Effects of water-salt coordinated regulation technique on ion distribution and antioxidant enzyme activities in tomato

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Abstract: In order to explore the effects of brackish water combined with regulated deficit irrigation of different growth periods, different levels of water, and salt synergistic stress on the characteristics of ion absorption, distribution, transport, and antioxidant enzyme activity of tomatoes, a greenhouse pot experiment was applied. The influencing factors were set as: irrigation water salinity (local shallow groundwater S0 1.1 g/L, S1 2.0 g/L, S2 4.0 g/L), different water deficit levels (W1 70%-80% field capacity (FC), W2 60%-70% FC, W3 50%-60% FC), and three growth stages (T1 seedling stage, T2 blossoming and bearing fruits stage, T3 mature picking stage). Monitoring and analyzing the dynamic changes of K^+ and Na^+ content in tomato root stems and leaves under water-salt stress and antioxidant enzyme activities of leaves at the seedling stage. The results showed the coordinated regulation of water and salt can significantly change the ion absorption of roots and the transfer between stems and leaves at different growth stages. The roots and leaves of tomatoes mainly accumulated K^+, and the stems mainly accumulate Na^+. The increase of irrigation salinity can reduce the ratio of K^+/Na^+ ratio in leaves. Under the coordinated regulation of water and salt, K^+ became the main osmotic adjustment ion of the leaves again. The activities of catalase, superoxide dismutase, and peroxidase in tomato leaves increased with the increase of water-salt stress. The results have great significance to the response of crops and to meet the requirements for water quality and quantity of brackish water under water-salt stress at different growth stages of tomatoes.

Keywords: brackish water irrigation, water and salinity stress, ion contents, antioxidant enzyme activity
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1 Introduction

Soil salinization is one of the world's serious environmental problems. It is the main environmental factor that restricts the growth and development of crops, which has a severe impact on agricultural production and development[1]. According to statistics, the global salinized soil area is 950 million hm^2, accounting for 7.26% of the land area. Salinized soil is widely distributed in China, with an area of about 37 million hm^2, accounting for 4.88% of the nationally available land area, of which the salinized area of cultivated land reaches 9 million hm^2, accounting for 6.62% of the national cultivated land area[2,3]. In order to improve agricultural productivity in salinized areas it is urgent to increase the salt tolerance of crops. In order to achieve this goal, it is essential to study physiology, biochemistry, and molecular mechanism of crop evolution in response to salt stress.

Soil salinization will inhibit the water absorption of crops, and the imbalance of ions in plants will eventually lead to ion poisoning and osmotic stress[4]. Ion channels in plant cells play a pivotal role in adapting and overcoming salt stress[5]. In order to resist salt stress, crops will restrict Na^+ entry through selective uptake by roots, or maintain ion balance through ion compartmentalization to improve salt tolerance[6], but different plants have different ion compartmentalization methods. For example, European hornbeam (*Carpinus betulus*) Na^+ is mainly concentrated in the stems, hornbeam (*C. turczanzinowii*) and Virginia oak (*Quercus virginian*) Na^+ is mainly concentrated in the roots[7]. K^+ has physiological functions such as regulating ion balance, regulating osmosis, and cell turbulence, and because Na^+ and K^+ have similar hydration energy and ionic radius, Na^+ competes for K^+ absorption sites and active sites under salt stress, The K^+ in root cells will be replaced by Na^+ and transported to the ground. As a result, K^+ absorption is reduced, K^+ dependent enzyme activities and metabolic processes are inhibited[8], so maintaining a higher K^+ content and a higher K^+/Na^+ ratio in the cell can reduce the salt damage to tissues and maintain normal life activities of the body[9]. Salt stress can promote the formation and accumulation of reactive oxygen species in plant cells[10]. Oxidative stress defense promotes antioxidant mechanisms by enzymes, including catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD), etc.[11] If the protection system cannot remove excessive reactive oxygen species in time, the biofilms of plant organs and cells will be damaged. Antioxidant enzymes can promote the protection system to act simultaneously, coordinate with each other and maintain balance under various adversities. To remove reactive oxygen species, reduce the damage to membrane structures, and

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promote cells' self-repair[12].

The nutrients in tomato fruits are rich and it has a wide planting area. It is one of the main facility vegetables and economic crops in China. Tomatoes are extremely sensitive to irrigation amount, have a long growth period, and there is a great difference in water requirements at different growth stages[13], combined with the current situation of China's agricultural water resource shortage and severe secondary salinization of soil, in this study, a fine irrigation model with brackish water deficit regulation and high water use efficiency was proposed for water saving and yield increase of the local economic crop, tomatoes. Water and salt stress is an objective problem that puzzles agricultural production. After combined water and salt stress, the change of soil solute potential has an impact on the distribution of salt ions and the activity of antioxidant enzymes in different organs of crops, how the soil water variation induced by combined water and salt stress indirectly affects the temporal and spatial distribution of salt, and the impact of uneven distribution of soil water and salt on plant physiology is not clear at present. Combined salt water with different salinity with regulated deficit irrigation mode for irrigation to study the effects of K^+, Na^+ absorption, transportation, and distribution characteristics and antioxidant enzyme activity in roots, stems, and leaves under NaCl stress, in order to explore the physiological basis of salt tolerance of tomatoes, provide a reference for tomato breeding in saline-alkali land and make up for the lack of plant physiological research under water and salt stress.

2 Materials and methods

2.1 Study area

The experiment was carried out in a greenhouse for agricultural water and soil engineering in the College of Agriculture and Food, Kunming University of Science and Technology, in June 2019, which is located in Chenggong District, Kunming City, Yunnan Province, China (24°50'56″N, 102°51'49″E). The soil for the experiment was selected from the surface soil in the greenhouse. The soil texture was clay loam. The basic physical and chemical properties of soil are listed in Table 1.

Table 1 Basic physical and chemical properties of soil

Soil property	Setting
Bulk density/g·cm^{-3}	1.285
pH	6.70
Field water holding capacity/%	22.56
Organic matter/g·kg^{-1}	10.11
Available nitrogen/mg·kg^{-1}	60.18
Available phosphorus/mg·kg^{-1}	30.05
Available K/mg·kg^{-1}	118.51
Soil depth/cm	0-30
Clay/%	16.50
Silt/%	29.18
Sand/%	54.32
Soil texture	Clay loam

2.2 Experimental materials

According to local planting mode, the test tomato 'Stone T1228' was purchased from Baoshan City, Yunnan Province, China. It is an unlimited growth type and is suitable to plant in a greenhouse in early spring or autumn. The size of the breeding plate was 16×10 grids. Humus and perlite were used as the substrate. The form of pot experiment was utilized. The flower pot for potting was a standard truncated cone, it was a plastic bucket with a top diameter of 28 cm, a bottom diameter of 22 cm, a height of 36 cm, and a volume of 17 756 cm^3. The plastic bucket was perforated at the bottom in consideration of water permeability and air permeability of the roots, a bucket has 5 holes with a diameter of 5 mm. The number of holes and size of each bucket were the same. In order to accurately control irrigation and the distribution of soil salinity, try to put the soil through a 2 mm sieve after natural air drying, and fill the soil with a natural bulk density of 1.285 g/cm^3. The soil weight per pot was calculated to be 22.8 kg. During the experiment period, the soil moisture content was measured by weight method every other day. The irrigation scheme adopts drip irrigation, and the dripping flow rate was 1 L/h, the water quantity was controlled by the water meter on the main pipe.

