

INTERACTIVE EFFECT OF FOLIAR APPLICATION OF NITRIC OXIDE (NO) AND SALINITY ON WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

The present experiment was conducted to assess the interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (*Triticum aestivum* L.). Wheat cv. S-24 was grown under non-stressed or salt stressed conditions (0 and 150 mM NaCl). Different levels of NO (water spray, 0.05, 0.10 and 0.15 mM) were applied as a foliar spray. Salinity applied through rooting medium significantly reduced growth attributes while foliar applied NO was found to be effective in amelioration of adverse effects of salt stress on growth parameters. Application of NO caused an increase in shoot fresh weight under non-saline or saline conditions. Photosynthetic rate of cultivar increased due to foliarly applied NO both under control and saline conditions. Furthermore, increase in growth due to exogenously applied NO may have been due to changes in photosynthesis. However, NO applied as a foliar spray did not change the sub-stomatal CO₂ concentration suggesting that stomatal factors were not the major controlling factors for photosynthesis. However, photosystem-II activity in our experiment did not change by foliar spray of NO. Overall, the adverse effects of salt stress could be alleviated by exogenous application of NO.

Introduction

Salinity is one of the major abiotic environmental stresses that reduce crop productivity due to disturbance in normal growth and metabolic processes (Gorai *et al.*, 2010; Shahbaz *et al.*, 2012). It is a major limiting factor to crop productivity particularly in arid and semi-arid regions of the world (Koyro, 2006; Nakashima *et al.*, 2000) that is 20% of the irrigated land area (Muhling & Lauchli, 2003). It has been reported that smaller and thicker leaves, shorter stems and roots are produced under high saline conditions (Rahman *et al.*, 2008). Salinity stress is involved in reduction of various growth morphological and physiological attributes of various crops like it reduces growth in rice (Shereen *et al.*, 2005; Shahbaz & Zia, 2011; Masood *et al.*, 2005) and maize (Zia *et al.*, 2011), while in case of wheat photosynthetic rate, photochemical capacity and water uptake are reported to be reduced (Gowing *et al.*, 2009; Perveen *et al.*, 2010; Hamayun *et al.*, 2010; Kanwal *et al.*, 2011).

Nitric oxide (NO) is a signaling molecule that is produced as a physiological response in plants under stress conditions. It is lipophilic free radical, volatile in nature and itself acts as an antioxidant in plants (Hayat *et al.*, 2010). Nitric oxide is a very attention-grabbing particle as it has role in signaling mechanism due to its chemical properties (Lamattina *et al.*, 2003). Nitric oxide is readily diffusible through membranes because of its gaseous diatomic nature and water solubility. It is involved in different morpho-physiological processes of plants (Misra *et al.*, 2011; Shaheen *et al.*, 2012). Under saline conditions, nitric oxide is produced as a signaling molecule by different physiological activities while act as an antioxidant in plants (Kopyra & Gwozdz, 2004). It is involved in stress tolerance and widely used against stresses since last few decades (Molassiotis *et al.*, 2010; Hamayun *et al.*, 2010a). Both in animals and plants nitric oxide (NO) is synthesized and participated in biological regulation (Pagnussat *et al.*, 2002). It enhances growth attributes under stress conditions when applied exogenously (Zhang *et al.*, 2006). Moreover,

nitric oxide (NO) is believed to be involved in improvement of defense system in plants which face different stresses (Uchida *et al.*, 2002). Different morpho-physiological parameters like growth biomass attributes, photosynthetic rate, photosystem II activity and gas exchange characteristics decreased under salt stress. All these adverse effects of salinity have been reported to be alleviated by foliar application of NO (Guo *et al.*, 2003; Gabaldon *et al.*, 2005). Similarly, different gas exchange characteristics like sub-stomatal CO₂ concentration (C_i), transpiration rate (E), stomatal conductance (g_s) and net photosynthetic rate (P_n), efficiency of photosystem-II (F_v/F_m) are improved under salt stress by exogenous application of NO at seedling stage in *Lycopersicon esculentum* Mill. (Wu *et al.*, 2011). On the other hand, foliar spray of NO showed better results under saline conditions as compared to non-saline conditions (Tian & Lie, 2006). Exogenous application of the NO reduced oxidative damage by enhancing gas exchange attributes in wheat (Tan *et al.*, 2008).

