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# Performance of Wheat Seedlings under Nitric Oxide and Potassium Treatments

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**Seed germination, growth, osmotic adjustment, antioxidant enzyme defense were studied in wheat (*Triticum aestivum* L. cv. Samma) under different concentrations of (i) $KCl_0$ mM + $NO_0$ mM (control), (ii) $KCl_{50}$ mM + $NO_0$ mM, (iii) $KCl_{100}$ mM + $NO_0$ mM, (iv) $KCl_{150}$ mM + $NO_0$ mM, (v) $KCl_{200}$ mM + $NO_0$ mM, (vi) $KCl_{250}$ mM + $NO_0$ mM, (vii) $KCl_0$ mM + $NO_{0.5}$ mM, (viii) $KCl_{50}$ mM + $NO_{0.5}$ mM, (ix) $KCl_{100}$ mM + $NO_{0.5}$ mM, (x) $KCl_{150}$ mM + $NO_{0.5}$ mM, (xi) $KCl_{200}$ mM + $NO_{0.5}$ mM, (xii) $KCl_{250}$ mM + $NO_{0.5}$ mM. Application K (50 and 100 mM) with or without NO significantly enhanced seed germination, growth (shoot fresh weight (Shoot FW), Root FW, shoot dry weight (Shoot DW), Root DW and area leaf$^{-1}$ (LA), and physiological and biochemical (Chlorophyll (Chl) *a*, *b* and Total Chl, content of proline (Pro), potassium (K), magnesium (Mg), calcium (Ca) and K/Na ratio (except Na) attributes of wheat plants. The higher concentration of K (150, 200, 250 mM) inhibited all these traits relative to the lower concentrations of K(50 and 100 mM) and controls. It appears from the results that application of NO enhanced the tolerance of plants to KCl-induced-salt stress by improving ions homeostasis and organic (Pro) and inorganic (K) osmoprotectants, and by reducing membrane damage. Also, application of NO with lower levels of K enhanced the performance of wheat plants as compared to the alone application of NO and K.**

**Key words:** Potassium, Nitric oxide, Photosynthetic pigments, Growth, Essential nutrients, Peroxidase, Proline, Wheat seedlings, *Triticum aestivum*.

The productivity of most crop plants is markedly affected by diminishing fresh water resources and increasing soil salinization in arid and semi-arid regions (Adolf *et al*., 2012). According to the FAO (2005) near about 800 million hectares of land throughout the world are salt-affected. Also, every year about 6 million arable lands are lost from agricultural uses due to increasing salinization (Ahire *et al.*, 2013). The presence of excessive amounts of soluble salt in growth medium that disturbs almost every aspects of plant metabolism which leads to a series of morphological, physiological and molecular changes in plants. Many physiological and biochemical processes such as seed germination, ion toxicity, mineral distribution, organic solutes/ osmolytic synthesis, enzymes activity and photosynthesis are substantially disturbed by salinity (Marschner, 2002; Siddiqui *et al.*, 2010).

The soil solution has variety of ions that causes salinity stress; the majority of studies have been focused on the effects of NaCl on plants. While few studies have been done on the phytotoxic effects of potassium chloride (KCl) on plants. According to Ahire *et al.* (2013) increasing concentrations of KCl reduced shoot length, fresh weight, dry weight, tissue water content and also increased lipid peroxidation in *Bacopa monnieri* (L.). Presence of excess K in soil affects osmotic

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pressure in soil, uptake of nutrients [nitrogen (N), magnesium (mg), calcium (Ca) and boron (B)]. However, as we know that potassium ($K^+$) as an essential element plays a central role in several physiological processes in plant cell (Nieves-Cordones *et al.*, 2012). It plays very important role in many regulatory functions in plants for their growth and development. It is well established that K has substantial role in enzymes activation, protein synthesis, photosynthesis, stomatal activity, transport of water, nutrients and sugars, cation-anion balance and stress resistance (Marschner, 2002).

