BIOLOGIA PLANTARUM 64: 258-265, 2020 DOI: 10.32615/bp.2019.091

Effects of potassium chloride and nitric oxide on growth and physiological characteristics of winter wheat under salt stress

Y.J. DONG[1], Q. ZHANG[1], X.L. DAI[2], and M.R. HE[2]*

College of Resources and Environment, Shandong Agricultural University, Tai'an 271018, P.R. China[1]
College of Agronomic Sciences, Shandong Agricultural University, Tai'an 271018, P.R. China[2]

Abstract

A hydroponic culture was conducted to evaluate the effects of KCl and sodium nitroprusside (SNP; a nitric oxide donor) in wheat seedlings under salt stress. Exposure to 100 mM NaCl for 7 d decreased biomass of wheat seedlings, root activity and H^+-ATPase activity, significantly increased free proline content, reactive oxygen species (ROS) accumulation and lipid peroxidation, and suppressed the activity of superoxide dismutase (SOD). Moreover, NaCl stress significantly decreased the K^+ and increased the Na^+ content. Addition of KCl or SNP led to the increase in root activity and soluble protein content, stimulated the activity of SOD, and decreased free proline content, superoxide anion radical generation rate, and lipid peroxidation. The increased K^+ and decreased Na^+ content in the leaves of treated seedlings indicated that suitable KCl and NO addition stimulated the selective transport of K^+ and Na^+ to the maintain K^+/Na^+ homeostasis.

Additional key words: chlorophyll, CAT, ion homeostasis, malondialdehyde, POD, proline, ROS, SOD, *Triticum aestivum*.

Introduction

Soil salinity is one of the main factors that affect plant growth and lead to crop yield reduction (Abd El Gawad *et al.* 2016). Salt stress inhibits plants growth mainly by causing ion imbalance and osmotic stress (Khan *et al.* 2012). There exists a continuous spectrum of resistance to salinity ranging from glycophytes, which are sensitive to salinity, to halophytes, which can survive and grow under high salt concentrations (Glenn *et al.* 1999). The high concentration NaCl can reduce the soil water potential and so water uptake by roots. At the same time, the stomata of leaves are closed and the chloroplasts are damaged, which results in the decrease of photosynthetic and production rate. Salt stress also causes an accumulation of large number of ROS, causing oxidative damage to lipids, proteins, and amino acids also resulting in decreased crop yield (Foyer *et al.* 2000, Abreu *et al.* 2013). Among different salt resistance mechanisms, regulation of ion homeostasis in various tissues and intracellular compartmentalization of various ions is very important (Bressan *et al.* 2008). During salt stress, Na^+ taken in by the cell must be extruded at plasma membrane and/or compartmentalized into the vacuole. This requires up-regulation of tonoplast and plasma membrane Na^+ transporters. Meanwhile, the plants under salt stress accumulate a number of compatible solutes, such as soluble sugars, proline, and glycine betaine. They can function in osmotic adjustment, or act as signalling molecules to activate some specific transduction pathways (Misra *et al.* 2009). Secondary stresses such as oxidative damage often occur (Mittler *et al.* 2002, Xu *et al*. 2015) as a consequence of excess production of ROS. To counteract the oxidative stress, both enzymatic and non-enzymatic antioxidants have evolved in plants (Zheng *et al.* 2009a, Lin *et al.* 2012).

Potassium fertilization which can improve crop yield under adverse conditions has been interpreted as evidence that K increases the resistance of plants to abiotic stresess (Cakmak *et al.* 2005, Marschner and Marschner 2012). Both K and Na are monovalent cations having many similar properties. Although Na could partially substitute the function of K, high amount Na is toxic to some metabolic reaction. During salt stress, there are a decrease in K^+ and an increase in Na^+ content in plant cells. The competition between ions is influenced by the properties of the membrane transporters and by the concentrations of different ions in solution (Marschner and Marschner 2012). Plant cells must regulate cation channels, pumps,

Submitted 7 January 2019, *last revision* 11 April 2019, *accepted* 12 July 2019.
Abbreviations: CAT - catalase; $HO^{\bullet}$ - hydroxyl radical; MDA - malondialdehyde; $O_2^{\bullet-}$ - superoxide anion radical; POD - peroxidase; ROS - reactive oxygen species; SNP - sodium nitroprusside; SOD - superoxide dismutase; TTF - triphenyl formazan.
Acknowledgments: This work was financially supported by the National Key Research and Development Support Program of China (2017YFD0201705), the Major Agricultural Application Technology Innovation Project of Shandong Province (SD2019ZZ021), and the Major Science and Technology Innovation Projects of Shandong Province (2017CXGC0301).
* Corresponding author; e-mail: mrhe@sdau.edu.cn

or transporters at the tonoplast and plasma membranes to maintain ion homeostasis. The sodium-proton antiporters and ion channels take distinguish part in the uptake and transmission of mineral element (Serrano *et al.* 2001). Most higher plants have developed high selectivity in the uptake of K^+ compared with Na^+ by roots and in its transport to shoots (Tian *et al.* 2015). A suitable K^+/Na^+ ratio is important for the adjustment of cell function. So the KCl application under saline condition needs more study.

Nitric oxide is one kind of small signalling molecules in plants, and it plays an important role in plant disease resistance (Singh *et al.* 2017). Furthermore, NO is also proved to have a mitigating effect on abiotic stresses, such as salinity, heat, and drought (Delledonne *et al.* 2005, Tian *et al.* 2015). The signalling of NO may be *via* a cGMP-dependent pathway or cGMP-independent pathway (Arasimowicz *et al.* 2007). In plants, NO signalling involves cGMP, cADP ribose, Ca^{2+}, salicylic acid (SA), and protein kinases often overlap and cross talk with H_2O_2 signalling (Crawford *et al.* 2005). The NO could induce the posttranslational modification of proteins, such as tyrosine nitration, methionine oxidation, and cysteine nitrosylation. Among them, the cysteine nitrosylation is a reversible modification that can modulate protein function (Dong *et al.* 2016). Sokolovski *et al.* (2004) showed that NO through S-nitrosylation regulates the activity of K channels in stomata guard cells. It has been also reported that exogenous NO stimulated the expression of plasma membrane H^+-ATPase under salt stress (Zhao *et al.* 2004, Zheng *et al.* 2009b).

