RESEARCH

Effects of ethephon on physiological characteristics and gene expression of Tartary buckwheat under salt stress

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ABSTRACT

Salt-tolerant 'Chuanqiao No.1' and salt-sensitive 'Chuanqiao No.2' Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) were used as materials. Effects of ethephon on physiological characteristics of Tartary buckwheat under salt stress were studied by different concentrations of ethephon (ETH) under 150 mM NaCl stress. Ethephon treatment could improve seed germination rate of both varieties under salt stress, and the effect of ethephon treatment on salt-sensitive variety was better. Ethephon treatment could improve physiological characteristics of Tartary buckwheat seedlings under salt stress, and appropriate concentrations of ETH root treatment in salt-tolerant and salt-sensitive variety were 1.0 and 1.5 mM, and for ETH spraying were 0.4 and 0.6 mM. The effect of ETH spraying on improving physiological characteristics of Tartary buckwheat under salt stress was better than that of ETH root treatment. Salt tolerance gene *FtNHX1* reached its maximum expression level at 24 h, which was increased by 277.77% and 251.17% in salt-tolerant and salt-sensitive variety compared with salt stress under ETH root treatment, while under ETH spraying treatment, which was increased by 232.39% and 190.91% in salt-tolerant and salt-sensitive variety compared with salt stress. Salt tolerance gene *FtSOS1* reached its maximum expression levels at 12 h, which was increased by 60.94% and 98.75% in salt-tolerant and salt-sensitive variety compared with salt stress under ETH root treatment, while under ETH spraying treatment, which was increased by 35.98% and 67.63% in salt-tolerant and salt-sensitive variety compared with salt stress.

Key word: Ethephon, physiological characteristics, salt stress, Tartary buckwheat.

INTRODUCTION

Buckwheat belongs to *Fagopyrum* Mill. of Polygonaceae. It was originated in China and is coarse grain with high nutritional value (Wu et al., 2018). The salt in the deep soil reaches the surface with water. After water evaporation, the salt content on the soil surface increases, leading to soil salinization (Li et al., 2012). Saline alkali land is a widely distributed soil type on the earth, with a total area of about 955 million hectares. It is distributed in the coastal and dry and arid areas of all continents in the world, accounting for about 25% of the total land area (Qin et al., 2005).

The impact of soil salinization on agriculture can be analyzed from two aspects: mild salinization worsens the quality of cultivated land and reduces the yield of common crops. This is mainly because the salt in the soil produces ion toxicity to plants, inhibits enzyme activity and protein synthesis in plants, resulting in metabolic disorder of plant physiology, meanwhile, the high concentration of salt in the soil reduces soil water potential, makes it difficult for roots to absorb water, leads to water shortage of plants, thus affects growth (Meng et al., 2014). In addition, the continuous expansion and deepening of salinization area is one of the important reasons for the reduction of cultivated land area in China (Li and Li, 2018).

Fagopyrum esculentum Moench and *F. tataricum* (L.) Gaertn. are two main cultivated buckwheat varieties. Tartary buckwheat (*F. tataricum*) contains many nutrients, such as polysaccharides, protein, lipids, vitamins, minerals, dietary fiber, etc. (Nie and Li, 2016). Tartary buckwheat contains flavonoids, which has a certain health care effect on human body (Tan et al., 2009). Rutin, which can improve blood vessel microcirculation and anti-aging, accounts for more than 70% of flavonoids. Modern medicine has proved that Tartary buckwheat has many pharmacological effects, such as hypoglycemic, anticancer, analgesic (Lin et al., 2011; Jia et al., 2012). In daily life, common buckwheat products include buckwheat tea, buckwheat flour, buckwheat beer, etc. (Cheng et al., 2018). In addition to buckwheat seeds and buckwheat malt, buckwheat husk can also be used as medicine pillow filling material to improve sleep quality (Wang, 2014).

