+, K^+/ Na^+ and soluble sugar(SS) decreased with the increasing of NaCl concentration. Conversely, the contention of Na^+, Proline and malondialdehyde (MDA), SOD, POD, CAT and APX activities and generation rate of $O_2^{\cdot-}$ increased. Growth of seedling shoots was suppressed by salt treatment. Osmotic adjustment substance play a critical role in the growth of tomato seedling under the condition of salt stress, and meanwhile the continual increasing bioactivity of antioxidant enzymes could scavenge reactive oxygen species (ROS) resulted from salt stress to exert the existence of tomato seedling.

Keywords: Tomato, Growth, Osmotic adjustment substance, Salt stress

1. Introduction

Salt stress is one of the mainly environmental factors interfering with the growth, development and biomass production of plants. Today, 20% of the world's cultivated land and nearly half of all irrigated lands are affected by salinity (Tanji, 1990, PP. 1-7). High concentrations of salts causes ion imbalance and hyperosmotic stress in plants. 300 million mu cultivated land in China is affected by salinity, which occupies 25% of arable land. With the quick development of industry, the leap of population, the acceleration of town construction, the sharp reduction of arable land area and unreasonable agricultural measures lead to secondary salinization of a large amount of arable land (Tester, 2003, PP. 503-507, Zhao, 1999). Destruction of arable land ascribed to saliniization seriously affected the civilization of remote antiquity and modern times at all times (Blumwald, 2004). Salinity has been one of the cardinal factors affecting the development of social economy (Shono, 2001, PP. 193-199). Tomato belongs to fruit vegetable of horticulture crops, and its flesh is applied not only as salad and ingredients of food, but also fresh food. It is one of the important routine fruits and vegetables. Study on the physiological responses of tomato seedlings to salt stress could give novel insight into the planting and modifying of tomato cultivars.

2. Materials and methods

2.1 Plant material and growth conditions

Tomato seeds (Zhongsu 5) were purchased from China Academy of Agriculture Science. Seeds of tomato were sterilized in 0.1% $HgCl_2$ for 10 min, and then rinsed with water. Plump kernels were selected for planting. Selected seeds were then germinated on moistened plastic basins containing thin sand. The seeds germinated after 3 days. The obtained seedlings were transferred to continuously aerated Hoagland solution in a greenhouse in which day and night temperature, everyday light, light intensity and relative humidity is 30-20℃, 15 hours, 700-900umol/m^2.s and 70-80%, respectively. Seedlings were subsequently transferred, at the stage of 4-5 fully expanded leaves (20 days later), in buckets filled with neat thin sand. Each basin four seedlings irrigated with aerated Hoagland solution. After planted for one month, concordant seedlings were selected for treatment. Tomato seedlings were treated with NaCl at the concentration ranged from 0 until 300 mM with the increasing rate of 50 mM every 12 hours, and subsequently irrigated with water every day. Irrigation quantity is 2 times of water capacity of thin sand, and relative physiological indices were investigated after a week.

2.2 Methods

2.2.1 Measure of FW and DW

The thin sand of roots was gently removed by tap water, quickly rinsed with distilled water to flush the dust in the surface. Then the roots were washed carefully and their surface water was completely dried by absorbent paper. FW was measured and then fresh seedlings were transferred to 110 ℃ oven in order to deactivation of enzymes and subsequently dried to constant weight at 80 ℃. DW was measured. Plant water content and degree of succulence were calculated as follows:

Plant water content=(FW-DW) /FW×100%

Degree of succulence=FW/DW

2.2.2 Content measure of osmotic adjustment substance

Dried plant samples were ashed in Muffle furnace at 550℃ after grinded and ash was extracted with HNO_3 with constant volume. Na^+ and K^+ in the seedling tissues was measured by Atomic Absorption Spectrophotometer (AAS) (Hitachi, Z-8000, Japan)(Zhang, 1990, PP. 259-260). Proline was measured as described by Chou etc.(Zhang, 1994, PP. 62-65, Chou, 1995, PP. 96-97, Zhu, 1990, PP. 249-252). MDA and SS content was measured as described by Zhang(Zhang, 2003, PP. 274-277, Zhao, 1994, PP. 207-210).Leaves of tomato seedling were rinsed distilled water to flush the dust in the surface , washed carefully and then their surface water was completely dried by absorbent paper. Samples prepared for treatment were immediately frozen in liquid nitrogen, stored at -80℃ for 15 min and subsequently lyophilized. Osmotic potential of obtained cell sap was measured by freezing point osmometer (Osmomat 030).