Wheat (*Triticum aestivum* L.) is one of the three major grain crops. It has higher nutritive value, however, it is usually considered to be very salt-sensitive. Salt stress always causes water deficit, Na^+ toxicity, and nutrient deficiency. Exposure to high salinity can affect photosynthesis, protein synthesis, as well as energy and lipid metabolism, leading to growth and yield reduction (Aftab *et al.* 2011). Khan *et al.* has shown that NO and $CaCl_2$ can reduce the impact of salt stress on wheat (2012). However, studies on the effects of application of NO and K complex on winter wheat seedlings under salt stress have not been reported yet. Therefore, in this study, the effects of exogenous NO and K on plant growth, chlorophyll content, root activity and H^+-ATPase in PMs, organic solutes, ROS, antioxidant enzymes, mineral nutrient, and K^+/Na^+ homeostasis in winter wheat seedling under NaCl stress were studied in hydroponic culture.

Materials and methods

Plants and treatments: Seeds of winter wheat (*Triticum aestivum* L. cv. Jimai 4) were surface sterilized with 10 % (m/v) NaClO solution for 10 min, then vigorously rinsed with distilled water. Sterilized seeds were sown in plug tray and placed in a *Spx-250ic* (*Shanghai Boxun Industry*, Shanghai, China) climate box under the day/night temperatures of 25/20 °C, an irradiance of 150 μmol m^{-2} s^{-1}, a 14-h photoperiod, and a 60 % relative humidity). Two fully expanded leaves were developed after 14 d. Then the seedlings were transplanted to glassware (6.0 × 3.5 × 10.0 cm^3) filled with half strength Hoagland solution (containing 2.5 mM K^+). The treatments were as follows: T1 - control (CK); T2 - 100 mM NaCl; T3 - 100 mM NaCl + 10 mM KCl; T4 - 0.10 mM SNP; T5 - 100 mM NaCl + 0.10 mM SNP; T6 - 100 mM NaCl + 10 mM KCl + 0.10 mM SNP. The nutrient solution was adjusted to pH 6.5 - 6.8. Each variant included fifty seedlings and represented one replicate, and there were three replicates per treatment. The nutrient solutions were changed every day to maintain constant NaCl, KCl, and SNP concentrations. The plants were sampled after 7 d of treatments, the shoots and roots were separated, and fresh and dry masses estimated. The water content (WC) was determined as [(fresh mass - dry mass)/fresh mass] × 100.

Pigment content was determined according to the method of Song *et al.* (2017). Fresh leaves (0.5 g) were extracted in 2 cm^3 of 95 % (v/v) ethanol for 24 h in the dark, and the solution was analyzed. The amounts of chlorophyll *a*, and *b*, and carotenoids were determined spectrophotometrically (*Shimadzu UV-2450*, Kyoto, Japan) by reading absorbances at 665, 649, and 470 nm.

Determination of root activity and H^+-ATPase in plasma membrane: As described in Duncan *et al.* (2004), the activity of the root can be expressed according to the amount of triphenyl formazan (TTF) resulting from 2,3,5-triphenyltetrazolium chloride (TTC) deoxidation.

A membrane fraction enriched in plasma membrane vesicles was prepared as described by Briskin *et al.* (1987) and ATP hydrolysis assays were performed. The H^+-ATPase activity was determined by measuring the release of Pi (Ohnishi *et al.* 1975).

Determination of proline, soluble sugars, and protein content: Free proline content was determined as described by Bates *et al.* (1973) with slight modification Proline amount was determined using the methods of Bates *et al.* (1973) and Bai *et al.* (2015). After extraction with 3 % (m/v) 5-sulfosalicylic acid at room temperature, proline content was determined from a standard curve and calculated on a fresh mass basis. The content of soluble sugars was determined according to Giannapoulis *et al.* 2007). Soluble protein content was determined as described by Bradford *et al.* (1976) by using the Coomassie brilliant blue G-250 reagent and bovine serum albumin as standard.

Determination of H_2O_2 and malondialdehyde content, and $O_2^{\bullet-}$ generation rate: For determination of H_2O_2 content, 1 g of fresh leaves or roots were homogenized in 2 cm^3 of ice-cold acetone and centrifuged at 4 000 *g* for 15 min. Titanium reagent [2 % (m/v) $TiCl_2$ in concentrated HCl] was added to a known volume of extract to give a Ti^{IV} concentration of 2 %. The Ti-H_2O_2 complex, together with unreacted Ti, was then precipitated by adding 0.2 cm^3 of 17 M ammonia solution for each 1 cm^3 of extract, then centrifuged at 4 000 *g* for 15 min, and the supernatant was discarded. The precipitate was washed five times with ice

acetone by resuspension, drained in 1 M H_2SO_4 (3 cm^3). The absorbance of the solution was measured at 410 nm against blank, which had been similarly prepared but without plant tissue (Liu *et al.* 2014).

For the measurement of $O_2^{\cdot-}$ generation rate, 0.3 g fresh leaves or roots were homogenized in 3 cm^3 of ice cold 50 mM phosphate buffer saline (PBS; pH 7.0) and the homogenate was centrifuged at 10 000 *g* for 10 min. Then, 0.5 cm^3 of the supernatant was added to 0.5 cm^3 of a 50 mM PBS (pH 7.8) and 0.1 cm^3 of 10 mM hydroxylamine hydrochloride. The mixture was incubated at 25 °C for 35 min and to 0.5 cm^3 of solution from the above mixture 0.5 cm^3 of 17 mM sulfanilamide and 0.5 cm^3 of 7.8 mM α-naphthylamine was added. After 20 min of reaction, 2 cm^3 of ether was added into the above solution, and mixed well. The solution was centrifuged at 1 500 g and 4 °C for 5 min. The absorbance of the pink supernatant was measured at 530 nm with the spectrophotometer. Absorbance values were calibrated to a standard curve generated with known concentrations of HNO_2 (Tian *et al.* 2015).