In order to improve the salt tolerance of crops timely and effectively, many experts and scholars focus on the application of exogenous substances (Guo et al., 2017), exogenous substances include glycinebetaine, salicylic acid (Gao et al., 2018; Yu et al., 2019) and phthalanilic acid (Zhang et al., 2018). Ethylene is one of the important hormones produced in plants. It not only plays an important role in seed germination, rooting, flowering, fruiting and senescence, but also plays an important role in plant stress tolerance and resistance. For example, ethylene plays an important role in plant response to drought, high salt, low temperature, disease and other stresses (Yu and Huang, 2013). Under normal conditions, the content of ethylene in plants is low, and it increases rapidly after stress, which stimulates the response to stress (Wright, 1974). Ethephon (ETH) can release ethylene and can be used as a substitute for ethylene in production. It was found that applying a certain concentration of ETH could improve the physiological characteristics of plants under stress and alleviate the damage of stress to plants (Wang, 2008). For example, soaking seeds with ETH before sowing can improve their drought resistance of wheat (Hu, 2017), and spraying ETH can alleviate the damage of heavy metal stress to *Houttuynia cordata* (Xia and Zhu, 2010).

In view of the widespread existence and low utilization rate of saline soil, in order to meet the needs of crop growth and ensure food security, it is of great significance to cultivate salt-tolerant crops or apply exogenous substances to improve crop salt tolerance, so as to realize the rational utilization of saline soil. Tartary buckwheat can be used as food and extract functional substances, it is one of the most suitable crops for planting in salinized soil (Lang, 1996). Compared with the period of cultivating new stress resistant varieties, the application of exogenous substances can improve the plant resistance in a shorter time. Appropriate concentration of aspartic acid (Zhang et al., 2020) and abscisic acid (Lu et al., 2021) could significantly improve the physiological characteristics of Tartary buckwheat under salt stress.

The transcriptome sequencing of Tartary buckwheat has been completed (Song et al., 2021), and the salt tolerance related genes *FtNHX1* (Liu et al., 2017a) and *FtSOS1* (Liu et al., 2017b) have been cloned. In this study, salt-tolerant 'Chuanqiao No.1' and salt-sensitive 'Chuanqiao No.2' (Liu et al., 2015) Tartary buckwheats were used as experimental materials, and ETH root treatment and leaf spraying were applied under NaCl stress. The effects of ETH on salt tolerance of Tartary buckwheat seedlings were investigated by measuring the physiological indexes and the expression of salt tolerance genes of *FtNHX1* and *FtSOS1*, which will provide the basis for the study of salt tolerance mechanism of buckwheat and the effective utilization of saline soil resources.

MATERIALS AND METHODS

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) 'Chuanqiao No.1' and 'Chuanqiao No.2' were provided by Alpine Crop Research Station, Xichang Department of Agriculture and Science, Liangshan State, Sichuan Province, China.

Seed treatment

The seeds were sterilized with 1 g L^{-1} $KMnO_4$ for 10 min, washed twice with appropriate amount of sterile water, aerated and inflated for 5 h in deionized water, and evenly placed in a culture dish with gauze and cultured in 26 °C incubator, each culture dish had 121 seeds. The appropriate amount of sterile water in the culture dish was used as the control (CK), and 150 mM NaCl solution with sterile water was used as salt stress treatment (NaCl). The concentration of ethephon (ETH) prepared by NaCl solution was 0.5, 1.0, 1.5 and 2.0 mM (NaCl+ETH), respectively. Three replicates were set for each treatment.

Seedling cultivation and treatment

The medium and soil were mixed 1:1 by volume, and put into a small plastic basin with the length, width and height of 6 × 6 × 10 cm. Each basin had six seeds, and the depth was 2 cm. After seeds germinated, the seedlings were irrigated with 1/2 Hoagland nutrient solution, natural light, routine management, day and night temperatures were 26 and 18 °C, relative humidity was 60%, and Tartary buckwheat seedlings were treated with NaCl and ETH at third leaf stage.

Root treatment: Take nutrient solution irrigation as control (CK), 150 mM NaCl solution prepared with nutrient solution as salt stress treatment (NaCl), ETH concentration prepared by NaCl solution was 0.5, 1.0, 1.5, 2.0 mM (NaCl+ETH), and relevant physiological indexes of seedlings were determined after 3 d. Three replicates were set for each treatment.