Wheat (*Triticum aestivum* L.) is known as “king of cereals” but locally known as “Gandum” and a leading world food crop. The use of wheat as an essential food stuff is the main reason for the distribution of wheat world-over particularly in the developing countries (Jaiswal, 2009). Presence of special kind of proteins known as ‘gluten’ within the seeds of the wheat made it unique among the different cereal crops.

Keeping in view the role of NO in plant growth, it is hypothesized whether or not foliar application of NO could be effective to improve growth under saline conditions. The main objective of current study was to observe NO induced modulation in growth and gas exchange attributes under saline conditions.

Materials and Methods

The present experiment was carry out to evaluate the interactive effect of exogenous application of NO and salt stress on wheat (*Triticumaestivum*L.). Single wheat cultivar S-24 was used in current study and experiment was performed in net house of Old Botanical Garden, Department of Botany. Seeds were obtained from Botany

Department, University of Agriculture, Faisalabad. Fifteen seeds were directly sown in each plastic pot containing 10 kg well washed sand and thinned 45 days old plants after germination up to six plants of equal size and Hoagland's nutrient solution of full strength was used for irrigation of the plants. Completely Randomized Design with four replications was applied. Salt stress was applied in Hoagland's nutrient solution through rooting medium after 58 days of sowing. Two salt levels [(control (0 mM) and 150 mM)] were used. Nitric oxide (NO) levels [Control (water spray), 0.05 mM, 0.10 mM and 0.15 mM] were applied as foliar spray after 64 days of sowing. Tween 20 at 0.1 % level was used as surfactant. After two weeks of NO application, the data for various attributes were recorded. Two plants from each replicate were harvested. After cutting them in shoot and root, their fresh biomass was recorded separately and means were used. Total leaf area per plant was calculated using following formula:

$$\text{Total leaf area per plant (cm}^2\text{)} = (\text{length} \times \text{width}) \times \text{Number of leaves} \times 0.75$$

0.75 = correction factor

Gas exchange characteristics: Various gas exchange attributes such as stomatal conductance, sub-stomatal conductance, net CO₂ assimilation rate and transpiration rate were measured on 3rd leaf from top. The readings were made from 11:30 am to 2:00 pm with the subsequent specification of the leaf chamber: leaf surface area 11.35 cm², ambient CO₂ level (Cref) 354.4 μmol mol⁻¹, leaf chamber temperature varied from 31.5 to 37.8°C, leaf chamber gas flow rate (v) 392.8 mL min⁻¹, molar flow of air per unit leaf area (Us) 404.84 mol m⁻² s⁻¹, ambient pressure (P) 99.2 kPa, water vapor pressure (e ref) into chamber ranged from 20.5 to 23.1 mbar and Qleaf was 1048 μmol m⁻² s⁻¹.

Photosystem II activity: Schreiber *et al.* (1986) and Genty *et al.* (1989) methods were used for the measurement of the photosystem II activity by Multi-Mode Chlorophyll Fluorometer (Model, OS5P Opti-Sciences, Inc. Winn Avenue Hudson, USA). Leaf samples were covered with the stopper for fifteen minutes because leaf must remained in darkness before the measurement of fluorescence performed. Four hundred μmol m⁻² s⁻¹ of actinic light while 8000 μmol m⁻² s⁻¹ of constant rhythm light were used. Parameters of the fluorescence photochemical quenching (*qP*), co-efficient of non-photochemical quenching (*qN*), electron transport ratio (*ETR*), non-photochemical quenching (*NPQ*) and efficiency of photosystem-II (*Fv/Fm*) were measured.

Statistical analysis: Data were recorded and analyses of variance for all the parameters were computed, using the COSTAT computer package (Cohort software Berkeley, California). The least significant differences between means were calculated (Snedecor & Cochran, 1989).

Results

Rooting medium salinity (150 mM) significantly decreased growth attributes like shoot and root fresh weights and leaf area per plant of wheat cultivar S-24, while foliar application of varying levels of nitric oxide NO were found to be effective in enhancing these growth parameters under both control and saline conditions (Table 1; Fig 1). However, NO-induced improvement of growth in wheat was more prominent under non-stressed conditions where as difference was slight under salt stress conditions. Among various levels of NO 0.10 mM was found to be most effective under non-stress conditions, while 0.05 mM under salt stressed conditions (Table 1; Fig 1).