The lipid peroxidation in fresh leaves was measured in terms of MDA content by the thiobarbituric acid reaction method (Heath and Packer 1968).

Antioxidant enzyme extraction and assays: For extraction of antioxidant enzymes, leaves were homogenized with 50 mM Na_2HPO_4 - NaH_2PO_4 buffer (pH 7.8) containing 0.2 mM EDTA and 2 % insoluble polyvinyl pyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 12 000 *g* for 20 min and the resulted supernatant was used for determination of enzyme activities. The whole extraction procedure was carried out at 4 °C. The SOD activity was assayed as described by Aftab *et al.* (2011) and Dong *et al*.(2019). One unit (U) of SOD activity was defined as the amount of the crude enzyme extract that is required for inhibiting the reduction rate of nitro blue tetrazolium by 50 %. The CAT activity was assayed as described by Shi *et al.* (2007). The POD activity was determined by the method described by Hammer *et al.* (1982) with some modifications. It was measured by monitoring the increase in absorption at 470 nm of a reaction mixture containing 0.25 % (v/v) guaiacol and 0.75 % (v/v) H_2O_2 as substrates and initiating the reaction by the addition of the crude enzyme extract.

Determination of mineral nutrients: Roots were soaked in 20 mM Na_2-EDTA for 15 min to remove metal ions adhering to root surfaces, and rinsed with deionized water several times. The root and shoot samples were oven-dried for 30 min at 105 °C, and then at 70 °C till the materials reach their constant mass. About 0.1 g was mineralized and completely digested with 5 cm^3 of 98 % (m/v) H_2SO_4 at 200 °C with a few drops of H_2O_2 (30 %, v/v). The content of mineral element were estimated by atomic absorbance spectrometry (*Persee TAS-990*, Beijing, China).

Statistical analysis: All the data presented were the mean values, and all the treatments were repeated three times. We used the *Excel 2003* software to process data and draw tables, *SPSS 19.0* software for statistics, and minimal significant difference method (LSD) for difference significance test ($P < 0.05$).

Results

Treatment with 100 mM NaCl significantly decreased the plant growth, with a 13.53 and 14.23 % reduction of dry mass of shoots and roots, respectively, as compared to the control (Table 1). Also water content of wheat seedlings treated with 100 mM NaCl decreased compared to the control. Addition of K^+ caused further inhibition of the shoots growth, but alleviated effects of NaCl on the roots. Moreover, the presence of SNP stimulated the growth under NaCl stress.

Wheat seedlings treated with 100 mM NaCl showed a significant increase in Chl *a*, *b* and *a*+*b* content per fresh mass unit in the leaves compared with the control (Table 1). Other treatments (except T6) also increased the chlorophyll content. However, the carotenoid content was decreased compared to the control under NaCl stress (Table 1).

The treatment with 0.10 mM SNP suppressed the root activity under non-saline conditions, but the detrimental effect faded out under salt stress. Compared with CK, the root activity decreased under salt stress but less after KCl addition (Table 2). Treatment with NaCl evidently decreased the H^+-ATPase activity. Addition of SNP improved the activities of H^+-ATPase both under non-saline and saline conditions, while the KCl addition had no effect (Table 2).

Under NaCl stress, significant increase of free proline content was recorded in wheat seedlings, especially in the leaves (Table 2). The content of free proline in the leaves treated with NaCl alone was 4.03-times higher than that in CK. In the leaves, the application of KCl only slightly aleviate increase in the proline content, but the addition of NO inhibited the proline production under salt stress. Compared to T1, the content of proline in roots and leaves of different treatments had a similar trend, but there was no significant difference in the content of proline in roots. Soluble sugar content increased in the roots treated with 100 mM NaCl or 0.10 mM SNP alone (Table 2), and the SNP and KCl addition to NaCl solution did not have significant effect on the sugar content. Compared to T1, the content of soluble sugar after different treatments of leaves and roots had a similar trend. The soluble protein content increased in the leaves and decreased in the roots under 100 mM NaCl stress (Table 2). The addition of 10 mM K^+ elevated the soluble protein content in the leaves under NaCl treatment. Furthermore, under combined SNP and KCl, there was highest soluble protein content than under other treatments (Table 2).

The H_2O_2 content of wheat seedlings increased under salt stress compared with CK (Table 3). The application of KCl alleviate effect of NaCl in leaves but enhanced it in roots. The SNP application increased the H_2O_2 content in roots and leaves under bot saline and non-saline conditions (Table 3). Under NaCl stress, $O_2^{\cdot-}$ generation

Table 1. Effects of KCl and SNP on fresh (f.m.) and dry masses (d.m.) [mg plant^{-1}], water content (WC) [%], and content of chlorophyll (Chl) *a* [mg g^{-1}(f.m.)], Chl *b* [mg g^{-1}(f.m.)], Chl *a*+*b* [mg g^{-1}(f.m.)], and carotenoids (Car) [mg g^{-1}(f.m.)] in wheat seedlings grown under NaCl stress; T1 - control, T2 - 100 mM NaCl, T3 - 100 mM NaCl + 10 mM KCl, T4 - 0.1 mM SNP, T5 - 100 mM NaCl + 0.1 mM SNP, T6 - 100 mM NaCl + 10 mM KCl + 0.1 mM SNP. Means ± SDs, n = 3. Different letters within the same column indicate significant differences at $P < 0.05$.