Nitric oxide (NO) is a small in size, no charge, short-lived, and highly diffusible across biological membranes. It has multifunctional roles in the regulation of remarkable spectrum of plant cellular mechanisms (Siddiqui *et al.*, 2011). It has multiple plant responses towards a variety of biotic and abiotic stresses and alleviating some consequences provoked by oxidative stresses (Delledonne, 2005). NO improves the plant tolerance to abiotic stress by alleviating the harmful effect of reactive oxygen species (ROS), and reacting with other target molecules, and regulating the expression of stress responsive genes (Qiao and Fan, 2008; Siddiqui *et al.*, 2011). The beneficial and harmful effect depends on the concentration and location of NO in plant cells (Qiao and Fan, 2008). NO molecule itself possesses antioxidant properties, has a cytoprotective role by reacting with ROS, hemes, thiols and proteins and generates biochemical signals that directly and indirectly regulate enzymatic activity (Siddiqui *et al.*, 2011).

Today, alleviating the adverse effects of salt stress is extremely important to fulfill the demand of the increasing population across the globe. In this complex situation, it is very crucial to understand physiological and biochemical mechanism by which plants could perform normally and produce better yield under different environmental conditions. Like other crops, globally wheat is very important crop and leading source of vegetable protein, and having a higher protein content than other major cereals, maize and rice. Since performance of plants are affected not only by $Na^+$ salts but also by excess $K^+$ salts present in soil. In view of the available information, the present study was designed to investigate the plausible role of NO in alleviation of $K^+$ toxicity by regulating antioxidant system and organic solute [proline (Pro) and inorganic osmoticum ($K^+$)] and ions homeostatsis in wheat and also to study the cumulative effect of NO and $K^+$ on the performance of wheat plants.

## MATERIALS AND METHODS

### Plant material and Treatments

The study was conducted in a growth chamber at the Department of Botany and Microbiology, King Saud University, Riyadh. The growth chamber illuminated with fluorescent lamps with light intensity of 325 mmol $m^{-2}$ $s^{-1}$. The photoperiod was 14 h light and 10 h dark and the air temperature was 20-24 °C. Seeds of wheat (*Triticum aestivum* L. cv. Samma) were obtained from a local market in Riyadh, Saudi Arabia. Healthy seeds were surface sterilized by using 1% sodium hypochlorite for 10 min then vigorously rinsed with sterilized double distilled water (DDW). Healthy seeds were divided into two groups: group one for percent germination and group two for physiological and biochemical characteristics of seedling measurement.

In group one five replicates of 25 seeds each were placed in Petri dishes (Size 12 cm) having two sheets of sterilized filter paper imbibed in treatment solutions (mentioned below). All Petri dishes kept in compete darkness at 26 $^0$C. The percent germination was counted number of seeds that showed radical emergence (at least 2 mm length).

In group two, healthy seeds were sown (25 seeds/pot) in plastic pots (25 cm in diameter, 25 cm height) filled with perlite and supplied with Raukura's nutrient solution (Smith et al., 1983). The pots were arranged in a simple randomized design in growth chamber with a single factor and four replicates. Seedlings were thinned after one weak and maintained healthy plants of uniform size in each pot. Pots were irrigated every two days with nutrient solution (100 ml) to keep the perlite moist.

When the plants were at the stage of two to three true leaves, treatment solution was added to the pots containing experimental wheat plants to attain the final concentration from 0.0 to 250 mM of added KCl. Treatments for both groups were given as follows: (i) $KCl_0$ mM + $NO_0$ mM

(control), (ii) $KCl_{50}$ mM + $NO_0$ mM, (iii) $KCl_{100}$ mM + $NO_0$ mM, (iv) $KCl_{150}$ mM + $NO_0$ mM, (v) $KCl_{200}$ mM + $NO_0$ mM, (vi) $KCl_{250}$ mM + $NO_0$ mM, (vii) $KCl_0$ mM + $NO_{0.5}$ mM, (viii) $KCl_{50}$ mM + $NO_{0.5}$ mM, (ix) $KCl_{100}$ mM + $NO_{0.5}$ mM, (x) $KCl_{150}$ mM + $NO_{0.5}$ mM, (xi) $KCl_{200}$ mM + $NO_{0.5}$ mM, (xii) $KCl_{250}$ mM + $NO_{0.5}$ mM. The source of NO and K was sodium-nitroprusside and potassium chloride (KCl) and the dose of sodium nitroprusside was selected for this study on the bases of an earlier experiment (Basalah *et al.*, 2013). At the end of treatments, plants were sampled to measure the growth characteristics shoot fresh (Shoot FW) and dry weight (Shoot DW) plant$^{-1}$, root fresh (Root FW) and dry weight (Root DW) plant$^{-1}$ and area leaf$^{-1}$ (LA) and physio-biochemical attributes [chlorophyll (Chl) *a* and b, Total Chl, proline (Pro) content, and content of some elements [potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg)], electrolyte leakage and the activity of antioxidant enzyme peroxidase (POD) of plants.