Table 1. Mean squares from analyses of variance (ANOVA) of data for growth and physiological attributes of wheat (*Triticum aestivum* L.) plants foliar-sprayed with nitric oxide under stressed and non-stressed conditions.

Source of variance	df	Shoot f. wt.	Root f. wt.	Leaf area/plant	A	E
Salinity (S)	1	606.8***	6.561***	1505***	44.98***	0.058***
NO Treatment (NO)	3	41.96***	0.567**	4669.0**	4.482**	0.085***
S × NO	3	29.78***	0.560**	3543.0*	1.447ns	0.012**
Error	24	2.39	0.086	8579.3	0.678	0.002
Source of variance	df	<i>g_s</i>	<i>C_i</i>	<i>A/E</i>	<i>C_i/C_a</i>	<i>qN</i>
Salinity (S)	1	11755***	24.26ns	0.000ns	2.003ns	0.01ns
NO Treatment (NO)	3	4726.1***	758.1ns	0.116ns	1.846ns	0.009ns
S × NO	3	303.7ns	1169.7ns	0.056ns	0.394ns	0.003ns
Error	24	136.11	1040.2	0.472	2.799	0.005
Source of variance	df	<i>qP</i>	<i>NPQ</i>	<i>Fv/Fm</i>	<i>ETR</i>	
Salinity (S)	1	0.002ns	0.002ns	0.002ns	1.28ns	
NO Treatment (NO)	3	0.0023ns	0.008ns	0.000ns	3.127ns	
S × NO	3	0.003ns	0.013ns	0.0001ns	0.341ns	
Error	24	0.0024	0.009	0.001	4.122	

*, ** and *** = significant at 0.05, 0.01 and 0.001 levels respectively; ns = non-significant

df= degrees of freedom; A= net CO₂ assimilation rate; E= transpiration rate; *g_s*= stomatal conductance; *C_i*= sub-stomatal carbon dioxide concentration; *A/E*= water use efficiency; *qN*= co-efficient of non-photochemical quenching; *qP*= photochemical quenching; *NPQ*= non-photochemical quenching; *Fv/Fm*= maximum quantum yield of PS-II; *ETR*= electron transport rate