	T1	T2	T3	T4	T5	T6
Shoot f.m.	331.94±56.37a	238.19±52.81b	183.91±20.15b	324.75±37.78a	310.47±19.34a	213.77±5.20b
Shoot d.m.	34.80±5.21a	30.09±4.71ab	23.08±1.04c	36.56±5.32a	35.95±1.71a	27.21±2.10bc
Shoot WC	89.49±0.23a	87.24±0.98b	87.39±0.90b	88.76±0.65a	88.41±0.44ab	87.28±0.71b
Root f.m.	78.34±12.93a	51.05±5.29b	68.11±2.74ab	70.68±15.95ab	77.52±23.99a	60.50±6.80ab
Root d.m.	7.59±0.96ab	6.51±0.62b	8.50±0.34ab	7.75±1.02ab	9.21±2.49a	7.70±0.42ab
Root WC	90.25±0.91a	87.22±1.02b	87.50±0.92b	88.86±1.24ab	88.00±0.82b	87.17±1.37b
Chl *a*	1115.06±73.53ab	1292.70±56.41a	1143.90±129.51ab	1122.36±152.13ab	1229.10±151.77ab	1094.74±48.13b
Chl *b*	339.95±25.71b	392.59±18.09ab	333.97±39.93b	347.33±32.47b	438.93±58.89a	337.17±27.00b
Chl *a*+*b*	1455.01±90.24a	1685.29±72.55a	1477.87±168.83a	1469.69±180.82a	1668.03±210.65a	1431.92±72.63a
Car	395.87±20.76a	286.69±13.85b	249.04±24.60b	305.57±88.08b	227.88±63.56b	234.15±10.95b

Table 2. Effects of KCl and SNP on root activity [μg g^{-1}(f.m.) h^{-1}], H$^+$-ATPase activity [μmol(Pi) mg^{-1}(protein)·h^{-1}), and content of free proline [μg g^{-1}(f.m.)], soluble sugars [mg g^{-1}(f.m.)], and soluble proteins [mg g^{-1}(f.m.)] in leaves and roots of wheat seedlings under NaCl stress; T1 - control, T2 - 100 mM NaCl, T3 - 100 mM NaCl + 10 mM KCl, T4 - 0.1 mM SNP, T5 - 100 mM NaCl + 0.1 mM SNP, T6 - 100 mM NaCl + 10 mM KCl + 0.10 mM SNP. Means ± SDs, n = 3. Different letters within the same column indicate significant differences at $P < 0.05$.

Treatment	Roots		Leaves			Roots		
	Root activity	H$^+$-ATPase	Proline	Sugars	Proteins	Proline	Sugars	Proteins
T1	37.30±5.57a	0.07±0.01bc	22.31±4.59c	6.49±0.84a	14.20±2.29c	8.41±0.77b	5.26±0.23b	7.07±0.18ab
T2	31.27±2.36a	0.06±0.00c	89.96±10.73a	5.90±0.14ab	18.24±0.97ab	25.91±13.12a	6.90±0.44a	3.36±1.09c
T3	36.03±1.50a	0.06±0.01bc	83.17±10.60a	6.02±0.50ab	19.12±2.26ab	17.06±5.01ab	4.37±0.26b	4.71±1.30c
T4	19.26±3.34b	0.08±0.01a	18.07±6.26c	5.84±0.08ab	16.45±2.30bc	12.08±1.83b	7.14±0.50a	5.31±1.43bc
T5	32.66±2.27a	0.07±0.00bc	59.92±5.26b	5.55±0.07b	17.03±1.26bc	17.83±4.40ab	5.14±0.81b	3.89±0.94c
T6	35.85±6.91a	0.07±0.00ab	53.40±2.71b	5.48±0.47b	20.55±1.319a	14.43±5.32b	4.67±1.00b	8.18±1.56a

rate significantly increased in the roots (Table 3). The highest values were recorded in the roots of wheat seedlings treated with NaCl alone, which were 3.21-times higher than in CK. However, the presence of SNP and KCl significantly inhibited the $O_2^{\cdot-}$ generation rate in leaves and roots compared with NaCl alone (Table 3). The highest values of MDA in this paper were also recorded both in leaves and roots treated with NaCl alone. Addition of SNP also increased the MDA production, but the combination of KCl + SNP decreased the MDA content to values similar to those in CK (Table 3).

The salt stressed leaves showed a significant decrease in the activity of SOD, but application of NO and KCl alleviated this decrease (Table 3). The decreased tendency of SOD activity was in accordance with the increase of $O_2^{\cdot-}$ generation rate. The activities of CAT and POD slightly increased under salt stress, in both roots and leaves. (Table 3).

Under NaCl stress, there was a decrease in K content in leaves and an increase in Na content in both leaves and roots as expected. However, a decrease of Mg and Zn content and an increase of Ca and Fe content were observed in wheat roots under 100 mM NaCl stress compared with CK. When PSN was added, the Na, Ca, Mg, Zn, Fe, and Cu content increased in leaves without NaCl stress. Under salt stress, the addition of NO did not have significant effect on the mineral element, while the presence of KCl decreased the content of the Na and Ca in leaves and Na, Zn, Mg, Cu, and Fe in the roots. Moreover, NaCl treatment increased leaf/root Na^+ content ratio and decreased leaf/root Na^+ content ratio compared with other treatments. When 10 mM KCl added to the saline solution, the wheat leaves got a higher K^+/Na^+ ratio compared with the NaCl-treatment alone. The combined NO and K treatment led to the highest K^+/Na^+ ratio in leaves and roots under salt conditions.

Discussion

NaCl treatment reduced the growth of wheat seedlings in the present study (Table 1), which might be consequence of unfavourable effect of salt stress on the various physiological processes such as photosynthesis, nutrient homeostasis, accumulation of compatible solutes, and activities of antioxidant enzymes (Aftab *et al.* 2011). The

Table 3. Effects of KCl and NO on content of H_2O_2 [μmol g^{-1} (f.m.)], superoxide anion ($O_2^{\bullet-}$) generation rate [μmol g^{-1}(f.m.) min^{-1}], content of malondialdehyde (MDA) [nmol g^{-1}(f.m.)], and activities of superoxide dismutase (SOD) [U g^{-1}(f.m.)], catalase (CAT) [μmol H_2O_2 mg^{-1}(f.m.) min^{-1}], and peroxidase (POD) [U g^{-1}(f.m.) min^{-1}] in leaves and roots of wheat seedlings under NaCl stress; T1 - control, T2 - 100 mM NaCl, T3 - 100 mM NaCl + 10 mM KCl, T4 - 0.1 mM SNP, T5 - 100 mM NaCl + 0.1 mM SNP, T6 - 100 mM NaCl + 10 mM KCl + 0.10 mM SNP. Means ± SDs, $n = 3$. Different letters indicate significant differences at $P < 0.05$.