**Morphological characteristics**

The shoot and root length of plant were measured by using a meter scale after removal from the pots. The fresh weight of the plant roots and shoots were recorded, then placed in an oven run at 60°C for 48 h. These dried plants were weighed to record the plant dry weight. Leaf area was measured using leaf area meter (LI.COR-MODEL LI-3000).

**Determination of physiological and biochemical parameters**

Chlorophylls were extracted from fresh leaves of wheat seedlings from group two and assayed by the DMSO method based on Barnes *et al.* (1992). Absorption of Chl in the extract was measured using (UV-VIS) spectrophotometer.

Adopting the ninhydrin method of Bates *et al*. (1973), Pro content was determined spectrophotometrically. 300 mg of fresh leaf samples was homogenized in sulphosalicylic acid, then added 2 mL each of acid ninhydrin and glacial acetic acid. The samples were heated at 100°C. The mixture was extracted with toluene, and the free toluene was quantified spectrophotometrically at 528 nm using L-proline as a standard.

Some mineral elements such as $Na^+$, $K^+$, $Ca^{++}$ and $Mg^{++}$ were determined according to the Association of Official Analytical Chemistry methods (AOAC, 1984) using Atomic Absorption Spectrophotometer AA-675 Series.

Determination of electrolyte leakage was determined as described by Lutts *et al.* (1995). Samples were washed three times with DDW to remove the surface contamination. The leaf discs were prepared by cutting the young leaves and were placed in a closed vial containing 10 mL of DDW and incubated on a rotatory shaker for 24 h; subsequently, the electrical conductivity of the solution (EC1) was determined. Samples were then autoclaved at 120°C for 20 min and then electrical conductivity (EC2) was measured after cooling the solution at room temperature. The electrolyte leakage was calculated as: Electrolyte leakage% = $(EC_1/EC_2) \times 100$.

**Determination of antioxidant enzymes activity**

**Preparation of enzyme extracts**

A crude enzyme extract was prepared by homogenizing 500 mg of leaf tissue in extraction buffer containing 0.5% triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15000g for 20 min at 4°C. The supernatant was used for the enzymatic assays.

Peroxidase POD (E.C. 1.11.1.7) activity was determined by the method of Chance and Maehly (1955). Five milliliters of the assay mixture for the POD activity comprised phosphate buffer (pH 6.8), 50 M of pyrogallol, 50 mM of $H_2O_2$ and 1 mL of the 20 times-diluted enzyme extract. This was incubated for 5 min at 25°C, after which the reaction was stopped by adding 0.5 mL of 5% (v/v) $H_2SO_4$. The amount of purpurogallin formed was determined by taking the absorbency at 420 nm. Specific activity of POD was calculated by purpurogallin formed $\mu g^{+1}$ protein $min^{+1}$.

**Statistical analysis**

Each pot was treated as one replicate and all the treatments were repeated four times. The data were analyzed statistically with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Means were statistically compared by Duncan's multiple-range test at $p<0.05$% level.

## RESULTS

In the present experiment, the plausible role of NO in alleviation of $K^+$ salinity and the cumulative effect of NO and $K^+$ on the performance

of wheat (*Triticum aestivum* L.) plant were assessed on the basis of seed germination, plant growth, physiological and biochemical attributes.

**Effect of NO and $K^+$ on growth characteristics**

The seeds of wheat were supplied with different dose of K (KCl: 0, 50, 100, 150, 200 and 250 mM) and observed that the percent seed germination was high with 100 mM of K (Fig. 1). However, a significant reduction in percent germination was observed with increasing concentration of K (150-250 mM). The combined application NO with K (NO + 50 and 100 mM of K) significantly enhanced the percent germination as compared to alone application of NO and K. Also, application NO with high dose of K (150, 200 and 250 mM) significantly improved percent seed germination (Fig. 1A).