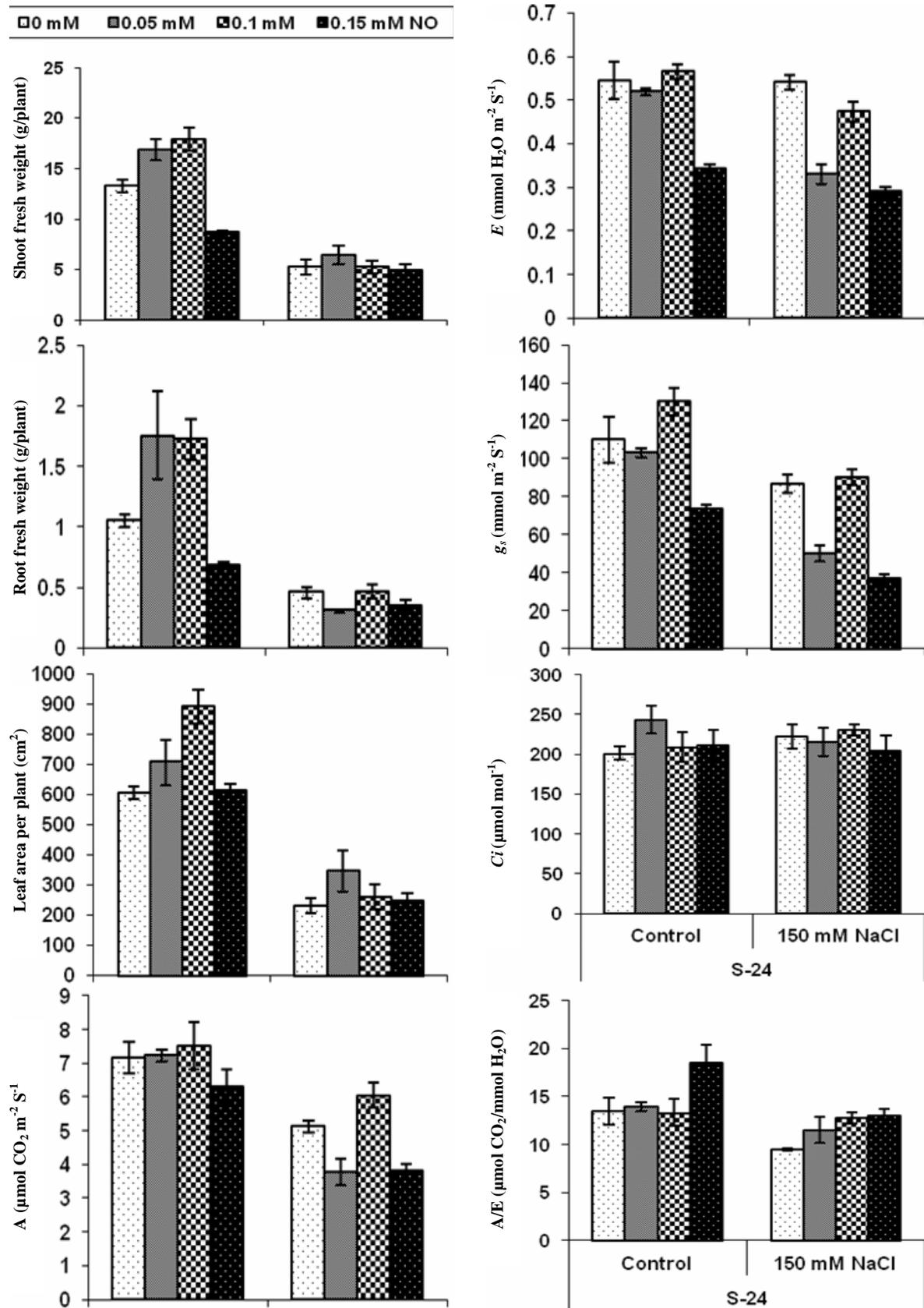


Fig. 1. Growth and gas exchange characteristics of wheat (*Triticum aestivum* L.) plants foliar-sprayed with nitric oxide under salt stressed and non-stressed conditions.

Net CO₂ assimilation rate of wheat was reduced significantly under saline conditions (Table 1; Fig 1). Application of NO level, 0.10 mM slightly increased photosynthetic rate while other NO levels decreased this attribute. Effect of NO was not prominent on photosynthetic rate when applied under non-saline conditions (Table 1; Fig 1). Data for stomatal conductance (g_s) and transpiration rate (E) revealed that salinity (150 mM NaCl) caused significant reduction in these attributes. Application of NO as foliar spray significantly decreased stomatal conductance (g_s) and transpiration rate (E) of wheat cultivar S-24 and maximum reduction was observed when NO was applied @ 0.15 mM under both non-stressed and salt stressed conditions (Table 1; Fig 1). In this study, both salinity stress (150 mM NaCl) and foliar application of different NO levels exerted a non-significant effect on sub-stomatal CO₂ concentrations (C_i), water use efficiency A/E and C_i/C_a ratio (Table 1; Figs. 1 & 2).

Chlorophyll fluorescence attributes like photochemical quenching (qP), co-efficient of non-photochemical quenching (qN), non-photochemical quenching (NPQ), electron transport ratio (ETR) and efficiency of photosystem-II (Fv/Fm) were not being affected by both salinity stress and foliar-applied different NO levels (Table 1; Fig. 2).

Discussion

In our experiment, salinity caused an adverse effect on shoot and root fresh weights and leaf area per plant. These results are in accordance with the some earlier studies in different cereal crops like wheat (Perveen *et al.*, 2011, 2012), rice (Arshadullah *et al.*, 2011), sorghum (Bashir *et al.*, 2011) and pearl millet (Hussain *et al.*, 2008). In this experiment sodium nitropruside was used as NO donor to study interactive effect of NO and salinity in wheat cultivar S-24. Our results showed that foliar spray of NO enhanced the salt stress tolerance of wheat (*Triticum aestivum* L.). Foliar application of NO increased shoot and root fresh weight and leaf area of wheat plants under non-saline conditions as compared to saline conditions suggested that NO is actively participating in the regulation of plant growth. This highly significant correlation showed that leaf area increased by application of NO which is responsible for increase in growth attributes. Previous studies on various crops like soybean, tomato, rice and maize have shown that foliar spray of NO increased the plant growth under salt stress conditions that might be due to high activities of antioxidant enzymes (Zhang *et al.*, 2004; Hu *et al.*, 2005; Farooq *et al.*, 2009; Wu *et al.*, 2011).