		H_2O_2	$O_2^{\bullet-}$	MDA	SOD	CAT	POD
Leaves	T1	20.81±1.46b	1.39±0.05cd	3.51±1.07cd	165.11±17.21c	2.08±0.09b	92.65±4.45c
	T2	23.69±2.42a	5.84±0.55a	13.13±0.97a	146.19±9.74d	2.23±0.02ab	101.24±3.83ab
	T3	20.62±1.72b	1.64±0.20cd	4.65±1.10c	186.61±8.15b	1.51±0.14c	96.97±4.14bc
	T4	21.29±0.52ab	2.30±0.15b	9.74±0.08b	116.74±3.85e	2.20±0.22b	90.27±7.26c
	T5	21.42±0.39ab	1.73±0.16c	12.52±0.85a	205.02±7.04a	2.47±0.07a	107.45±2.81a
	T6	21.77±0.74ab	1.19±0.13d	3.06±0.53d	199.43±8.83ab	2.32±0.22ab	103.03±4.37ab
Roots	T1	10.20±0.32c	3.14±0.23c	3.61±0.82b	131.54±15.33b	0.40±0.09bc	78.89±2.16c
	T2	10.33±0.69c	23.35±2.73a	14.47±0.60a	79.00±10.86c	0.33±0.04c	100.03±6.03a
	T3	12.92±1.54ab	10.86±1.58b	4.96±0.39b	134.27±8.61b	0.37±0.02bc	103.95±7.61a
	T4	13.36±1.51a	4.92±0.69c	4.93±0.37b	129.96±11.70b	0.46±0.01ab	89.12±5.58b
	T5	10.28±0.35c	12.85±0.93b	4.42±1.12b	123.75±13.05a	0.53±0.03a	86.14±4.02bc
	T6	11.55±0.29bc	11.23±0.37b	3.81±1.60b	187.11±6.25b	0.55±0.13a	102.81±7.13a

Table 4. Effects of KCl and SNP on content of K, Na, Cu, Zn [mg kg^{-1}(d.m.)],Fe, Ca, and Mg [g kg^{-1}(d.m.)], in wheat seedlings under NaCl stress; T1 - control, T2 - 100 mM NaCl, T3 - 100 mM NaCl + 10 mM KCl, T4 - 0.1 mM SNP, T5 - 100 mM NaCl + 0.1 mM SNP, T6 - 100 mM NaCl + 10 mM KCl + 0.10 mM SNP. Means ± SDs, $n = 3$. Different letters within the same column indicate significant differences at $P < 0.05$.

Treatment		K	Na	K/Na	Ca	Mg	Zn	Fe	Cu
Leaves	T1	18.58±1.85a	4.52±1.35d	2.54±0.62a	1.79±0.20b	2.18±0.18a	137.9±13.5a	48.54±7.35b	25.62±1.87ab
	T2	15.47±0.23b	31.58±3.38a	0.29±0.03c	2.45±0.52ab	2.16±0.10a	136.0±7.6a	56.39±4.05ab	21.51±0.62bc
	T3	18.14±1.35a	17.93±2.52b	0.60±0.04bc	1.44±0.33b	2.02±0.11a	170.3±53.9a	60.44±7.44a	22.80±2.33bc
	T4	17.87±2.01ab	11.75±1.90c	0.91±0.18b	3.49±1.21a	2.21±0.25a	159.7±17.4a	53.16±8.81ab	28.72±2.69a
	T5	16.75±1.77ab	20.59±2.16b	0.48±0.05bc	1.41±0.44b	2.10±0.13a	132.6±12.8a	59.46±5.03ab	19.71±4.31c
	T6	17.20±0.85ab	17.42±0.95b	0.99±0.05bc	1.49±0.14b	2.13±0.08a	154.3±8.4a	60.72±4.63a	23.39±0.78bc
Roots	T1	17.54±2.06a	7.00±1.32c	1.49±0.14a	0.29±0.10ab	1.07±0.15a	169.1±24.5a	220.67±35.39b	37.76±2.16ab
	T2	16.76±2.04a	27.91±3.48a	0.37±0.08cd	0.30±0.10b	0.91±0.11ab	159.5±32.3a	294.43±56.03a	40.37±7.49a
	T3	17.84±0.85a	25.47±1.09a	0.45±0.02cd	0.34±0.04ab	0.71±0.07cd	144.2±10.8a	247.57±44.61ab	31.01±0.96b
	T4	17.58±2.27a	16.73±3.34b	0.63±0.13b	0.31±0.08ab	0.83±0.02bc	156.9±12.1a	224.68±16.39b	39.52±5.39ab
	T5	15.86±3.91a	25.74±3.63a	0.37±0.09d	0.28±0.14b	0.71±0.15cd	163.4±12.7a	245.88±30.80ab	39.13±6.47ab
	T6	16.88±1.98a	18.983.53b	0.53±0.04bc	0.45±0.06a	0.63±0.01d	171.4±42.4a	236.97±28.75ab	39.14±5.08ab

salt-induced reduction in growth parameters is generally found in many different crops (Khan *et al.* 2012, Liu *et al.* 2014). However, treatment with NO donor PNS increased the fresh and dry masses of shoots and roots (Table 1). Similarly, NO alleviated Cu-stress in tomato, Ni-stress in canola, and Cd-stress in ryegrass (Cui *et al.* 2010, Kazemi *et al.* 2010, Wang *et al.* 2013). When 10 mM KCl was added to 100 mM NaCl solution, shoot growth was more suppressed but root growth less suppressed than under NaCl alone (Table 1). This might be considered as adaption of wheat seedlings by increasing the root/shoot ratio.