2.3 Experimental designs

Three influencing factors were set up in the tomatoes brackish water regulated deficit irrigation experiment, namely the salinity of the irrigation water, different irrigation amount, and the growth stages of the water deficit. The salt concentration of irrigation water was set to 3 levels, local shallow groundwater S0, medium concentration salt stress S1, and high concentration salt stress S2, the irrigation water salinity is 1.1 g/L, 2.0 g/L, and 4.0 g/L respectively. The salinity of local shallow groundwater is 1.1 g/L, and irrigation water kinds with others salinities were configured by mixing NaCl with local shallow groundwater. Three kinds of water treatments were set up in this experiment, normal water treatment W1 (soil moisture content was 70% to 80% Field Capacity (FC)), mild water stress treatment W2 (soil moisture content was 60% to 70% FC), moderated water stress treatment W3 (soil moisture content was 50% to 60% FC). The water deficit occurred in the T1 (seedling stage), T2 (blossoming and bearing fruits stage), and T3 (mature picking stage). A control group was set up for each treatment with different salinity, and the control group was fully irrigated during the whole growth period, except for the three control groups (S0W1, S1W1, and S2W1), the other treatments were only irrigated with water deficit in one growth period and fully irrigated in the other two growth periods (Table 2). This experiment is a three-factor completely orthogonal experiment, so 18 treatments were set, and under the condition of 3 kinds of salt concentrations, a full irrigation treatment during the whole growth stage was set as a control treatment. Therefore, a total of 21 treatments were set up in this experiment, with 7 repetitions for each treatment. Table 2 shows the description of the treatment settings for the irrigation test.

2.4 Agricultural Measures

In this experiment, the growth stage of tomato was divided into three stages: seedling stage, blossoming and bearing fruits stage, and mature picking stage. On May 18, 2019, plump and disease-free tomato seeds were sown on a foam board with a well-divided grid (3 grains per grid) and contained nutrient medium (humus + perlite) for seed germination. The floating board was placed in clear water for suspension breeding (Figure 1a). When the seedlings grow to four euphylla times, the seedlings with the same growth were selected for transplantation (Figure 1b). Tomato seedlings were transplanted to the greenhouse and the seedlings would be fixed after 7 d of transplantation. Tomato seedling stage (July): from planting to the opening of the first flower of the first inflorescence; Flowering and fruit setting period (August September): from the first flower of the first inflorescence to the first fruit of the first inflorescence to the size of a table tennis ball (diameter up to 3 cm); Mature picking stage (September to November): from the first fruit of the first inflorescence to the size of ping-pong ball, to the end of fruit harvest until seedling pulling (Figure 1c). The topping treatment started at 5 to 6 panicles per plant. According to the planting experiences of local farmers, 3 fruits were left in the first ear, 4 fruits in the second to fifth ears, and 5 fruits in the sixth ear. Fertilizer was applied 3 times during the whole growth stage of the tomatoes. The first time, the base fertilizer was applied before the tomato planting, the second time and the third time were applied when the fruits of the first and second ears swelled, application amounts were 3.18 g urea (N), 1.81 g potassium sulfate (K), 4.62 g enzyme active phosphate fertilizer (P) every time, the same amount of fertilizer for 3 times, using the local agronomic measures to control pests and weeds.

Table 2 Test treatment designs of tomato brackish water regulated deficit irrigation

Salinity	Treatments	Irrigation amount	Growth stages of tomatoes		
			Seedling stage T1	Blossoming and bearing fruits stage T2	Mature picking stage T3
S0 (1.1 g/L)	S0W1	Normal irrigation	W1	W1	W1
	S0W2T1	Moderate RDI	(60%-70%) FC	W1	W1
	S0W2T2		W1	(60%-70%) FC	W1
	S0W2T3		W1	W1	(60%-70%)FC
	S0W3T1	Heavy RDI	(50%-60%) FC	W1	W1
	S0W3T2		W1	(50%-60%) FC	W1
	S0W3T3		W1	W1	(50%-60%)FC
S1 (2 g/L)	S1W1	Normal irrigation	W1	W1	W1
	S1W2T1	Moderate RDI	(60%-70%) FC	W1	W1
	S1W2T2		W1	(60%-70%) FC	W1
	S1W2T3		W1	W1	(60%-70%)FC
	S1W3T1	Heavy RDI	(50%-60%) FC	W1	W1
	S1W3T2		W1	(50%-60%) FC	W1
	S1W3T3		W1	W1	(50%-60%)FC
S2 (4 g/L)	S2W1	Normal irrigation	W1	W1	W1
	S2W2T1	Moderate RDI	(60%-70%) FC	W1	W1
	S2W2T2		W1	(60%-70%) FC	W1
	S2W2T3		W1	W1	(60%-70%) FC
	S2W3T1	Heavy RDI	(50%-60%) FC	W1	W1
	S2W3T2		W1	(50%-60%) FC	W1
	S2W3T3		W1	W1	(50%-60%) FC

Note: RDI: Regulated deficit irrigation; FC: Field Capacity; S0: local shallow groundwater; S1: Medium concentration salt stress; S2: High concentration salt stress; W1: 70%-80% FC; W2: 60%-70% FC; W3: 50%-60% FC; T1: Seedling stage; T2: Blossoming and bearing fruits stage; T3: Mature picking stage, the same as below.

a. Grow seedlings b. Planting in seedling stage c. Mature picking stage

Figure 1 Photos of treatment of grow seedlings, planting in seedling stage, and mature picking stage

2.5 Monitoring of meteorological data

The determination of temperature and humidity: using the small meteorological instrument to observe the temperature and humidity in the greenhouse. The determination of water surface evaporation: The E-601 evaporating dish was used for the measurement, and the observation time was 8:00 every morning (Figure 2).

2.6 Basic physical properties of soil samples for testing

The bulk density of soil and the field water holding capacity were determined by the indoor ring knife method. Soil pH was measured with phs-3c pH meter. Soil organic matter was determined by the excess potassium dichromate volumetric method. The soil available phosphorus was extracted by 0.5 mol/L $NaHCO_3$, the soil available potassium was determined by sodium tetraphenylborate turbidimetry, and the determination of alkali hydrolyzable nitrogen in the soil was carried out by alkali diffusion method.