Spraying treatment: Take nutrient solution irrigation as control (CK), 150 mM NaCl solution prepared with nutrient solution as salt stress treatment (NaCl). On the basis of salt stress, 0.2, 0.4, 0.6 and 0.8 mM ETH (NaCl+ETH) was sprayed on leaves once a day, and relevant physiological indexes of seedlings were measured 3 d later. Three replicates were set for each treatment.

Germination was recorded at the same time every day for five consecutive days, and seed germination rate was calculated: Germination rate (GR) = n/N × 100%, n is germination number, and N is total number of seeds. Malondialdehyde (MDA) content was determined according to the method of Li et al. (1983). Chlorophyll content was evaluated with SPAD (soil and plant analysis development) value, which was determined by chlorophyll content determinator (PJ-4N, Henan, China).

RNA extraction

RNA was extracted by hexadecyltrimethylammonium bromide (CTAB) method. Preparation of RNA extraction buffer: adding reagents other than Tris and sterile water according to the formula, sterilizing with damp heat at 121 °C for 120 min, cooling to 65 °C, adding Tris and concentrated hydrochloric acid to adjust pH, extracting, cleaning and dissolving. The concentration and purity of RNA were measured by micro spectrophotometer and stored at -80 °C.

Obtaining cDNA by reverse transcription

The first step was to remove genomic DNA, the second step was to reverse transcription, and the reverse transcription system was prepared according to Table 1. Reverse transcription kit: PrimeScript RT reagent kit with gDNA Eraser (Takara Bio Inc., Kusatsu, Japan). After reaction, 40 µL RNase free dH_2O was added to dilute and store at -20 °C for real-time quantitative PCR.

Gene expression determination by real-time quantitative PCR

The primers used in real-time quantitative PCR (RT-qPCR) are shown in Table 2. Real time fluorescent quantitative PCR reagent: ChamQTM Universal SYBR qPCR Master Mix was used (Vazyme Biotech, Nanjing, China). The real-time fluorescence quantitative reaction system is shown in Table 3. The above reaction system was well mixed and centrifuged. This process should be carried out under weak light. The reaction procedure of real-time fluorescent quantitative PCR (Stratagene Mx3000P; Agilent Technologies, Santa Clara, California, USA) was set up according to Table 4. The data were saved after running. The relative expression level of detecting genes was quantified by the $2^{\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Data processing

The significant differences between two Tartary buckwheat varieties were analyzed by ANOVA and student's t-test (Wang et al., 2010).

Table 1. gDNA reverse transcription system.

Step 1	5×gDNA Eraser Buffer	2.0 µL	PCR instrument, 42 °C for 3 min, 4 °C for storage
	gDNA Eraser	1.0 µL	
	Total RNA*	[a]	
	RNase Free dH_2O	[a]	
Step 2	Reaction solution of step 1	10.0 µL	PCR instrument, 42 °C for 15 min, 85 °C for 5 s, 4 °C for storage
	PrimeScript RT Enzyme Mix I	1.0 µL	
	RT Primer Mix	1.0 µL	
	5×PrimeScript Buffer II (for Real Time)	4.0 µL	
	RNase Free dH_2O	4.0 µL	

[a]In the first step, the total RNA amount was calculated according to the formula RNA concentration (ng μL^{-1}). Total RNA (µL) = 1500. Finally, RNase Free dH_2O was added to make the final volume 10 µL.

Table 2. Primers for real-time quantitative PCR.

Primers	Sequence (5'→3')
FtNHX1-F	CGTTGCTAGGACGCAATGTTCCA
FtNHX1-R	ACAGTCCACGTCGGATGCCTTAT
FtSOS1-F	CCTTACACCGTACCCGCTC
FtSOS1-R	CCGGAAGAAACACAGCCAACA
Actin-F	GCTGGATTTGCTGGAGATGATGC
Actin-R	CTTCTCCATGTCATCCCAGTTGCT

Table 3. Reaction system of real-time quantitative PCR.