The growth of some crop plants can be enhanced by low concentrations of NaCl (10 - 40 mM). Under high salinity, previous studies have showed increase or decrease chlorophyll content per fresh mass unit (Hanada *et al.* 1994, Santos *et al.* 2004), which depend on the tolerance of different plants to the salt stress. García-Valenzuela *et al.* (2005) proved that chlorophyll accumulation increased in response to osmotic stress in graminaceous chlorophyllic cells. In addition, some halophytes accumulate high amount of Na^+ for osmotic adjustment and their chloroplasts function well. It can be supposed that high salinity induced osmotic stress is the signal for the increased chlorophyll accumulation (García-Valenzuela *et al.* 2005). In plants, carotenoids play a role in protecting plants against photooxidative processes. The carotenoids act as a ROS-scavenging compounds, which could prevent the production of the $^1O_2$ (Müller *et al.* 2011). NaCl stress decreased the Car content in wheat seedlings (Table 1) and so their antioxidant capacity.

Root systems play an important role in controlling plant growth and development because of their importance in

the absorption of water and nutrients (Qi *et al.* 2012). Root activities of NO-treated wheat seedlings were decreased under non-saline conditions and increased under saline condition (Table 2). The enzymatic reduction of TTC by cellular redox systems to insoluble red formazan has been used to measure viability of roots (Duncan *et al.* 2004). The NO donor, SNP contains CN^- that may inhibit the cytochrome pathway. Furthermore, NO could affect the activities of antioxidant enzymes and the ROS accumulation under salt stress (Table 3), that suggests that NO can participate in the response of wheat to salt stress by regulating the activity of antioxidant enzymes and the amount of ROS. The plasma membrane H^+-ATPase is a tightly bound and integral transmembrane protein. It is well known that H^+-ATPase in plasma membrane plays an important role in the transport of multiple ions (Zhao *et al.* 2004, Wang *et al.* 2013). NO could induce H^+-ATPase activity (Table 2), which is a benefit for the selective uptake of mineral elements. However, 10 mM KCl had no significant effect on root activity. In maize, K^+ activates membrane H^+-ATPase and so facilitates the transport of different ions (Gibrat *et al.* 1990). Under NaCl stress, the KCl+SNP treatment alleviated the adverse of salt stress to H^+-ATPase activities. Similar result was reported in cotton seedlings under NaCl stress (Liu *et al.* 2014).

Accumulation of organic solutes in the cytosol and organelles is a good way of osmotic adjustment. Abiotic stress always results in the accumulation of proline, which was also found under salt stress in this study (Table 2). Proline accumulation is one of the most frequently reported modifications induced by salt stress in plants, and it is often considered to be a stress resistance mechanism (Misra *et al.* 2009). The decrease in the content of proline was observed with the addition of PSN to NaCl solution compared with NaCl alone. Reduction in proline content by NO pretreatment has been reported in canola under Ni-stress and in ryegrass under Cd-stress (Kazemi *et al.* 2010, Wang *et al.* 2013). Lopez-Carrion *et al.* (2008) reported that NO reduced proline accumulation in cabbage under salt stress and suggested that the reduction in the proline content was related to increased activity of proline dehydrogenase. In addition, in the present study, the soluble protein content increased under NaCl stress in the wheat leaves, which suggests that salt stress could induce the expression of soluble protein or produce new protein to suit the saline conditions. Soluble sugar content in the KCl or SNP treatments was lower compared with NaCl alone treatment.

ROS play an important role in redox signal transduction, although they are toxic. In this study, NaCl treatment increased $O_2^{\cdot-}$ accumulation and lipid peroxidation in wheat seedlings (Table 3). Amongst the different ROS, H_2O_2 is taken as the most stable one, so that its content is typical for the redox status. H_2O_2 is also studied as a signalling molecule (Zhang *et al.* 2013, Qiao *et al.* 2014), however, higher content of H_2O_2 is toxic and leads to programmed cell death; so it is crucial for plants to regulate H_2O_2 intracellular content (Egbichi *et al.* 2013). The accumulation of MDA under saline conditions reflects oxidation of membrane-bound fatty acids causing propagation of lipid peroxidation. The SOD, POD, CAT, and other antioxidant are present in different organelles in plants. The results show, that treatment with KCl or SNP can increase the activity of SOD (Table 3) and so decrease in the content of $O_2^{\cdot-}$ (Table 3). The decrease of root SOD activity under NaCl treatments was the main reason for the significant increase of $O_2^{\cdot-}$. Moreover, the combination of KCl +SNP showed a synergism on ROS metabolism and SOD function in wheat seedlings.

The NO is an unusual signal; it is a reactive, volatile, and lipophilic free radical that could be cytotoxic (Crawford *et al.* 2005). Under salt stress, the activities of SOD, POD, and CAT maintained rather high, and the content of MDA, H_2O_2 and O_2^- decreased when treated with NO, KCl, or NO+KCl. The NO has been recognized to detoxify ROS either with direct interaction with superoxide or may enhance the antioxidant capacity of cell by increasing the activities of antioxidant enzymes (Khan *et al.* 2012). The effect of NO on ROS metabolism has been reported in many other studies (*e.g.* White *et al.* 1999, Shi *et al.* 2007). The reduction of ROS decreases lipid peroxidation and protein damage. On the other hand, KCl treatment also affect ROS metabolism because KCl treatment promotes absorption of K^+ and inhibits absorption of Na^+, which reverses toxicity of the single ion.