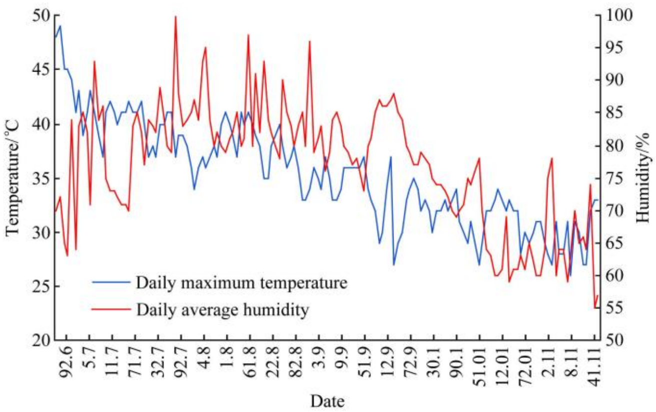


Figure 2 Climate variables (daily maximum temperature and relative humidity) during the growing seasons of tomatoes in 2019

2.7 Determination of Na^+ and K^+ content

The ion content in tomato roots, stems, and leaves was measured every 15 d. The tomato roots, stems, and leaves were sampled separately, green removed at 105℃, dried at 70℃, crushed and sifted through a 1 mm sieve, and accurately weighed 1.5 g into a cone and added 5 mL of concentrated HNO_3 and 1 mL of 30% H_2O_2 to the bottle, and sealed it overnight. Using an electric hot plate to heat and digest until colorless or clear. Continuing heating to drive out HNO_3 and H_2O_2. Then adding a small amount of water to dissolve the sample. After cooling, the digester liquor was fixed capacity to 25 mL with ethyl acetate. At the same time as each batch of samples were digested, a blank test was performed to correct the errors in reagents and methods. Na^+ and K^+ ions were quantified using a flame photometer (Jenway Model PEP7, USA).

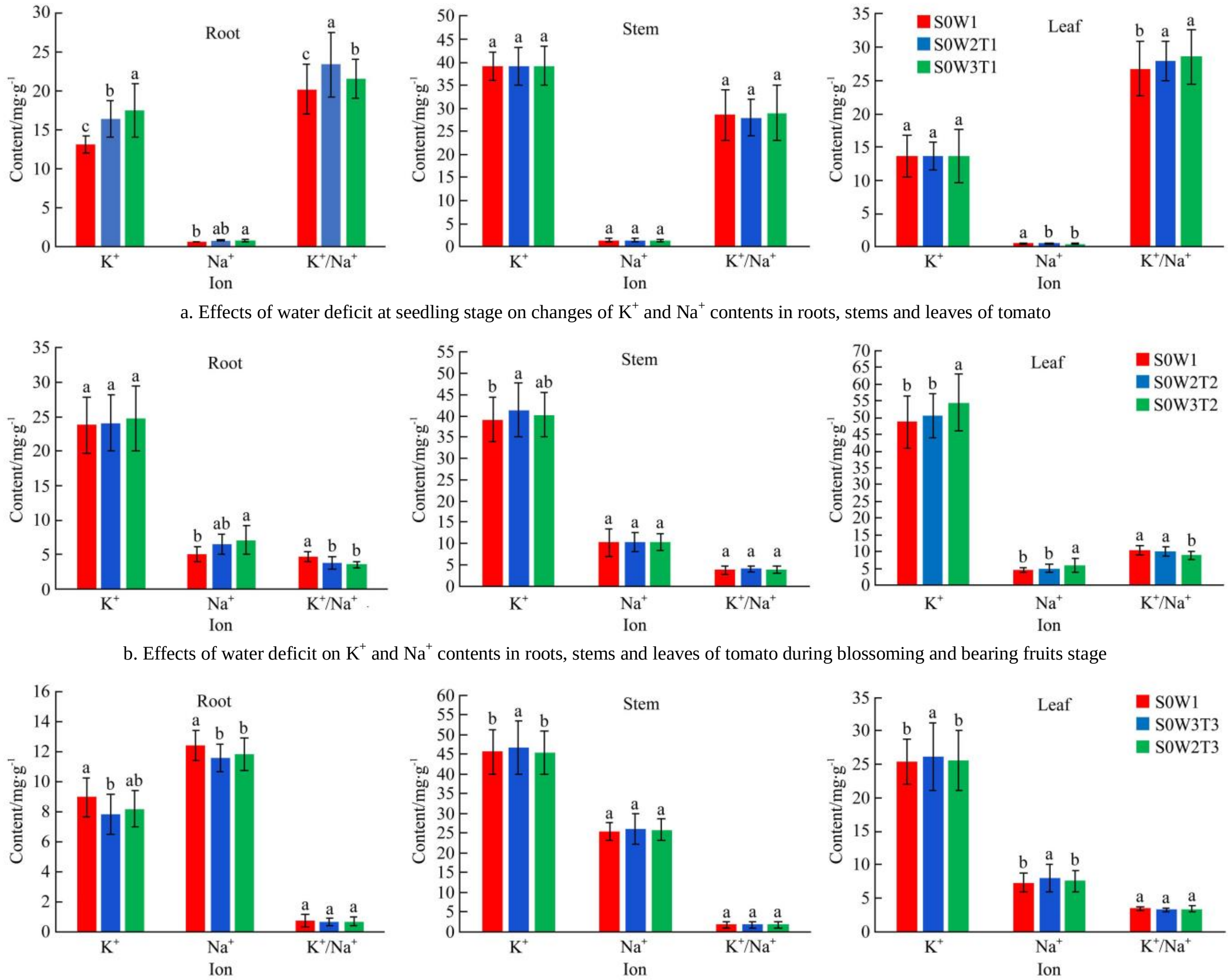
2.8 Determination method of resistance index

CAT activity was measured by potassium permanganate titration, SOD activity was measured by NBT reduction method, and POD activity was measured by guaiacol colorimetric method[14].

3 Results

3.1 Effect of water deficit regulation on ion content in roots, stems, and leaves at different growth stages

Effects of water deficit regulation on the changes of K^+, Na^+, and K^+/Na^+ ratio in roots, stems, and leaves of tomato at the seedling stage are shown in Figure 3a. It can be seen that K^+ and Na^+ in roots were sensitive to water stress at the seedling stage, and the two ions showed significant differences. In stems and leaves, the effects of different water stress on K^+ and Na^+ were small, and there was no significant difference among the treatments. Water deficit at the seedling stage could significantly increase the contents of K^+ and Na^+ in roots, and the greater the degree of water deficit was, the more the ion content increased. Under mild and moderate water deficit, K^+ increased by 24.56% and 32.31%, respectively, compared with the control treatment S0W1, Na^+ increased by 7.69% and 24.61%, respectively, and K^+/Na^+ increased by 15.67% and 6.64%, respectively, compared with the control treatment S0W1.



a. Effects of water deficit at seedling stage on changes of K^+ and Na^+ contents in roots, stems and leaves of tomato

b. Effects of water deficit on K^+ and Na^+ contents in roots, stems and leaves of tomato during blossoming and bearing fruits stage

c. Effects of water deficit at tomato fruit stage on changes of K^+ and Na^+ contents in roots, stems, and leaves

Note: FC: Field Capacity; S0: local shallow groundwater; W1: 70%-80% FC; W2: 60%-70% FC; W3: 50%-60% FC; T2: Blossoming and bearing fruits stage; T3: Mature picking stage, the same as below.