We measured seven growth characteristics (Shoot FW, Root FW, Shoot DW, Root DW and LA) (Fig. 1 B and C; Table 1). All these growth attributes increased with increasing concentration of K up to 100 mM, while increasing concentration of K (150, 200 and 250 mM) showed inhibitory effect. However, application of NO with K (50 and 100 mM) increased further growth characteristics. Also, NO improved growth characteristics by alleviating inhibitory effect of increasing concentrations of K.

**Table 1.** Effect of potassium and nitric oxide on leaf area,chlorophyll*a* (Chl *a*), Chl*b* and Total Chl. Values followed by the same letter do not differ statistically at P < 0.05 (Duncan Multiple Range Test)

| Treatment | Area leaf$^{-1}$ ($cm^2$) | Chl *a* (mg $g^{-1}$ FW) | Chl *b* (mg $g^{-1}$ FW) | Total Chl (mg $g^{-1}$ FW) |
|---|---|---|---|---|
| 0 mM KCl + 0 mM NO | 6.67±0.27$^{e}$ | 9.82±0.02$^{e}$ | 4.38±0.07$^{e}$ | 14.20±0.08$^{e}$ |
| 50 mM KCl + 0 mM NO | 7.72±0.17$^{d}$ | 10.84±0.26$^{d}$ | 4.58±0.20$^{de}$ | 15.42±0.27$^{d}$ |
| 100 mM KCl + 0 mM NO | 9.18±0.17$^{c}$ | 12.85±0.14$^{c}$ | 5.04±0.10$^{cd}$ | 17.89±0.24$^{c}$ |
| 150 mM KCl + 0 mM NO | 4.38±0.24$^{f}$ | 8.62±0.11$^{g}$ | 2.69±0.16$^{g}$ | 11.31±0.15$^{g}$ |
| 200 mM KCl + 0 mM NO | 3.92±0.62$^{fg}$ | 7.51±0.06$^{h}$ | 1.78±0.11$^{hi}$ | 9.28±0.11$^{h}$ |
| 250 mM KCl + 0 mM NO | 2.24±0.29$^{h}$ | 6.58±0.22$^{i}$ | 1.38±0.20$^{i}$ | 7.95±0.13$^{i}$ |
| 0 mM KCl + 0.5 mM NO | 9.01±0.17$^{c}$ | 12.75±0.18$^{c}$ | 4.97±0.08$^{c}$ | 17.72±0.18$^{c}$ |
| 50 mM KCl + 0.5 mM NO | 10.33±0.24$^{b}$ | 13.97±0.21$^{b}$ | 5.55±0.22$^{b}$ | 19.52±0.20$^{b}$ |
| 100 mM KCl + 0.5 mM NO | 11.54±0.45$^{a}$ | 15.20±0.08$^{a}$ | 6.04±0.16$^{a}$ | 21.24±0.17$^{a}$ |
| 150 mM KCl + 0.5 mM NO | 6.66±0.23$^{e}$ | 9.19±0.14$^{f}$ | 3.15±0.05$^{f}$ | 12.34±0.09$^{f}$ |
| 200 mM KCl + 0.5 mM NO | 4.25±0.13$^{f}$ | 8.25±0.06$^{g}$ | 2.69±0.02$^{g}$ | 10.94±0.07$^{g}$ |
| 250 mM KCl + 0.5 mM NO | 3.35±0.18$^{g}$ | 7.91±0.21$^{i}$ | 1.84±0.15$^{h}$ | 8.75±0.29$^{i}$ |

**Table 2.**Effect of potassium and nitric oxide on the content of K, Na, Mg and Ca , and K/Na ratio. Values followed by the same letter do not differ statistically at P < 0.05 (Duncan Multiple Range Test)