2×ChamQ Universal SYBR qPCR Master Mix	5.0 μL	Single sample reaction system
Primer-F	0.4 μL	
Primer-R	0.4 μL	
Template cDNA	1.0 μL	
ddH_2O	3.2 μL	
Total	10.0 μL	

Table 4. Reaction program of real-time quantitative PCR.

Step		Number of cycles	Temperature	Duration time
			°C	s
Step 1	Pre-denaturation	1	95	30
Step 2	Cyclic reaction	40	95	10
			55	30
			72	30
			60	60
Step 3	Dissociation curve		95	15

RESULTS AND DISCUSSION

Effect of ethephon treatments on seed germination under salt stress

Table 5 shows seed germination rate of Tartary buckwheat under different concentrations of ETH treatment. Under salt stress, seed germination rate of 'Chuanqiao No.1' and 'Chuanqiao No.2' was decreased significantly, which was decreased by 15.81% and 36.24% compared with the control. Under ethylene treatment, seed germination rate of 'Chuanqiao No.1' and 'Chuanqiao No.2' was obviously increased. Under 1.5 mM ETH treatment, 'Chuanqiao No.1' was increased by 13.92% compared with salt stress, and restored to the control level. Under 1.0 mM ETH treatment, 'Chuanqiao No.2' was increased by 19.15% compared with salt stress.

Seed germination rate is an important index for salt tolerance identification of crops (Meng et al., 2015). Liu et al. (2015) found that the germination rate of buckwheat seeds was decreased under salt stress. In this study, the seed germination rate of two Tartary buckwheat varieties was decreased significantly under salt stress. Under appropriate concentration of ETH treatment, that of two Tartary buckwheat varieties was increased significantly, and the increase of 'Chuanqiao No.2' was significantly larger, suggesting ETH treatment has better effect on promoting seed germination of salt-sensitive variety under salt stress.

Table 5. Seed germination rate of Tartary buckwheat under different concentrations of ethephon (ETH).

			NaCl+ETH (mM)			
Varieties	CK	NaCl	0.5	1.0	1.5	2.0
	%					
Chuanqiao No.1	92.15 ± 5.26a	76.34 ± 4.23bc	79.17 ± 4.11b	82.05 ± 4.26b	90.26 ± 4.53a	83.12 ± 4.57b
Chuanqiao No.2	93.62 ± 5.17a	57.38 ± 3.21d	61.29 ± 3.87d	76.53 ± 4.06bc	70.91 ± 4.12c	68.46 ± 3.98c

Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Effect of ethephon treatments on MDA content under salt stress

Malondialdehyde is the product of membrane lipid peroxidation (Tian et al., 2009), and MDA content can reflect the degree of membrane lipid peroxidation to some extent and the strength of plant response to adversity (Cao et al., 2005). Table 6 shows the MDA content of Tartary buckwheat under ETH root treatment. Under salt stress, leaf MDA content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was increased significantly, which was increased by 94.76% and 167.75% compared with the control. Under ETH root treatment, leaf MDA content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was decreased significantly. Under 1.0 mM ETH root treatment, 'Chuanqiao No.1' was decreased by 34.89% compared with salt stress. Under 1.5 mM ETH root treatment, 'Chuanqiao No.2' was decreased by 31.01% compared with salt stress. Table 7 shows the MDA content of Tartary buckwheat under ETH spraying treatment. Under salt stress, the leaf MDA content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was increased significantly. Under ETH spraying treatment, the leaf MDA content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was decreased significantly. Under 0.4 mM ETH spraying treatment, 'Chuanqiao No.1' was decreased by 46.22% compared with salt stress, and restored to the control level. Under 0.6 mM ETH spraying treatment, 'Chuanqiao No.2' was decreased by 46.64% compared with salt stress.