Figure 3 Effects of water deficit at different growth stages on changes of K^+ and Na^+ contents in roots, stems, and leaves of tomato and K^+/Na^+ ratio

As shown in Figure 3b, the laws reflected in the blossoming and bearing fruit stage were different from those in the seedling stage. When the water deficit occurred in the blossoming and bearing fruits stage, the roots, stems, and leaves of tomato had ionic responses to water stress. The Na^+ in roots were most significantly affected by water stress. Compared with the control S0W1 treatment, the Na^+ in roots under mild and moderate water stress were increased by 28.63% and 40.16%, respectively, while

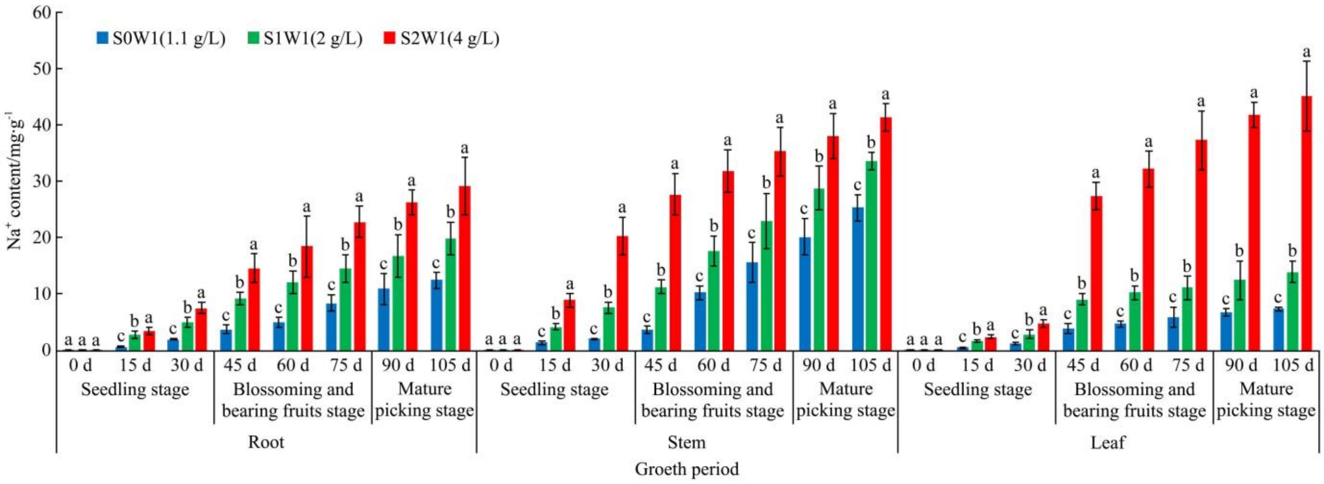
K^+ were the main regulatory response ions in tomato stems. The K^+ content under mild water stress was higher than that under moderate water stress (the K^+ accumulation contents under mild and moderate water stress were increased by 5.72% and 2.63%, respectively, compared with the control group), indicating that when water stress was severe, cations would be distributed according to the needs of osmotic adjustment in different parts, mainly accumulated in important parts such as roots and leaves, and thus reduced the content of stems. At the same time, it was also found that the response of leaves was more sensitive. K^+ and Na^+ increased significantly with water stress. Compared with the control treatment S0W1, the contents of K^+ and Na^+ increased by 3.65% and 11.71%, 8.37%, and 28.97%, respectively.

It can be seen from Figure 3c that the water deficit treatment at the fruiting stage had little effect on the contents of K^+ and Na^+ in tomato leaves, and there was no significant difference in the different degrees of water deficit. Only in roots, K^+ and Na^+ showed significant differences among different treatments. Different from the other two periods, the water deficit did not increase the contents of K^+ and Na^+ in tomato roots at the fruiting stage, and the ion content was the highest under full irrigation. In the mature picking stage, under no salt stress and mild water deficit condition (S0W2T3), K^+ and Na^+ contents in tomato roots decreased by 13.01% and 4.34%, respectively.

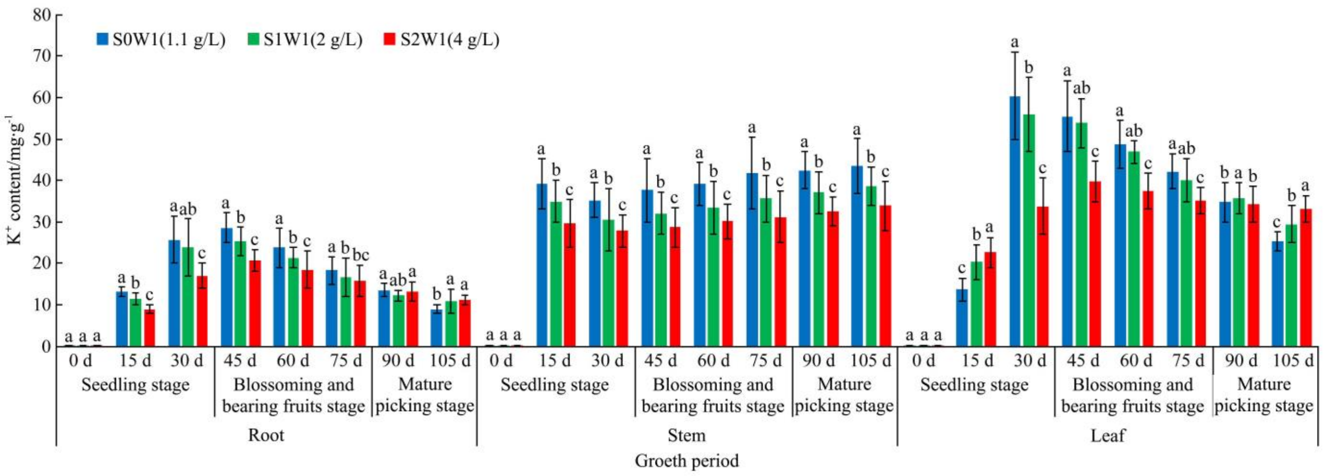
Comparing the K^+/Na^+ratio of the three growth stages, it can be found that the K^+/ Na^+ ratio of root and leaf of tomato increased only in seedling stage, so 'stone' tomato had the best water stress resistance in seedling stage, and water stress was the best in tomato seedling stage.