| Treatment | $K^+$mg $g^{-1}$ DW | $Na^+$mg $g^{-1}$ DW | $K^+/Na^+$ | $Mg^{2+}$mg $g^{-1}$ DW | $Ca^{2+}$mg $g^{-1}$ DW |
|---|---|---|---|---|---|
| 0mM KCl + 0mM NO | 50.61±0.83$^{l}$ | 3.98±0.09$^{a}$ | 12.76±0.45$^{j}$ | 4.34±0.07$^{d}$ | 15.62±0.62$^{f}$ |
| 50mM KCl + 0mM NO | 79.70±0.92$^{j}$ | 2.88±0.03$^{b}$ | 27.69±0.16$^{i}$ | 4.72±0.08$^{c}$ | 21.32±0.24$^{c}$ |
| 100mM KCl + 0mM NO | 119.55±1.07$^{h}$ | 1.81±0.02$^{f}$ | 65.98±1.15$^{e}$ | 5.03±0.10$^{b}$ | 23.66±0.31$^{b}$ |
| 150mM KCl + 0mM NO | 143.16±0.89$^{f}$ | 2.28±0.05$^{d}$ | 62.91±1.67$^{ef}$ | 3.66±0.06$^{ef}$ | 19.91±0.57$^{cd}$ |
| 200mM KCl + 0mM NO | 159.90±1.63$^{d}$ | 2.61±0.04$^{c}$ | 61.27±1.43$^{f}$ | 3.42±0.04$^{fg}$ | 16.27±1.69$^{ef}$ |
| 250mM KCl + 0mM NO | 186.60±2.34$^{b}$ | 2.40±0.01$^{d}$ | 77.88±0.83$^{c}$ | 2.49±0.08$^{h}$ | 15.32±0.95$^{f}$ |
| 0mM KCl + 0.5mM NO | 64.14±1.58$^{k}$ | 1.73±0.04$^{fg}$ | 37.22±1.74$^{h}$ | 4.64±0.04$^{c}$ | 24.16±0.97$^{b}$ |
| 50mM KCl + 0.5mM NO | 92.02±0.60$^{i}$ | 1.62±0.02$^{g}$ | 56.92±0.89$^{g}$ | 5.00±0.07$^{b}$ | 25.66±0.48$^{b}$ |
| 100mM KCl + 0.5mM NO | 127.80±1.64$^{g}$ | 1.27±0.03$^{h}$ | 100.42±1.19$^{a}$ | 5.51±0.07$^{a}$ | 29.66±0.56$^{a}$ |
| 150mM KCl + 0.5mM NO | 153.73±0.71$^{e}$ | 2.01±0.02$^{e}$ | 76.60±0.96$^{c}$ | 4.36±0.08$^{d}$ | 19.64±0.43$^{cd}$ |
| 200mM KCl + 0.5mM NO | 169.50±0.94$^{c}$ | 2.34±0.02$^{d}$ | 72.38±0.79$^{d}$ | 3.89±0.24$^{e}$ | 18.85±0.62$^{cd}$ |
| 250mM KCl + 0.5mM NO | 203.83±2.27$^{a}$ | 2.32±0.03$^{d}$ | 87.98±1.78$^{b}$ | 3.15±0.05$^{g}$ | 18.13±0.95$^{de}$ |

**Influence of NO and K on physiological and biochemical parameters**

The membrane damage and alteration were assessed by electrolyte leakage in leaves treated with NO and different concentrations of K (Fig. 2A). The seedlings treated with 50 and 100 mM of K showed lesser electrolyte leakage than other treatments of K. However, inclusion of NO in medium containing higher doses of K (150, 200 and 250 mM) significantly improved the electrolyte leakage.