Salt stress can directly or indirectly lead to a series of metabolic and functional changes in plants, which can be used as indexes to identify salt tolerance and salt tolerance screening (Li et al., 2008). Under salt stress, the balance of the active oxygen metabolism system in plants is affected, and more active oxygen accumulation in body can cause membrane lipid peroxidation. Malondialdehyde is the main product of membrane lipid peroxidation, and MDA content is closely related to the degree of membrane lipid damage (Zhan et al., 2009). In this study, leaf MDA content of two Tartary buckwheat varieties was increased significantly under salt stress, suggesting the membrane lipid of Tartary buckwheat is damaged. Under ETH root and spraying treatment, that of Tartary buckwheat was decreased significantly, and the decrease under spraying treatment was more than that under root one, suggesting ETH spraying treatment has better effect on improving physiological characteristics of Tartary buckwheat under salt stress.

Effect of ethephon treatments on chlorophyll content under salt stress

Table 8 shows the chlorophyll content of Tartary buckwheat under ETH root treatment. Under salt stress, leaf chlorophyll content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was decreased significantly, which was decreased by 28.87% and 41.87% compared with the control. Under ETH root treatment, leaf chlorophyll content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was increased significantly. Under 1.0 mM ETH root treatment, 'Chuanqiao No.1' was increased by 39.47% compared with salt stress, and restored to the control level. Under 1.5 mM ETH root treatment, 'Chuanqiao No.2' was increased by 45.31% compared with salt stress. Table 9 shows the chlorophyll content of Tartary buckwheat under ETH spraying treatment. Under salt stress, leaf chlorophyll content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was decreased significantly. Under ETH spraying treatment, leaf chlorophyll content of 'Chuanqiao No.1' and 'Chuanqiao No.2' was increased significantly. Under 0.4 mM ETH spraying treatment, 'Chuanqiao No.1' was increased by 43.22% compared with salt stress, and restored to the control level. Under 0.6 mM ETH spraying treatment, 'Chuanqiao No.2' was increased by 64.68% compared with salt stress, and also restored to the control level.

Table 6. Malondialdehyde content of Tartary buckwheat under ethephon (ETH) root treatment.

			NaCl+ETH (mM)			
Varieties	CK	NaCl	0.5	1.0	1.5	2.0
	μmol g^{-1} FW					
Chuanqiao No.1	23.46 ± 1.82f	45.69 ± 2.85c	38.64 ± 2.56d	29.75 ± 1.97e	35.69 ± 2.12d	36.38 ± 2.31d
Chuanqiao No.2	25.12 ± 1.79f	67.26 ± 3.93a	58.15 ± 3.03b	53.02 ± 3.11b	46.40 ± 2.72c	55.23 ± 3.25b

Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Table 7. Malondialdehyde content of Tartary buckwheat under ethephon spraying.

			NaCl+ETH (mM)			
Varieties	CK	NaCl	0.2	0.4	0.6	0.8
	μmol g^{-1} FW					
Chuanqiao No.1	23.46 ± 1.82f	45.69 ± 2.85b	33.46 ± 1.96d	24.57 ± 1.68f	29.39 ± 1.85e	30.48 ± 1.87de
Chuanqiao No.2	25.12 ± 1.79f	67.26 ± 3.93a	47.62 ± 2.51b	40.13 ± 2.13c	35.89 ± 2.12d	46.41 ± 2.90b

Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Table 8. Chlorophyll content of Tartary buckwheat under ethephon root treatment.

Varieties	CK	NaCl	NaCl+ETH (mM)			
			0.5	1.0	1.5	2.0
	SPAD value					
Chuanqiao No.1	43.20 ± 2.57a	30.73 ± 1.65d	37.29 ± 2.16b	42.86 ± 2.43a	36.34 ± 2.04b	35.60 ± 2.13bc
Chuanqiao No.2	41.65 ± 2.41a	24.21 ± 1.52f	27.32 ± 1.64e	30.16 ± 1.58d	35.18 ± 2.11bc	32.13 ± 1.82cd

Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Table 9. Chlorophyll content of Tartary buckwheat under ethephon spraying treatment.