3.2 Effect of irrigation salinity on ion content in roots, stems and leaves at different growth stages

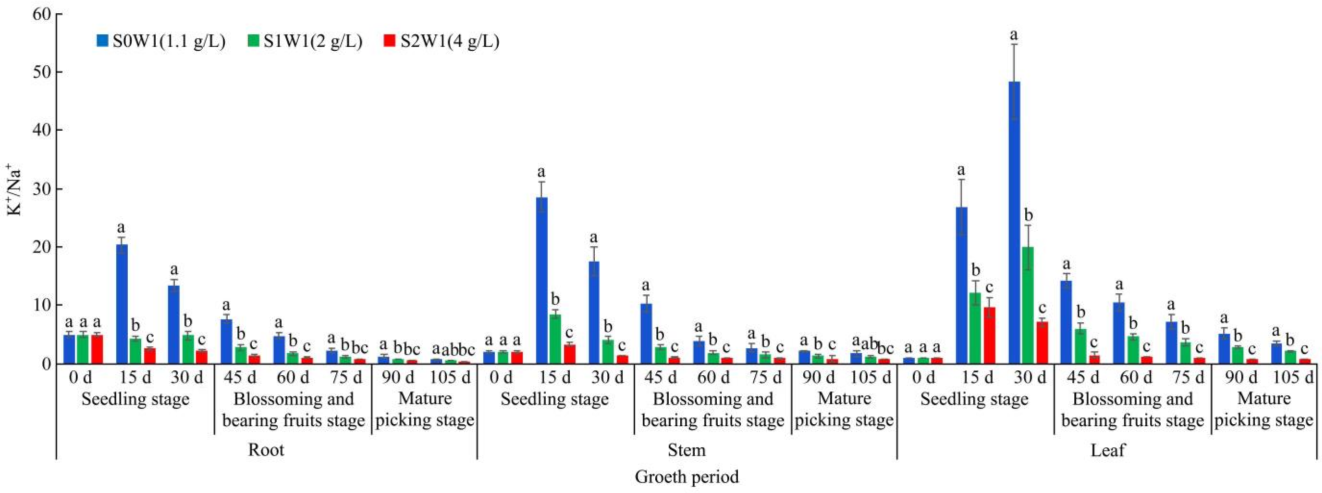
The changes of inorganic ions in tomato roots, stems, and leaves with growth period under three mineralization degrees in each full irrigation treatment were shown in Figure 4a. The result showed that the accumulation of Na^+ in roots, stems, leaves, and fruits of tomato gradually increased with the increase of salt concentration and stress time. Especially when the salt content reached 4 g/L, the Na^+ content in each part increased. Under fresh water treatment, the accumulations of Na^+ in various organs of tomato at seedling stage were in a descending order of root, stem, and leaf; and the accumulation of Na^+ varied with the growth of tomato. In the middle stage of flowering and fruiting, the accumulation of Na^+ in stem began to exceed that in root, and the accumulations of Na^+ in various organs of tomato at mature stage were in descending order of stem, root, and leaf. Compared with the fresh water treatment, when the irrigation water salt concentrations were 2 g/L and 4 g/L, the accumulation of Na^+ of tomato seedlings changed in descending order as stem, root, and leaf. This indicated that the content of Na^+ in the stem, leaf, and root system changed with the change in salt concentration. The stem of tomato preferentially absorbed and accumulated a large number of Na^+ to adapt to salt stress, Na^+ accumulated in the root was slightly less to ensure the normal growth of crops.



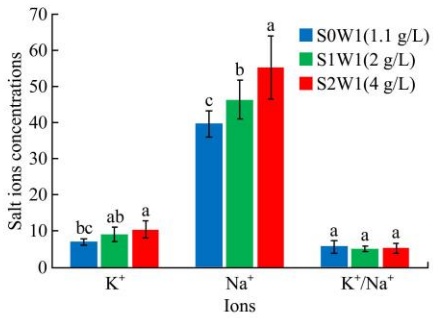
a. Distribution of Na^+ content in roots, stems, and leaves



b. Distribution of K^+ content in roots, stems, and leaves



c. K^+/Na^+ ratio



d. Salt ion content in fruits

Note: FC: Field Capacity; S0: local shallow groundwater; S1: Medium concentration salt stress; S2: High concentration salt stress; W1: 70%-80% FC, the same as below.

Figure 4 Changes of different kinds of inorganic ions in tomato roots, stems, leaves, and fruits with the growth period under three salinity treatments

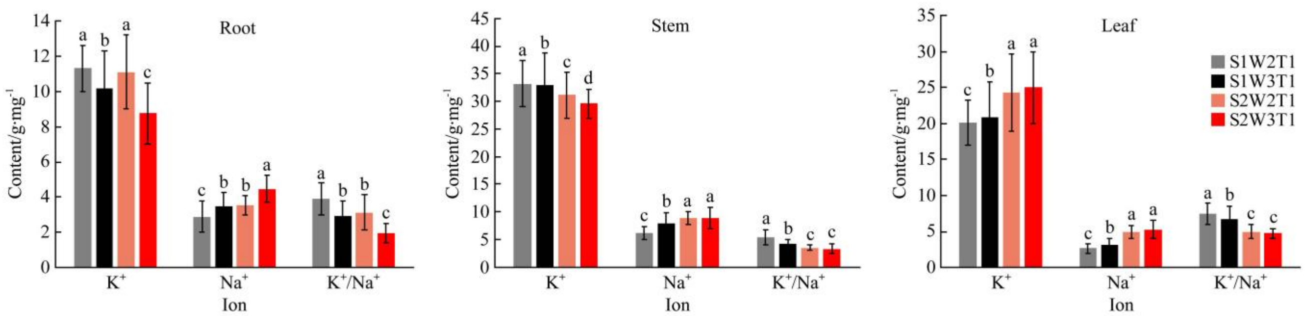
It can be seen from Figure 4b that the effect of salt stress on the K^+ content in various organs of tomato plants is relatively complex. K^+ content in tomato root increased with the growth and development at the seedling stage, decreased at the flowering and fruit-setting stage, and decreased with the increase of stress intensity in the tomato stem. The regularity of other parts is not strong. When the salt concentration was less than 4 g/L, the roots, stems, and leaves of tomato could accumulate more K^+ in the body through their own regulation and response mechanisms such as selective absorption, so as to alleviate the inhibitory effect of salt stress on growth. When the salt concentration reached 4 g/L, the accumulation of K^+ in roots and stems decreased significantly, while the accumulation of K^+ in leaves showed an increasing trend, which is also the regulation mechanism of tomatoes under salt stress. Through the selective transport of absorbed K^+ to the leaves to maintain normal growth and metabolism of plants, 4 g/L salt concentration may be the threshold of tomato self-regulation.