2.2.3 Measure of antioxidant enzyme activities

The enzyme activities of SOD, CAT and POD were assayed as described by Li(2000, PP. 167-169), Li(2000, PP. 165-167) and Zhang(2003, PP. 154-155), respectively; APX activity was assayed as described by Nakano and Asada(1981, PP. 867-880); generation rate of $O_2^{\cdot -}$ was measured as described by Wang(1990, PP. 55-57).

3. Results

3.1 Effects of salt stress on the growth

In the treatments of different saline contention, the growth of tomatoes varied obviously and suppressed by salt stress, which was associated with salt content. In the treatment of 300 mM NaCl, leaves of tomato suffered greatly damage with all yellow leaves and growth was almost arrested. With the increasing of NaCl, FW and DW of tomato seedlings reduced gradually, while water content and degree of succulence increased obviously. Results showed that the growth of tomato seedlings was suppressed by salt treatment, but survived due to the water content and degree of succulence. Osmotic adjustment substance might play a critical role in the growth of tomato seedling under the condition of salt stress.

3.2 Effects of salt stress on the content of osmotic adjustment substance

Na^+ ions both in leaves and roots increased gradually with the increasing of salt concentration, and the increasing degree of Na^+ ions was higher in leaves than that in roots. Conversely, K^+ ions both in leaves and roots decreased gradually with the increasing of salt concentration, and the decreasing degree of K^+ ions was higher in roots than that in leaves. K^+/ Na^+ decreased gradually in leaves and roots, and the decreasing degree was obviously higher in roots than that in leaves. All the results showed that Na^+ and K^+ ions was correlated with the physiological responses of tomato seedlings to salt stress as one of cardinal factors attributed to salt stress.

Proline content increased gradually by increasing NaCl concentration, and was 12.36 and 22.29 times of the control at the treatment of 100 and 300 mM NaCl, respectively, with great significance.

SS content decreased by increasing the NaCl concentration. SS content of leaves decreased by 17.38% compared to the control at the treatment of 100mM NaCl. At the treatment ranged from 200 to 300 mM NaCl, SS content decreased by 65.85%-74.7% compared to the control. At the treatment of 100-200 mM NaCl, SS levels were progressively decreased.

MDA content increased by increasing the NaCl concentration. MDA content of leaves increased by 4.6% compared to the control at the treatment of 100mM NaCl. At the treatment ranged from 200 to 300 mM NaCl, MDA content increased by 10.38%-23% compared to the control. When NaCl concentration was above 100mM, MDA content was obviously higher than the control.

Osmotic potential increased with the increasing of NaCl concentration. Osmotic potential of leaves increased by 43.55% compared to the control at the treatment of 100mM NaCl. At the treatment ranged from 200 to 300 mM NaCl, MDA content increased by 91.94%-116.13% compared to the control. At all treatments, osmotic potential was obviously higher than the control, which indicated that with the enhancement of salt stress tomato accumulated large amount of osmotic adjustment substances in order to preserve adequate water necessary for existence.

3.3 Effects of salt stress on antioxidant enzyme activities

SOD activity increased by 9.9% at the treatment of 100 mM NaCl compared to the control, while at the treatment of 200-600 mM NaCl, SOD activity decreased obviously. With the increasing of NaCl concentration, POD activity decreased gradually at the concentration of 100-500 mM and reduced by 2.338-40.92 times than the control. At the concentration of 600 mM, POD activity increased and was 9.72 times of that at the concentration of 500 mM. CAT activity increased gradually which was 2.5 times of the control at the concentration of 100 mM, and then decreased gradually.

The generation rate of $O_2^{\cdot -}$ in leaves increased with the increasing of NaCl concentration and raised by 28.6%, 85.7% and 142.86%, respectively, compared to the control.

4. Discussions

Whether halophyte or non-halophyte, during the period of adapting to saline, in order to avoid water loss, the most efficacious measure is osmotic adjustment (Xu, 1996, PP. 249–257). With the accumulation of Na^+ in vacuoles, cells kept the osmotic equilibrium between cytoplasm and vacuole through osmotic adjustment. This adjustment relied on synthesizing or accumulating some solutes, namely inorganic salts and organic solutes, which did not affect the biochemistry reactions in cells.