Varieties	CK	NaCl	NaCl+ETH (mM)			
			0.2	0.4	0.6	0.8
	SPAD value					
Chuanqiao No.1	43.20 ± 2.57a	30.73 ± 1.65d	35.46 ± 2.09c	44.01 ± 2.49a	38.62 ± 2.13bc	33.51 ± 1.92c
Chuanqiao No.2	41.65 ± 2.41ab	24.21 ± 1.52e	29.52 ± 1.72d	32.30 ± 1.78cd	39.87 ± 2.21b	35.97 ± 2.06c

Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Chlorophyll content affects plant photosynthesis, which is an important index to reflect the strength of leaf photosynthesis (Loh et al., 2000). In this study, leaf chlorophyll content of two Tartary buckwheat varieties was decreased significantly under salt stress. Under ETH root and spraying treatment, that of two Tartary buckwheat varieties was increased significantly, and 'Chuanqiao No.2' was increased more under ETH spraying treatment, suggesting ETH spraying treatment has better effect on increasing leaf chlorophyll content of salt-sensitive variety.

Effect of ethephon treatments on *FtNHX1* gene expression under salt stress

NHX is a Na^+/H^+ antiporter; NHX1 can cause compartmentalization of Na^+ into vacuoles and reduce salt stress damage in cytoplasm. Studies have shown that overexpression of NHX protein can significantly improve plant salt tolerance (Shu et al., 2013). Figure 1 shows that the relative expression of *FtNHX1* gene in Tartary buckwheat leaves was first increased and then decreased, and then decreased to the control level at 48 h under salt stress. After 6, 12 and 24 h of salt stress, the relative expression of *FtNHX1* gene was significantly increased, that of 'Chuanqiao No.1' was increased by 331.04%, 413.89% and 118.55% compared with the control, and reached the maximum expression level at 12 h, that of 'Chuanqiao No.2' was increased by 222.89%, 163.54% and 90.00% compared with the control, and reached the maximum expression level at 6 h. Under ETH root treatment, the relative expression of *FtNHX1* gene in 'Chuanqiao No.1' was increased significantly at 6, 12, 24 and 48 h, which were 132.05%, 38.58%, 277.77% and 108.79% higher than those under salt stress, that in 'Chuanqiao No.2' was significantly increased at 12, 24 and 48 h, which were 65.54%, 251.17% and 122.56% higher than those under salt stress. 'Chuanqiao No.1' and 'Chuanqiao No.2' reached their maximum expression levels at 24 h. Under ETH spraying treatment, the relative expression of *FtNHX1* gene in 'Chuanqiao No.1' was increased significantly at 24 and 48 h, which was increased by 232.39% and 138.95% compared with salt stress, that in 'Chuanqiao No.2' was increased significantly at 12, 24 and 48 h, which was increased by 32.45%, 190.91% and 111.43% compared with salt stress. Both varieties reached their maximum expression levels at 24 h.

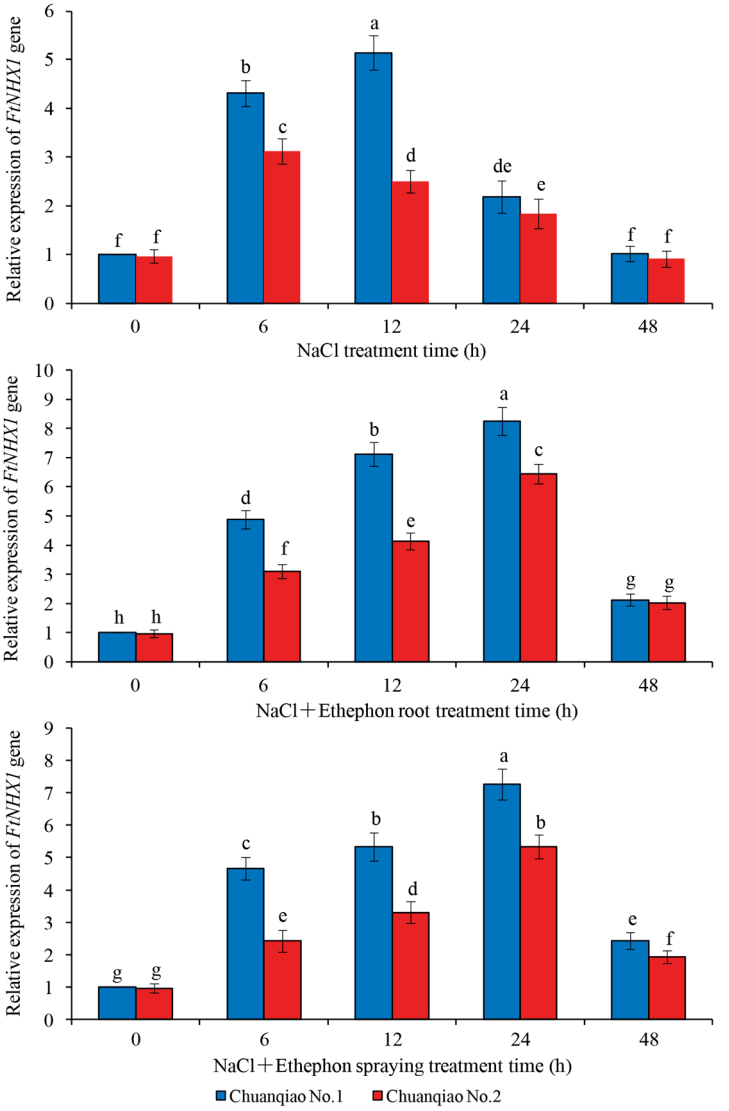
Effect of ethephon treatments on *FtSOS1* gene expression under salt stress