The maintenance of a high K^+/Na^+ ratio can reduce salt damage, which is a necessary prerequisite to ensure the normal life activities of plants. Therefore, the decrease in the K^+/Na^+ ratio is a typical manifestation of the destruction of ion balance in plant cells under salt stress. It can be seen from Figure 4c that with the growth of tomato, the K^+/Na^+ ratio in roots, stems, and leaves of tomato decreases, the seedling stage is greater than the flowering and fruit setting stage and the mature picking stage is greater than the result stage. After salt stress, the K^+/Na^+ ratio of leaves, stems, and roots of tomato decreased with the increase of NaCl concentration, the decreasing trend of stems and roots was relatively large, and the change range of roots was relatively small. Taking the treatment time of 15 d as an example, comparing with the S0W1 (1.1 g/L) treatment, the K^+/Na^+ ratio of roots stems and leaves under S1W1 (2 g/L) treatment and S2W1 (4 g/L) treatment were decreased by 83.07%, 81.17%, 54.71% and 87.30%, 92.57%, 63.86%, respectively. It could be seen that the greater the salt concentration, the greater the decrease range of the K^+/Na^+ ratio, indicating that salt stress increases Na^+ content in tomato leaves, stems, and roots, and the K^+ extravasation broke the original ion balance. The root system of the tomato was the first part that was subjected to suffered salt stress, and the K^+/Na^+ ratio decreased significantly compared with the stem under salt stress. The K^+/Na^+ ratio of leaves and fruits changed a little under salt stress. When salt stress reached a certain level (salinity was 4 g/L), for maintaining normal growth, the declining trend of the K^+/Na^+ ratio of stems was higher than roots. The K^+/Na^+ ratio decreased slowly, and the Na^+ accumulated preferentially in the stem.

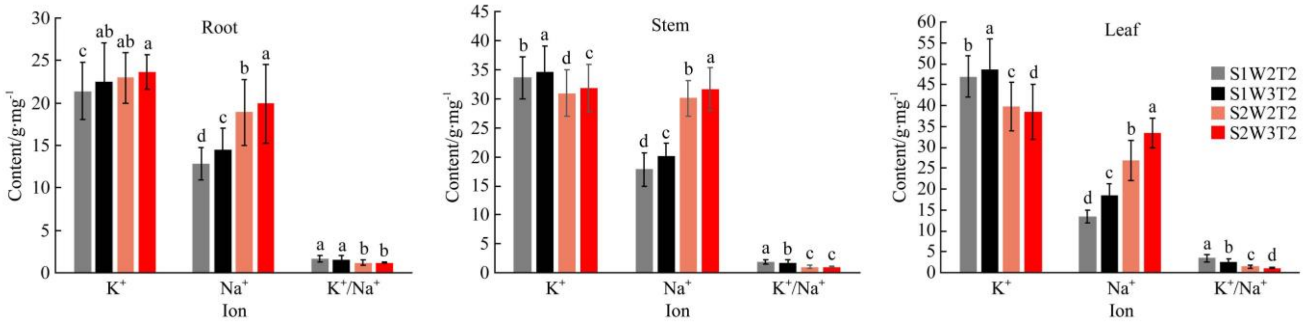
Tomato is a kind of fruit and vegetable with high K^+ content, as shown in Figure 4d. Tomato fruit is the part with the highest K^+ content among all organs, and the demand for K^+ is increased at the fruiting stage. In this experiment, due to the influence of salt content, the growth of tomatoes was inconsistent when picked at 135 d, the ripeness of the fruit was different, and the K^+ content changed greatly.

3.3 Effects of water and salt stress on ion content in roots, stems, and leaves at different growth stages

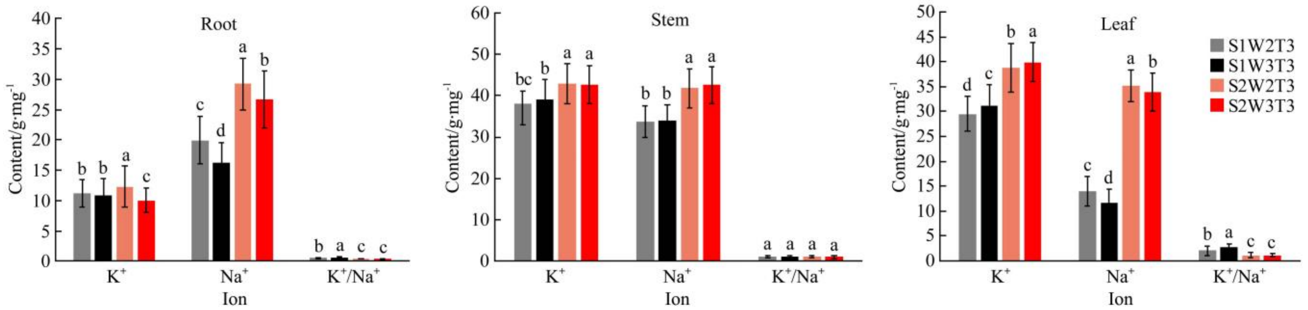
It can be seen from Figure 5a that the K^+ contents in tomato roots were basically no different under mild water-regulated deficit treatments (S1W2T1, S2W2T1). With the increase in the degree of regulation deficit, the accumulation content of K^+ in each treatment of moderate water regulation deficit decreased significantly. Compared with the mild water regulated deficit treatment S1W2T1, under the moderate water regulated deficit treatments (S1W3T1, S2W3T1), the K^+ accumulation decreased by 10.27% and 22.57%, respectively. It can be seen that brackish water combined with moderate water regulated deficit irrigation can significantly reduce the accumulation of K^+ in tomato roots at seedling stage, while brackish water combined with mild water regulated deficit irrigation has no significant effect on the accumulation of K^+ in tomato roots. On the contrary, the accumulation of Na^+ in the root system increased with the increase of water stress and salt stress. In general, the coordinated regulation of water and salt at the seedling stage can increase the accumulation of Na^+ and reduce the content of K^+ in roots. The greater the degree of water and salt regulation, the more obvious the accumulation effect was. And moderate water regulated deficit had a significant effect on the addition of ion accumulation under salt stress. Because the stem was not the main accumulated organ of osmotic-regulated ions at the seedling stage, the difference in K^+ and Na^+ content in the stem was not significant under different water and salt collaborative stress treatments. The contents of the four kinds of ion were significantly influenced by water and salt collaborative stress, which can be clearly found that with the increase of irrigation salinity, K^+ and Na^+ contents had obviously increased. Especially when the salinity reached 4 g/L, the K^+ content of S1W3T1, S2W2T1, and S2W3T1 treatment increased by 3.98%, 20.68%, and 24.20% compared with S1W2T1 treatment, respectively. It can be seen that the same degree of water regulated deficit treatment has little effect on the contents of various ions, and the ion content was slightly higher under moderate water stress than under mild water stress treatment. It can be seen that the accumulation of ions in leaves is mainly affected by salt content.



a. Effects of synergistic regulation of water and salt at seedling stage on changes of K^+ and Na^+ contents in roots, stems and leaves of tomato



b. Effect of synergistic regulation of water and salt on the changes of K^+ and Na^+ contents in roots, stems and leaves of tomato during flowering and fruit-setting stage



c. Effect of synergistic regulation of water and salt on the changes of K^+ and Na^+ contents in roots, stems and leaves of tomato at fruiting stage

Note: FC: Field Capacity; S1: Medium concentration salt stress; S2: High concentration salt stress; W2: 60%-70% FC; W3: 50%-60% FC; T1: Seedling stage; T2: Blossoming and bearing fruits stage; T3: Mature picking stage, the same as below.