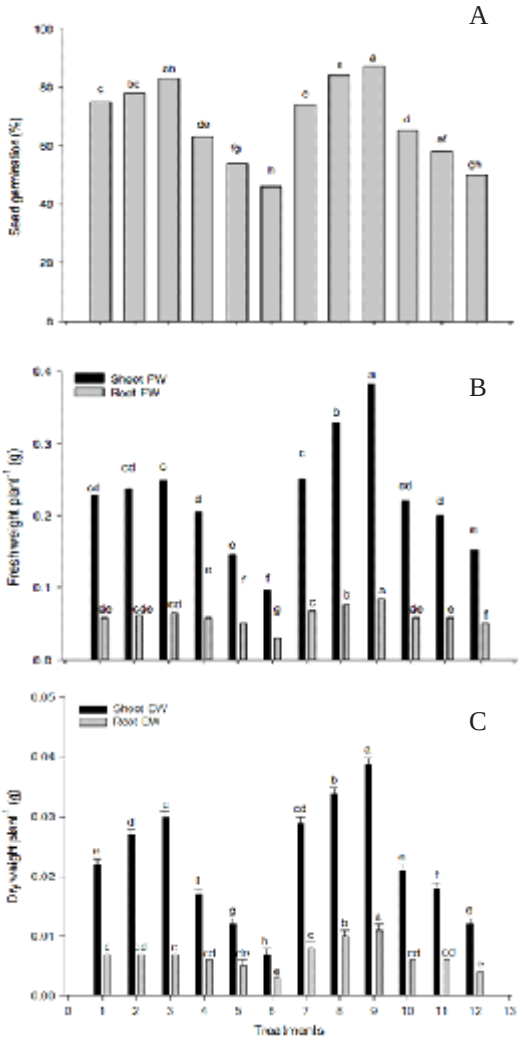


**Fig. 1.** Effect of potassium and nitric oxide on (A) seed germination (%), (B) shoot and root FW, and (C) shoot and root DW. Bars followed by the same letter do not differ statistically at $P < 0.05$ (Duncan Multiple Range Test). [(i) $KCl_0$ mM + $NO_0$ mM (control) (T1), (ii) $KCl_{50}$ mM + $NO_0$ mM (T2), (iii) $KCl_{100}$ mM + $NO_0$ mM (T3),(iv) $KCl_{150}$ mM + $NO_0$ mM (T4), (v) $KCl_{200}$ mM + $NO_0$ mM (T5),(vi) $KCl_{250}$ mM + $NO_0$ mM (T6), (vii) $KCl_0$ mM + $NO_{0.5}$ mM (T7), (viii) $KCl_{50}$ mM + $NO_{0.5}$ mM (T8), (ix) $KCl_{100}$ mM + $NO_{0.5}$ mM (T9), (x) $KCl_{150}$ mM + $NO_{0.5}$ mM (T10), (xi) $KCl_{200}$ mM + $NO_{0.5}$ mM (T11), (xii) $KCl_{250}$ mM + $NO_{0.5}$ mM (T12)

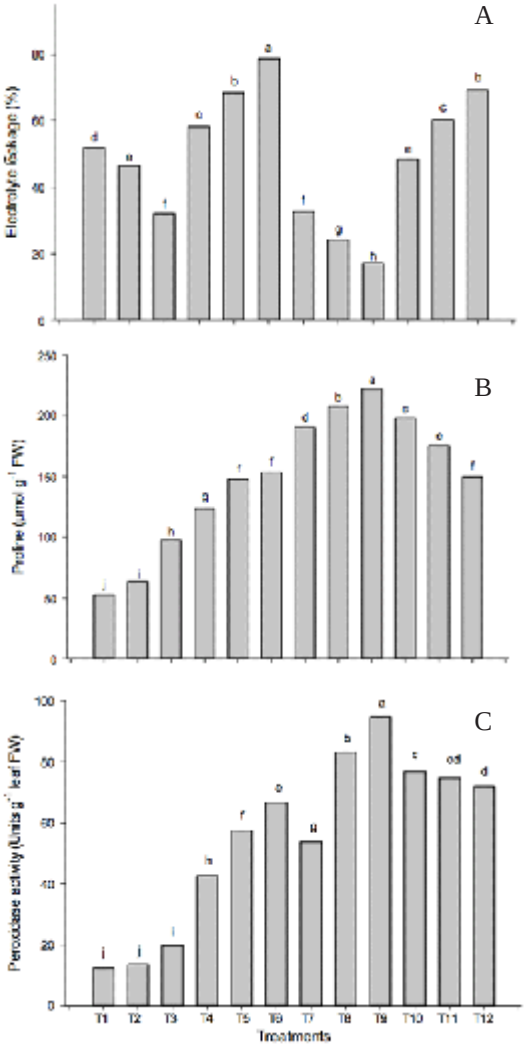


**Fig. 2.** Effect of potassium and nitric oxide on electrolyte leakage, proline content and peroxidase activity. Bars followed by the same letter do not differ statistically at $P < 0.05$ (Duncan Multiple Range Test). [(i) $KCl_0$ mM + $NO_0$ mM (control) (T1), (ii) $KCl_{50}$ mM + $NO_0$ mM (T2), (iii) $KCl_{100}$ mM + $NO_0$ mM (T3),(iv) $KCl_{150}$ mM + $NO_0$ mM (T4), (v) $KCl_{200}$ mM + $NO_0$ mM (T5),(vi) $KCl_{250}$ mM + $NO_0$ mM (T6), (vii) $KCl_0$ mM + $NO_{0.5}$ mM (T7), (viii) $KCl_{50}$ mM + $NO_{0.5}$ mM (T8), (ix) $KCl_{100}$ mM + $NO_{0.5}$ mM (T9), (x) $KCl_{150}$ mM + $NO_{0.5}$ mM (T10), (xi) $KCl_{200}$ mM + $NO_{0.5}$ mM (T11), (xii) $KCl_{250}$ mM + $NO_{0.5}$ mM (T12)