SOS1 gene encodes Na^+/H^+ antiporter on cell membrane, which can release Na^+ and alleviate salt stress, and plays an important role in salt tolerance mechanism of plants. Figure 2 shows that the relative expression of *FtSOS1* gene in Tartary buckwheat leaves was first increased and then decreased, and then decreased to the control level at 48 h under salt stress. After 6, 12 and 24 h of salt stress, the relative expression of *FtSOS1* gene was significantly increased, that of 'Chuanqiao No.1' was increased by 131.04%, 423.98% and 119.61% compared with the control, that of 'Chuanqiao No.2' was increased by 102.52%, 266.23% and 178.05% compared with the control. Both 'Chuanqiao No.1' and 'Chuanqiao No.2' reached their maximum expression levels at 12 h. Under ETH root treatment, the relative expression of *FtSOS1* gene was increased significantly at 6, 12, 24 and 48 h, that in 'Chuanqiao No.1' was 203.03%, 60.94%, 127.28% and 99.18% higher than that under salt stress, that in 'Chuanqiao No.2' was 71.27%, 98.75%, 61.87% and 125.58% higher than that under salt stress. Both varieties reached their maximum expression levels at 12 h. Under ETH spraying treatment, the relative expression of *FtSOS1* gene was increased significantly at 6, 12, 24 and 48 h, that in 'Chuanqiao No.1' was 122.08%,

35.98%, 114.42% and 138.03% higher than that under salt stress, that in 'Chuanqiao No.2' was 36.08%, 67.63%, 24.37% and 109.87% higher than that under salt stress. Both varieties reached their maximum expression levels at 12 h.