Figure 5 Effects of synergistic regulation of water and salt at different growth stages on changes of K^+ and Na^+ contents in roots, stems, and leaves of tomato and K^+/Na^+ ratio

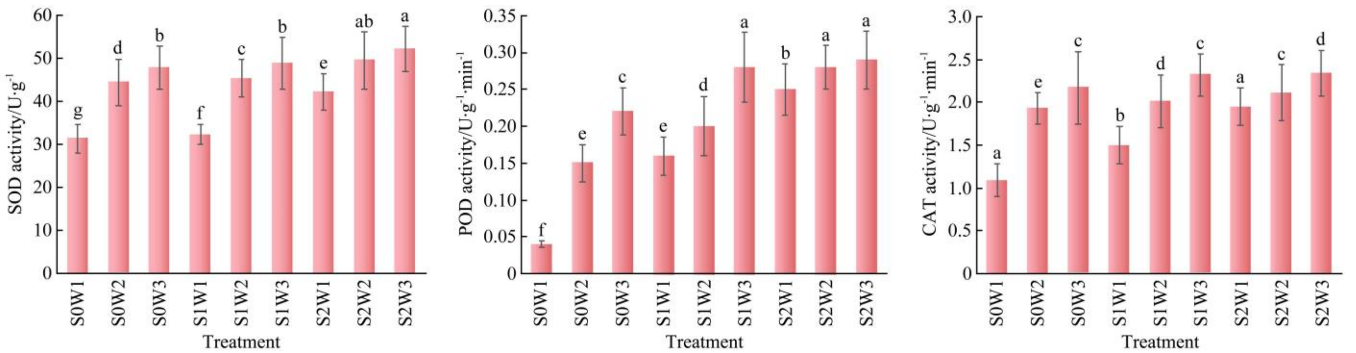
At the blossoming and bearing fruits stage, the contents of K^+ and Na^+ in tomato roots reached the maximum under S2W3T1 treatment, K^+ reached 23.69 g/mg and Na^+ reached 19.91 g/mg. As shown in Figure 5b, the accumulation of ions increased with the deepening of collaborative stress of water and salt. K^+ in the stem has different change rules. Under the same mineralization degree, the K^+ accumulation under mild water regulated deficit irrigation is greater than that under moderate water deficit treatment. Na^+ is opposite to K^+. When the salinity increased to 4 g/L, the accumulation of Na^+ in stem was significantly higher than that when the salinity of irrigation water was 2 g/L. On the one hand, it is related to the amount of irrigation water, on the other hand, it may be related to the accumulation of ions in different parts under stress. The change rules of K^+ and Na^+ in leaves were different.

Na^+ accumulate and increase with the depth of the water and salt collaborative stress, while K^+ accumulation decrease with the depth of water and salt collaborative regulation degree except for S1W3T2 treatment.

As shown in Figure 5c, in the fruiting stage, under three salinity levels and different degrees of water regulated deficit irrigation, K^+ content under mild water regulated deficit treatment was higher than moderate water regulated deficit treatment, and the change rule of Na^+ content and K^+ content was same in roots. This shows that after the salt stress in seedling stage and flowering and fruit setting stage, a large number of salt ions have accumulated in tomato root system to regulate osmotic. At this time, when water deficit occurred again, especially in severe water deficit, the root system would transport salt ions to other parts to ensure the activity of root system and continue to absorb water to maintain the growth and development of tomato. Therefore, ions at moderate water deficit began to decrease in the root system and migrated to the stem. K^+ in leaves increased significantly with the increase in the degree of water-salt synergistic regulation. S1W3T3, S2W2T3, and S2W3T3 increased by 5.45%, 31.34%, and 35.13% respectively compared with S1W2T3 treatment. Na^+ also showed an upward trend, indicating that most of the salt ions in irrigation water were absorbed by leaves, while the increase of K^+ indicated that when the degree of synergistic regulation of water and salt was high, leaves re-accumulated K^+ to resist Na^+ toxicity.

3.4 Effects of synergistic regulation of water and salt on antioxidant enzyme activity in tomato leaves at seedling stage

The activity of SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) in tomato leaves showed the same trend and increased continuously under the collaborative stress of water and salt. Among them, with the increase of the degree of water regulated deficit, there was max increase degree of antioxidant enzymes activity in tomato leaves of seeding stage, under salt-free stress. Taking CAT as an example, compared with S0W1, the CAT activity increased by 77.06% and 99.08%, respectively, under the treatment of S0W2 and S0W3. Under the collaborative regulation of water and salt, the activity of antioxidant enzymes in leaves increased, but the increasing degree was smaller than that under water stress only. For example, compared with S1W1 treatment, the CAT increased by 34.00% and 54.67%, respectively, under S1W2 and S1W3 treatments. Compared with S2W1 treatment, CAT increased by 8.21% and 20.00%, respectively, under S2W2 and S2W3 treatments.



a. Effects of collaborative stress of water and salt on superoxide dismutase (SOD) activity in tomato leaves at seedling stage

b. Effect of collaborative stress of water and salt on peroxidase (POD) activity in tomato leaves at seedling stage

c. Effect of collaborative stress of water and salt stress on catalase (CAT) activity in tomato leaves at seedling stage

Note: FC: Field Capacity; S0: local shallow groundwater; S1: Medium concentration salt stress; S2: High concentration salt stress; W1: 70%-80% FC; W2: 60%-70% FC; W3: 50%-60% FC; CAT: catalase; SOD:superoxide dismutase; POD: peroxidase, the same as below.

Figure 6 Effects of collaborative stress of water and salt on antioxidant enzyme activity in tomato leaves at seedling stage

4 Discussion

4.1 Effects of water and salt stress on ion content in different organs of tomato

Salt stress often leads to oxidative stress, osmotic stress, and nutritional deficiency. Therefore, maintaining the ion content balance in the plant body and the cell is essential to maintain the normal growth of plants[15]. The absolute content of salt ions in plants and the priority of ion distribution in various organs are closely related to the salt tolerance of crops[16]. The salt tolerance of crops directly affects their growth, yield, and quality in saline soil. The regional distribution of ions in different organs of tomato is co-determined by selective absorption of roots and selective transportation among various organs[17]. In this study, the ‘Stone’ tomato with strong salt tolerance was selected as the experimental material to analyze the distribution characteristics of Na^+ and K^+ in roots, stems, and leaves and the salt tolerance of tomatoes under water and salt stress at different growth stages. According to the accumulations of ions in root, stem, leave, and fruit at different growth stages under salt stress, the distribution rules of Na^+ content in different organs were in descending order of stem, root/leaf, and fruit. Na^+ is mainly accumulated in the stem of tomato, which is due to the tomato belonging to non-helophytic plants, and its salt tolerance is relatively weak. Stem, as a conducting tissue and supporting organ, has relatively weaker metabolic activity than roots and leaves. The salt ions that accumulated in stems can alleviate the damage to functional organs[18]. Previous studies showed that with the increase of salt tolerance of crops, the accumulation of parts of Na^+ increased accordingly. The higher the salt tolerance level, the higher the main accumulation parts of Na^+[19].