The content of fresh weight (FW), dry weight (DW), K^+, K^+/ Na^+ and soluble sugar (SS) decreased with the increasing of NaCl. Conversely, the contention of Na^+, Proline and malondialdehyde (MDA) and osmotic potential increased. Growth of seedling shoots was suppressed by salt treatment. Osmotic adjustment substance plays a critical role in the growth protection of tomato seedling under the condition of salt stress.

Zhu (2001, PP. 66-71) noted that high concentrations of salts cause ion imbalance and hyperosmotic stress in plants. As a consequence of these primary effects, secondary stresses such as oxidative damage often occur. Generation and scavengeing of free radials coexisted in plants, and was normally in a balance state. Once plants was suffered salt stress, this balance system would be broken, free radicals would accumulate, membrane permeability discrepancy lost, which lead to the enhancement of membrane pore and permeability and metabolic disorder, and thus plants were suffered injury(Zhao, 1993, PP. 519-525). Enzymatic protection system existed in plants to scavenge free radicals. SOD, POD, CAT and APX is mainly components of this system in which SOD could disproportionate $O_2^{\cdot -}$ into O_2 and H_2O_2.

High concentration H_2O_2 in tissues was mainly scavenged by CAT which led to a low level of H_2O_2, while low concentration H_2O_2 was mainly scavenged by POD during the period of oxidation of relative substances. When SOD, POD, CAT and APX were consistent and in harmony with one another, free radicals from ROS in plants could be kept at a low level which exerted the plant grow and metabolize naturally (Jiang, 1999, PP. 229 - 234).

Investigation showed that the content of FW, DW decreased with the increasing of NaCl. Conversely, the activities of SOD, POD, CAT and APX and generation rate of $O_2^{\cdot -}$ increased. Growth of seedling shoots was suppressed by NaCl treatment. Continual increasing bioactivity of antioxidant enzymes could scavenge reactive oxygen species (ROS) resulted from salt stress to make the existence of tomato seedlings.

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Table1. Effects of salt stress on the grows of tomato

	0	100	200	300
FW	17.5	15.8	14	12.2
DW	5.5	4.7	3.2	2.3
Water content	68.57%	70.25%	77.14%	81.15%
Degree of succulence	3.18	3.36	4.38	5.3

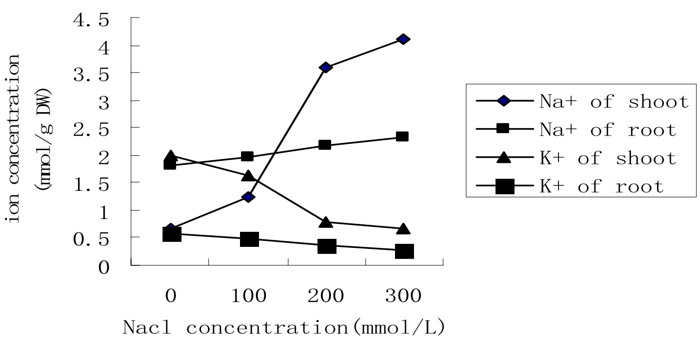

Figure 1. Effect sof salt stress on the Na+ and K+ contents of shoot and root of tomato