Under NaCl stress, there was a decrease in K^+ uptake in the wheat seedlings and an increase in Na^+ influx (Table 4). The increase in Na^+ accumulation is in accordance with results obtained in other crop plants (Khan *et al.*. 2012, Liu *et al.* 2014). Electrophysiological studies have shown that the voltage-insensitive monovalent-cation channel (VIC) or nonselective cation channel (NSCC) are responsible for the bulk of Na^+ influx into plant cells (White *et al.* 1999). Excess of Na^+ is toxic to the plant cells and K^+ is the major ion contributing to osmotic pressure and ionic strength (Serrano *et al.* 2001). In this experiment, the content of K^+ and the K^+/Na^+ ratio decreased under salt stress (Table 4). To maintain normal cell metabolism, a suitable K^+/Na^+ ratio is important for the adjustment of cell osmoregulation, pressure potential, stomatal function, activation of enzymes, protein synthesis, and photosynthesis (Shabala *et al.* 2003, Zheng *et al.* 2008). Our results showed that NO evidently alleviated effect of NaCl on wheat seedlings. The presence of NO could inhibit the Na^+ transport and improve the K^+ transport from root to shoot. So, the leaves of wheat seedlings get more K^+ and less Na^+ and so improving the K^+/Na^+ ratio when treated with SNP under saline condition. Under 100 mM NaCl, the Mg content in the roots slightly decreased, whereas the Fe content increased significantly (Table 4). That was the most usual phenomenon under salt stress, the ionic imbalance, which was different among various environments and plants. Though the content of Ca, Mg, Zn, Fe,Cu, and K in wheat seedlings were influenced by KCl and NO, K^+ played the key role in ion homeostasis under NaCl stress. The suitable K^+/Na^+ ratio in nutrient solution promoted the selective capacity for K^+, suppressed the uptake of Na^+ in root system, as well, K^+/Na^+ ratio in root cells and affected the transport of Na^+ and K^+ from roots to leaves.

In conclusion, salt stress depressed plant growth, inhibited root activity, decreased protein synthesis, caused oxidative stress, and inhibited uptake of nutrient elements. The addition of 10 mM KCl and/or 0.1 mM SNP could alleviate the NaCl-induced suppression in activities of H^+-ATPase and antioxidant enzymes, especially SOD, decrease the $O_2^{\cdot-}$ generation rate and lipid peroxidation under salt stress. Exogenous NO had an effect on the capacity of K^+ and Ca^{2+} uptake and could promote the ratio of K^+/Na^+ in the leaves. The 10 mM KCl added to 100 mM NaCl solution improved the K^+/Na^+ ratio in nutrient solution, thus enhanced the selective uptake of K^+ compared with Na^+. Furthermore, the combination of KCl and SNP had some positive effects on organic solutes, ROS metabolism, SOD function and K^+/Na^+ ratio and can be recommend for alleviation of adverse effect of salt stress in wheat seedlings.

References

AbdElgawad, H., Zinta, G., Hegab, M.M., Pandey, R., Asard, H., Abuelsoud, W.: High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Front.. Plant Sci. **7**: 276, 2016.

Abreu, I.A., Farinha, A.P., Negrão, S., Gonçalves, N., Fonseca, C., Rodrigues, M., Batista, R., Saibo, N.J.M., Oliveira, M.M.: Coping with abiotic stress: proteome changes for crop improvement. - J. Proteomics **93**: 145-168, 2013.

Aftab, T., Khan, M.M.A., da Silva, J.A.T., Idrees, M., Naeem, M., Moinuddin: Role of salicylic acid in promoting salt stress tolerance and enhanced artemisinin production in *Artemisia annua* L. - J. Plant Growth Regul. **30**: 425-435, 2011.

Arasimowicz, M., Floryszak-Wieczorek J.,.: Nitric oxide as a bioactive signalling molecule in plant stress responses. - Plant Sci. **172**: 876-887, 2007.

Bai, X.Y., Dong,Y.J., Wang,Q.H., Xu, L.L., Kong, J., Liu,S.: Effects of lead and nitric oxide on photosynthesis, antioxidative ability, and mineral element content of perennial ryegrass. - Biol. Plant.**59**: 163-170, 2015.

Bates, L.S., Waldren, S.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. - J. Plant Soil **39**: 205-207, 1973.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Bressan, R.A., Bohnert, H.J., Hasegawa, P.M.: Genetic engineering for salinity stress tolerance. - Adv. Plant Biochem. mol .Biol **1**: 347-384, 2008.

Briskin, D.P., Leonard, R.T., Hodges, T.K.: Isolation of the plasma membrane: markers and general principles. - Methods Enzymol. **148**: 542-558,1987.

Cakmak, I.: The role of potassium in alleviating detrimental effects of abiotic stresses in plants. - J. Plant Nutr. Soil Sci. **168**: 521-530, 2005.

Crawford, N.M., Guo, F.: New insights into nitric oxide metabolism and regulatory functions. - Trends Plant Sci. **10**:195-200, 2005.

Cui, X.M., Zhang, Y.K., Wu, X.B., Liu, C.S.: The investigation of the alleviated effect of copper toxicity by exogenous nitric oxide in tomato plants. - J. Plant Soil Environ. **6**: 274-281, 2010.

Delledonne, M.: NO news is good news for plants. - Curr Opin. Plant Biol. **8**: 390-396, 2005.

Dong, Y.J., Chen, W.F., Xu, L.L., Kong, J., Liu S., He, Z.L.: Nitric oxide can induce tolerance to oxidative stress of peanut seedlings under cadmium toxicity. - Plant Growth Regul. **79**:19-28, 2016.

Dong,Y.J., Wan, Y.S., Liu, F.Z., Zhuge, Y.P.: Effects of exogenous SA supplied with different approaches on growth, chlorophyll content and antioxidant enzymes of peanut growing on calcareous soil. - J. Plant Nutr. **42**: 1869-1883, 2019.

Duncan, D.R., Widholm, J.M.: Osmotic induced stimulation of the reduction of the viability dye 2,3,5-triphenyltetrazolium chloride by maize roots and callus cultures. - J. Plant Physiol. **16**: 397-403, 2004.