K^+ is a necessary element for plant growth. Tomato, as a non-helophytic crop, has stronger selective absorption of K^+ than Na^+ [17]. The study by Jia et al.[20] on the effects of salt stress on the growth and physiological characteristics of Primula forbesii Franch showed that the K^+ absorbed by the roots was transported to the leaves, thereby maintaining the high K^+/Na^+ ratio in the leaves. In this study, the K^+ content in tomato roots increased with the increase of salt content, which was not only related to the K^+ absorption selectivity of roots, but also related to the K^+ content in the soil. The decrease of K^+ content in tomato stems was mainly inhibited by Na^+ content, while the K^+ content in leaves was maintained to ensure photosynthesis and normal growth of tomato. In order to ensure the normal physiological activities of the plant, the minimum value of K^+/Na^+ ratio in the cytoplasm of plants is about 1, while K^+/Na^+ ratio in the saline environment is far less than 1. Plants maintain a high K^+/Na^+ ratio in the cytoplasm by increasing K^+ absorption selectivity, which becomes one of the determining factors of plant salt adaptation[21]. In this study, the ratio of K^+/Na^+ ratio in tomato roots was less than 1, indicating that the increase of Na^+ content in soil affected the growth of tomato plants to a certain extent. Low salt content had no obvious inhibiting effect on the aboveground parts of plants (K^+/Na^+ ratio >1), and high salt content had a significant effect on the growth and development of plants.

4.2 Effects of water and salt stress on antioxidant enzyme activity at tomato seedling stage

In normal conditions, reactive oxygen species (ROS) generation and scavenging reactions exist in the oxygen metabolism of plants, and the two reaction types are in a balanced state. The protective enzyme systems for ROS scavenging in plant cells include SOD, POD, and CAT. When crops are subjected to drought stress or salt stress, the initial response is often manifested that the $O_2^{\bullet}$ is disproportionated to O_2 and H_2O_2 by SOD. High concentration of H_2O_2 in tissues is mainly eliminated by CAT, so H_2O_2 is controlled at a low level. A low concentration of H_2O_2 is mainly digested by POD during corresponding substrate oxidated. The synergistic effect of SOD, POD, and CAT reduced the accumulation of ROS and prevented the transformation of $O_2^{\bullet}$ H_2O_2 to highly destructive OH through Fenton-type Haber-Weiss reaction, thus avoiding or reducing oxidative damage, so as to achieve the purpose of adaptation to adversity[22,23]. However, long-term and high-intensity water and salt stress will break the original balance of the active oxygen metabolism in plants, and in a short time, a large number of accumulated reactive oxygen species will seriously affect the activity of reactive oxygen scavengers such as SOD, POD, and CAT. Reactive oxygen scavengers in plants cannot cope with the surge of booming reactive oxygen species in a short time, resulting in a significant decline in the ability of plants to scavenge reactive oxygen species[24]. Some research results showed that POD activity in organs of Ulmus pumila L increased significantly with the increase of NaCl concentration and water stress intensity[25]. The ability to maintain high POD activity under adversity is the embodiment of plant adaptability to salt stress. The contents of POD and SOD in plants are relatively lower under normal growth conditions, but they will remain at a high level under stress, especially under drought or salt stress. At the same time, the active oxygen scavenging system can protect the cell membrane structure and function from damage to ensure they can maintain normal physiological activities[26]. In this study, the activity of SOD, POD, and COD of ‘Stone’ tomato seedlings showed an upward trend under water and salt stress, indicating that crops have the potential to exert stress adaptability.

It is very difficult for young organs of plants to respond to high-intensity stress by changing cell structure in a short time. It is the plant's protective enzyme system that plays an important role, and its activity can judge the salt tolerance of seedlings and even crops[27]. In the research of salt tolerant breeding, Liu et al.[28] found that the activity of CAT, POD, and CAT enzymes of ‘Miaohong’ tomato seedlings increased under the NaCl stress, the solution concentration range of NaCl was 50-200 mmol/L, and the treatment concentration range could be used as the optimal concentration of 'Miaohong' tomato for salt tolerance screening. In this experiment, the antioxidant enzymes activity and content of seedlings showed an upward trend under the collaborative treatment of NaCl stress and water regulated deficit. When the salinity of salt solution reached 4 g/L, the enzyme activity still showed an upward trend but the rising degree was not obvious. In this experiment, under the synergistic treatment of NaCl stress and water deficit, the antioxidant enzyme activity content of seedlings showed an upward trend. Compared with the results of Liu et al.[28], in this experiment, the resistance physiological indexes of ‘Stone’ tomato showed an upward trend and maintained at a high level, indicating that the tested cultivars had good salt tolerance. The irritability of plants to salt stress was mobilized due to the increase of osmotic adjustment substance content. The crop stabilizes the osmotic level in the cells of various organs through its own physiological reaction, and achieves the purpose of maintaining normal physiological activities. However, how long this ability to stabilize osmotic balance can be maintained under different salt stress levels remains to be further studied.

5 Conclusions

1) The collaborative regulation of water and salt will significantly change the ion absorption of roots and the transfer to stems and leaves at different growth stages. The content of the main salt toxic ion, Na^+, increases with the increase of salt content and the degree of water deficit regulation. With the change in the salt content, Na^+ preferentially accumulated in tomato stems, K^+ accumulated in tomato leaves. Tomato leaves maintained high K^+ concentrations and reduced the damage of Na^+ on plant photosynthetic organs to maintain normal growth. The water regulated deficit treatment at the seedling stage was helpful for the accumulation of K^+ in tomato leaves, the ability of salt resistance and drought resistance in the tomato seedling stage was stronger than that in other growth stages.

2) The K^+/Na^+ ratio decreased with the increase in salt concentration and the collaborative regulation of water and salt. The greater the salt concentration was, the greater the K^+/Na^+ ratio decreased degree. The tomato fruits had the highest content of K^+ among all tomato organs. The demand for K^+ increased at the fruiting stage, and the K^+/Na^+ ratio in fruit was not significantly affected by salt.

3) The activity of CAT, SOD, and POD in leaves of 'Stone' tomato increased with the increase of NaCl concentration and water stress, and maintained at a high level, indicating that the tested varieties had good salt tolerance.

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