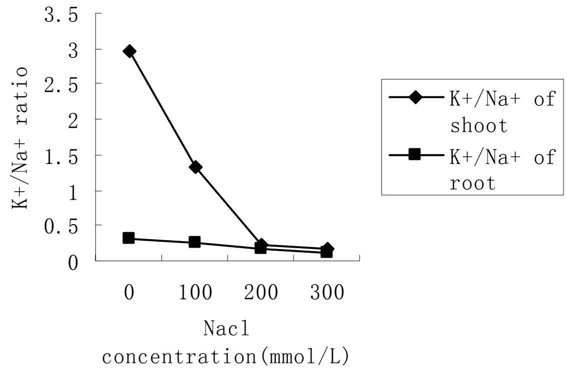

Figure 2. Effects of salt stress on the K^+/ Na^+ of shoot and root of tomato

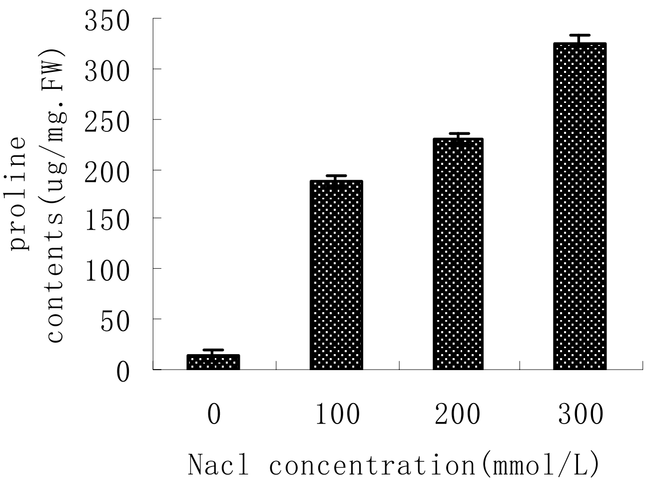

Figure 3. Effects of salt stress on the proline content of tomato

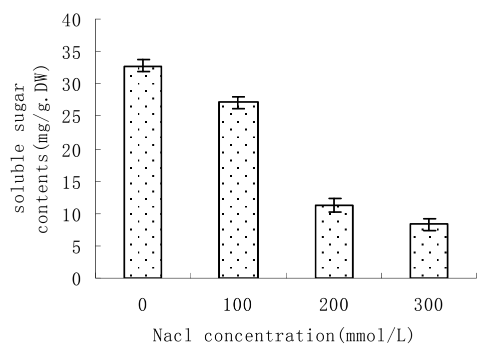

Figure 4. Effects of salt stress on SS contents of tomato

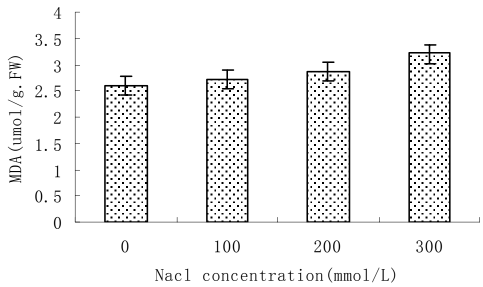

Figure 5. Effects of salt stress on MDA content of tomato

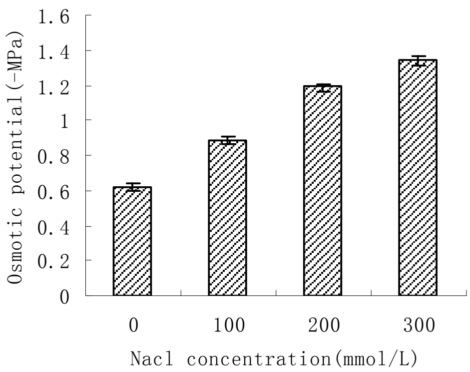

Figure 6. Effects of salt stress on osmotic potential of tomato leaves

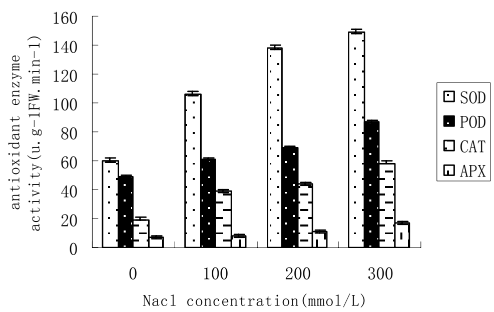

Figure 7. Effects of salt stress on antioxidant enzyme activities of tomato

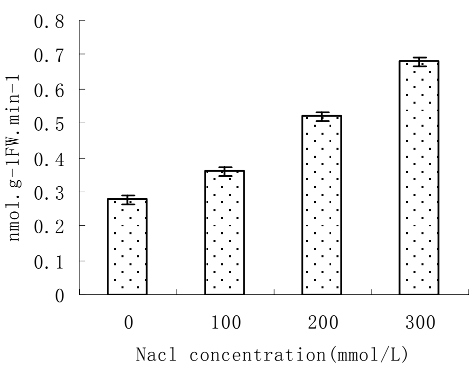

Figure 8. Effects of salt stress on the O_2^- content of tomato