Egbichi, I., Keyster, M., Jacobs, A., Klein, A., Ludidi, N.: Modulation of antioxidant enzyme activities and metabolites ratios by nitric oxide in short-term salt stressed soybean root nodules. - South Afr. J. Bot. **88**: 326-333, 2013.

Foyer, C.H., Noctor, G.: Oxygen processing in photosynthesis: regulation and signaling. - New Phytol. **146**: 359-388, 2000.

García-Valenzuela, X., García-Moya, E., Rascón-Cruz, Q., Herrera-Estrella, L., Aguado-Santacruz, G.A.: Chlorophyll accumulation is enhanced by osmotic stress in graminaceous chlorophyllic cells. - J. Plant Physiol. **162**: 650-661, 2005.

Giannakoula, A., Moustakas, M., Mylona, P., Ioannis, P., Traianos, Y.: Aluminium tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline and decreased levels of lipid peroxidation and Al accumulation. - J. Plant Physiol. **165**:385-396, 2007.

Gibrat, R., Grouzis, J.P., Rigaud, J., Grignon, C.: Potassium stimulation of corn root plasmalemma ATPase. - J. Plant Physiol. **93**: 1183-1189, 1990.

Glenn, E.P., Brown, J.J., Blumwald, E.: Salt tolerance and crop potential of halophytes. - Crit. Rev. Plant Sci. **18**: 227-255, 1999.

Hammer, S.R., Nuckles, E.M., Kuć, J.: Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrchum lagenarium*. - J. Physiol. Plant Pathol. **20**: 73-82,1982.

Hanada, A.M., El-Enany, A.E.: Effect of NaCl salinity on growth, pigment and mineral element contents, and gas exchange of broad bean and pea plants. - Biol. Plant. **36**: 75-81,1994.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Kazemi, N., Khavari-Nejad, R., Fahimi, H., Saadatmand, S., Nejad-Sattari, T.: Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. - Sci. Hort. **126**: 402-407, 2010.

Khan, M.N., Siddiqui, M.H., Mohammad, F., Naeem, M.: Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. - J. Nitric Oxide **27**: 210-218, 2012.

Lin, Y., Liu, Z., Shi, Q., Wang, X., Wei, M., Yang, F.: Exogenous nitric oxide (NO) increased antioxidant capacity of cucumber hypocotyl and radicle under salt stress. - Scientia Hort. **142**: 118-127, 2012.

Liu, S., Dong, Y.J., Xu, L.L., Kong, J.: Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. - Plant Growth Regul. **73**: 67-78, 2014.

Lopez-Carrion, A.I., Castellano, R., Rosales, M.A., Ruiz, J.M., Romero, L.: Role of nitric oxide under saline stress: implications on proline metabolism. - Biol. Plant. **52**: 587-591, 2008.

Marschner, P., Marschner,S.: Mineral Nutrition of Higher Plants. 3rd Ed. - Science Press, Beijing 2012.

Misra, N., Saxena, P.: Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. - J. Plant Sci. **177** :181-189, 2009.

Mittler, R.: Oxidative stress, antioxidants and stress tolerance. - Trends Plant Sci. **7**: 405-410, 2002.

Müller, L., Fröhlich, K., Böhm, V.: Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. - Food Chem. **129**: 139-148, 2011.

Ohnishi, T., Gall, R.S., Mayer, M.L.: An improved assay of inorganic phosphate in the presence of extra labile phosphate compounds: application to the ATP-ase assay in the presence of phosphocreatine. - Anal. Biochem. **69**: 261-267, 1975.

Qi, W.Z., Liu, H.H., Liu, P., Dong, S.T., Zhao, B.Q., So, H.B., Li, G., Liu, H.D., Zhang, J.W., Zhao, B.: Morphological and physiological characteristics of corn roots from cultivars with different yield potentials. - Eur. J. Agron. **38**: 54-63, 2012.

Qiao, W., Li, C., Fan, L.M.: Cross-talk between nitric oxide and hydrogen peroxide in plant responses to abiotic stresses. - Environ. exp. Bot. **100**: 84-93, 2014.

Santos, C.V.: Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. - Scientia Hort. **103**: 93-99, 2004.

Serrano, R., Rodriguez-Navarro, A.: Ion homeostasis during salt stress in plants. - Curr. Opin. Plant Biol. **13**: 399-404, 2001.

Shabala, S.N., Shabala, L., Van, E.V.: Effect of calcium on root development and root ion fluxes in salinised barley seedlings. - Funct. Plant Biol. **30**: 507-514, 2003.

Shi, Q., Ding, F., Wang, X., Wei, M.: Exogenous nitric oxide protects cucumber roots against oxidative stress induced by salt stress. - Plant Physiol. Biochem. **45**: 542-550, 2007.

Singh, A.P., Dixit, G., Kumar, A., Mishra, S., Kumar, N., Dixit, S., Singh, P.K., Dwivedi, S., Trivedi, P.K., Pandey, V., Dhankher, O.P., Norton, G.J., Chakrabarty, D., Tripathi, R.D.: A protective role for nitric oxide and salicylic acid for arsenite phytotoxicity in rice (*Oryza sativa* L.). - Plant Physiol. Biochem. **115**: 163-173, 2017.

Sokolovski, S., Blatt, M.R.: Nitric oxide block of outward-rectifying K+ channels indicates direct control by protein nitrosylation in guard cells. - J. Plant Physiol. **136**: 4275-4284, 2004.

Song, Y. L., Dong, Y J., Tian, X.Y., Kong, J., Bai, X.Y., Xu, L.L., He, Z.L.: Role of foliar application of 24-epibrassinolide in response of peanut seedlings to iron deficiency. - Biol. Plant. **60**: 329-342, 2016.

Song, Y.L., Dong, Y.J., Tian, X.Y., Wang, W.W., He, Z.L.: Effects of nitric oxide and Fe supply on recovery of Fe deficiency induced chlorosis in peanut plants. - Biol. Plant. **61**: 155-168, 2017.