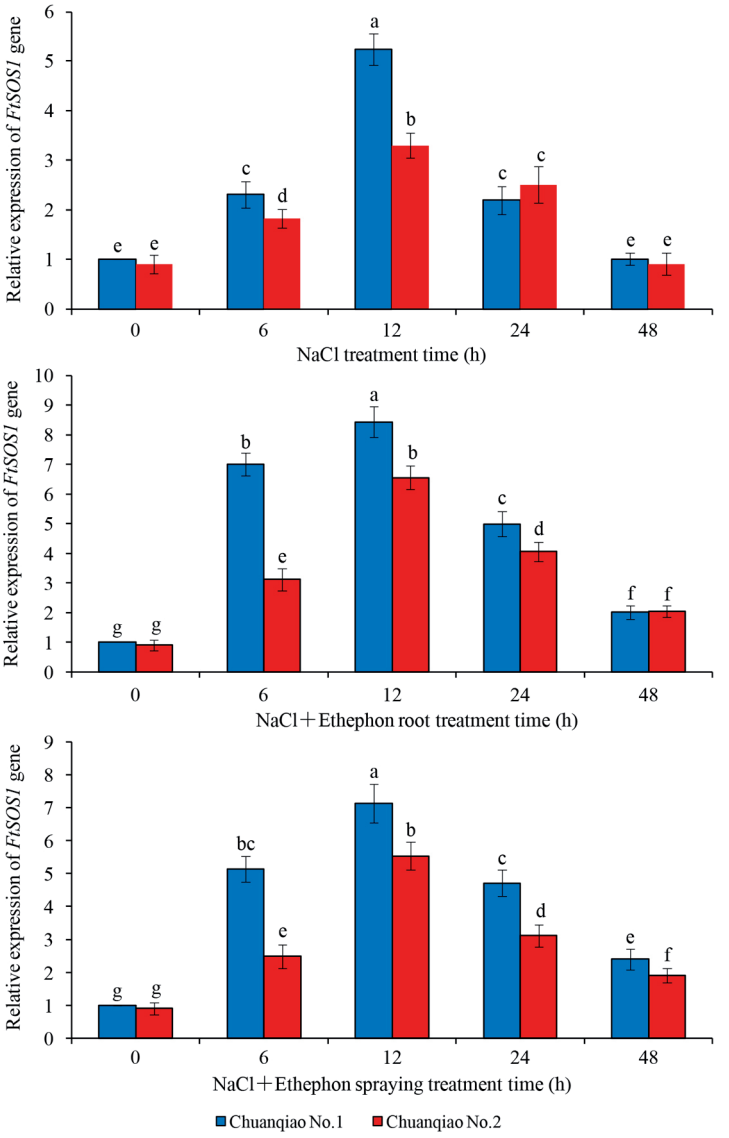
Since high Na^+ concentration can compete to inhibit the specific absorption of K^+ in roots, so it is necessary for cells to maintain high K^+/Na^+ in the cytoplasm of plants. The strategies for maintaining K^+/Na^+ balance in cytoplasm are: Na^+ extrusion, reduction or inhibition of Na^+ entering cells and compartmentalization of Na^+ in vacuole (Zhu and Ni, 2016). NHX (Na^+/H^+ antiporter) can compartmentalize Na^+ in cytoplasm into vacuole, thus reducing salt stress damage. Under salt stress, the root length and weight of soybean overexpressing *GmNHX1* gene were increased significantly, which enhanced the tolerance of soybean to salt stress (Wang et al., 2011). *SOS1* (salt overly sensitive 1) encodes plasma membrane Na^+/H^+ antiporter. Arabidopsis *SOS1* mutant is hypersensitive to salt, which indicates that *SOS1* plays an important role in plant salt tolerance mechanism (Yue et al., 2011). The proteins encoded by salt tolerance genes *FtNHX1* and *FtSOS1* can promote compartmentalization and extrusion of Na^+, thus reducing the damage of Tartary buckwheat to salt stress (Liu et al., 2017a; 2017b; Lu et al., 2017). In this article, the relative expression of *FtNHX1* and *FtSOS1* gene in Tartary buckwheat was significantly increased under salt stress. After ETH treatment, the relative expression of *FtNHX1* and *FtSOS1* was increased more significantly, indicating that ETH treatment can significantly promote the expression of salt tolerance genes in Tartary buckwheat.

Figure 1. Effect of ethephon root and spraying treatment on *FtNHX1* gene expression under salt stress.



Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

Figure 2. Effect of ethephon root and spraying treatment on *FtSOS1* gene expression under salt stress.



Values followed by different letters are different at the 0.05 probability level (values are mean ± SD, n = 3).

CONCLUSIONS

Ethephon (ETH) treatment could improve the physiological characteristics of Tartary buckwheat seedlings under salt stress, and the appropriate concentration of ETH root treatment in salt-tolerant and salt-sensitive variety varies with 1.0 and 1.5 mM, and that of ETH spraying with 0.4 and 0.6 mM. Under salt stress, the effect of ETH spraying on improving physiological characteristics of Tartary buckwheat is better than that of ETH root treatment. Appropriate concentration of ETH treatment could up regulate the expression of salt tolerance genes *FtNHX1* and *FtSOS1* more than that under salt stress.

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