The content of Chl *a*, *b* and Total Chl increased with increasing concentrations of K up to 100 mM, while the higher doses of K (150, 200 and 250 mM) inhibited the synthesis of Chl (Table 1). However, application of NO with 50 and 100 mM of K significantly improved the content of Chl *a*, *b* and Total Chl as compared to alone application of NO and K. Also, at higher K levels (150, 200 and 250 mM), NO showed alleviating effect of salt stress by increasing photosynthetic pigments.

All concentrations of K significantly increased the concentration of Pro in leaves relative to the control, although effects were most notable with NO alone and when NO and K were combined (Fig. 2B).

The concentration of ions increased with increasing concentrations of K up to 100 mM except K and Na (Table 2). The content of K increased with increasing concentrations of K, while Na concentration decreased with increasing concentrations of K. Values for K, Mg and Ca content was significantly higher than the control and alone NO. Application of NO with K significantly improved the content nutrients except Na. Also, Increased K/Na ratio up to 100 mM levels of K was noticed though it declined slightly thereafter. While, application of NO with K significantly improved the K/Na ratio.

The application of K and NO alone as well as in combination significantly influenced the activity of POD (Fig. 2C). However, application of NO with higher levels of K increased further POD activity.

## DISCUSSION

In the present experiment, the application of different levels of K (low and high dose) and a concentration of NO, individually as well as in combination to seeds and seedlings of wheat influenced seed germination, growth, physiological and biochemical characteristics (Table 1 & 2; Figs. 1 & 2), however, high dose of K caused salinity stress in plants.

High percent seed germination was recorded at 50 and 100 mM of K as compared to 150, 200 and 250 mM of K (Fig. 1A). This may be explained by the role of K in plants as it activates many enzymes (almost 60 enzymes) involved in the synthesis of high molecular weight compounds (starch, proteins and cellulose) (Bhandal and Malik, 1988). As we know that hydrophilic groups of proteins and carbohydrates attract the dipolar water molecules to form hydrated shell around these macromolecules. This helps the swelling of the seed coat and also makes it more permeable to oxygen and water. This natural phenomenon is obviously responsible for the increase in the water content of the seeds. Also, K is responsible for regulating the salt concentration in cells and the cytoplasm resulting in increased water retention and uptake. Also, seeds treated with NO and K alone as well as in combination enhanced seed germination. Interestingly, application of NO was found effective in alleviating harmful effect of K (higher doses, 150, 200 and 250 mM). This and other studies (Hua *et al*., 2003, Sarath *et al.*, 2006; Šírová *et al.*, 2011) clearly indicate that NO enhances seed germination, and also alleviates adverse effect of salt stress induced by excess K.

**Plant growth characteristics**

In the present study, the growth attributes (shoot FW, root FW, shoot DW, root DW and LA) of wheat seedlings increased with the application of lower levels of K (50 and 100 mM), but at higher levels of K (150, 200 and 250 mM), it was severely affected (Table 1; Fig. 1 A, B & C). According to Besford and Maw (1975) the growth of plants depends on the adequate supply of K. The adequate supply of K improves the photosynthesis by providing ATP to transport system to transport the photosynthates from leaf to other parts of plants (Wang *et al.*, 2013). Also, K plays an important role in the regulation of many enzymes and protein synthesis that is responsible for all growth processes. We found that growth characteristics of plants were declined at high the concentration of K. It may be due to osmotic and ionic stress. Egan and Ungar (1998) reported that growth characteristics of *Atriplex prostrate* declined in the present of excess K that lowered the osmotic potential. Also, excess of K is more toxic than Na for root growth (Kinraide, 1999). However, application of NO alleviated the toxic effect of K by improving all growth characteristics of wheat (Table 1; Fig. 1 A, b & C). It may be due to the role of NO in alleviating the oxidative damage due to salinity and also contributing better equilibrium between carbon and nitrogen metabolism by inducing total soluble protein

synthesis and activities of endopeptidase and carboxypeptidase in plants under salt stress (Zheng *et al.*, 2010, Siddiqui *et al.*, 2011).

It is well established that organic solutes (such as proline, glycinebetaine, polyol, sugar etc.) play an important role in osmotic adjustment under different environmental stresses. They are compatible to plants because they are easily soluble and non-toxic even at high concentration. Among these compatible solutes, investigated Pro content increased with increasing concentration of K when compared with the controls (Fig. 2B). The accumulation of Pro was found to be high with NO+K application. The hyper accumulation of this organic solute due to the combined application of NO and K enabled the cells to enhance tolerance to salinity stress by maintaining turgor pressure despite low water potential (Siddiqui *et al.*, 2008; Al-Whaibi *et al.*, 2011; Khan *et al.*, 2012). Pro acts as antioxidant and a source of energy, and also regulates gene expression for osmotic adjustment Matysik *et al.* 2002; Iyer and Caplan 1998). Also, Pro serves as a storage sink for carbon and nitrogen and as a reactive oxygen species scavenger (Flores *et al.*, 2007). In the present experiment, The high Pro accumulation might be due application of K and NO that could provide energy in the form of ATP for the synthesis of Pro is an energy dependent process (Ahire *et al.*, 2013)..

In the present study, Chl *a*, *b* and Total Chl were notably influenced by the combined and alone application of K and NO (Table 1). However, these photosynthetic pigments were severely affected by the higher dose of K (150, 200 and 250 mM). It may be due to membrane (Fig. 2A) and reaction centers damage (Kyle, 1987), and imbalance supply of nutrients leading to disturbance of protein synthesis (Khan *et al.*, 2012). While, application of NO with or without K improved chlorophyll synthesis. The increase in photosynthetic pigments synthesis may be due the reduced electrolyte leakage (Fig. 2A) and improved antioxidant enzyme (POD) (Fig. 2C)). Also, NO maintains iron homeostasis and improves internal iron transport, thereby inducing pigments synthesis and chloroplast development. These results corroborate previous findings of Popova and Tuan, 2010; Khan *et al*., 2012 that NO triggers photosynthetic pigments biosynthesis under salt stress.

The results clearly reveal that the content of nutrients (K, Mg and Ca) in plants was significantly and positively correlated with tolerance of K toxicity, except Na. In the present experiment, we observed that the content of these nutrients (Mg and Ca) increased with increasing concentrations of K up to 100 mM except K and Na. However, K and Na content increased and decreased with increasing concentration of K, respectively (Table 2). Application of NO with K increased further nutrients content (except Na) when compared with the controls. It is well established that K plays a key role in many biological process, including enzymatic activation, turgor formation, regulation of stomatal movement and maintenance of osmotic and ions homeostasis (Shabala *et al.* 2003). Also, Ca is a very essential macronutrient and induced the activities of $H^+$-ATPase and $H^+$-PPase, and also increases the tolerance of plant to biotic and abiotic stress (Khan *et al.*, 2012). According to Khan *et al.* (2012) and Siddiqui *et al.* (2013) Ca plays an important role in many physiological processes by inducing several enzymes and antioxidant enzymes activities, and by affecting oxidative signal transduction under different environmental conditions. The tolerance of plant to stress depends on the maintenance of higher concentration of K and Ca, and K/Na ratio in cell for normal metabolic functions of plants. In this study, application of NO maintained the content of K, Mg, Ca, and K/Na ratio under higher dose of K (Table 2).

## CONCLUSION

In conclusion, the present study reveals that influence of KCl was dose dependent. At low concentrations of KCl, K enhanced seed germination, growth, physiological and biochemical characteristics. However, at higher concentrations of KCl, K induced salt stress resulted in decreased seed germination, growth, physiological and biochemical attributes of wheat. Increased photosynthetic pigments (Chl*a*, *b* and Total Chl) and nutrients content of NO-treated plants and a parallel enhancement in synthesis of Pro and antioxidant enzyme POD activity may be responsible for the enhanced LA and biomass production in plants under sufficient and excess KCl conditions. The results demonstrate that

application of NO with KCl augmented the plants to tolerate salt stress by improving ions homeostasis and organic (Pro) and inorganic (K) osmoprotectants, and by reducing membrane damage. Application of NO with lower concentration of K relative to the application of NO and K alone was found effective and significantly improved seed germination, growth, physiological and biochemical characteristics of wheat.

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