High NaCl levels reduced photosynthetic efficiency and eventually growth of plants was also affected (Munns, 2002, Narusaka *et al.*, 2003). The photosynthetic performance was investigated by the changes in gas exchange characteristics and photosystem II activity. Photosynthetic performance was decreased under salt stress which might be due to limitation in stomatal conductance and/or non-stomatal conductance (Debez *et al.*, 2008). Salinity can reduce net CO₂ assimilation rate due to stomatal closure, a cause for decrease in photosynthetic performance. In the current study, net CO₂ assimilation rate, transpiration rate and stomatal conductance

were reduced in wheat plants under saline conditions, however, the reduction in these attributes can be alleviated by foliar spray of NO. On the other hand, changes in sub-stomatal conductance were not connected with reduction in net CO₂ assimilation rate, transpiration rate and stomatal conductance. Photosynthetic rate (A) and stomatal conductance (g_s) increased by application of NO and highly significant correlation observed in our experiment between growth attributes and photosynthetic rate (shoot fresh weight and A ($r = 0.8196^{**}$), root fresh weight and A ($r = 0.8381^{***}$), leaf area and A ($r = 0.8083^{**}$) and between growth attributes and stomatal conductance (g_s) [(shoot f. wt. and g_s ($r = 0.7797^*$) and leaf area and g_s ($r = 0.7162^*$). There was a significant correlation ($r = 0.921^{***}$) between photosynthetic rate and stomatal conductance (g_s). This indicated that growth attributes are increased by application of NO due to increased photosynthetic rate and stomatal conductance (g_s). Increase in photosynthetic performance is a comprehensive outcome of increased CO₂ fixation, assimilation, transportation and light use efficiency (Wang *et al.*, 2010). NO reduces transpiration rate as well as stomatal conductance under saline conditions. Neill *et al.* (2008) suggested that foliar spray of NO induced stomatal closure and protects cells against stress injury.

The limitation of photochemical activity is one of the non-stomatal factors that caused decrease in photosynthesis (Souza *et al.*, 2004). In fact, chlorophyll fluorescence study gives information on the capability of a plant to bear environmental stresses and the degree to which those stresses injured the photosynthetic machinery (Maxwell & Johnson, 2000). The value of Fv/Fm is frequently used as a sign of stress injury or photoinhibition to the PS-II activity (Calatayud & Barreno, 2004). In the present study, salinity caused significant inhibition in PS-II activity by decreasing the quantum yield (Fv/Fm) of PS-II. It is investigated from the current study that less decrease in Fv/Fm ratio occurred under saline conditions that might be due to NO foliar spray. This suggested that photosystem II efficiency improvement by exogenous NO spray in stressed plants was linked with decrease in inhibition of PS-II efficiency. There are some contradictory evidences on effects of foliar applied NO on photosynthetic efficiency. Yang *et al.* (2001) suggested that NO treatment alone decreased photosystem II activity. Improved photosynthetic efficiency by foliar spray of NO may be due to improved gas exchange characteristics and PS-II activity when plants were subjected towards salt stress conditions like as observed in barley (Zhang *et al.*, 2006) and tomato (Wu *et al.*, 2011). It has been reported that NO play an important role in plant growth under salt stress (Wu *et al.*, 2007). However, in our experiment NO alone applied to wheat seedlings under non-saline conditions showed slight effect on photosynthetic efficiency.

In conclusion, salt stress exerted adverse effects on growth attributes of wheat plants. However, exogenous application of NO proved to be quite effective in enhancing stress tolerance of wheat plants by increasing growth (shoot and root fresh weights and leaf area per plant) and photosynthetic performance under saline conditions, while NO application did not alter efficiency of photosystem-II under both saline and non-saline condition.

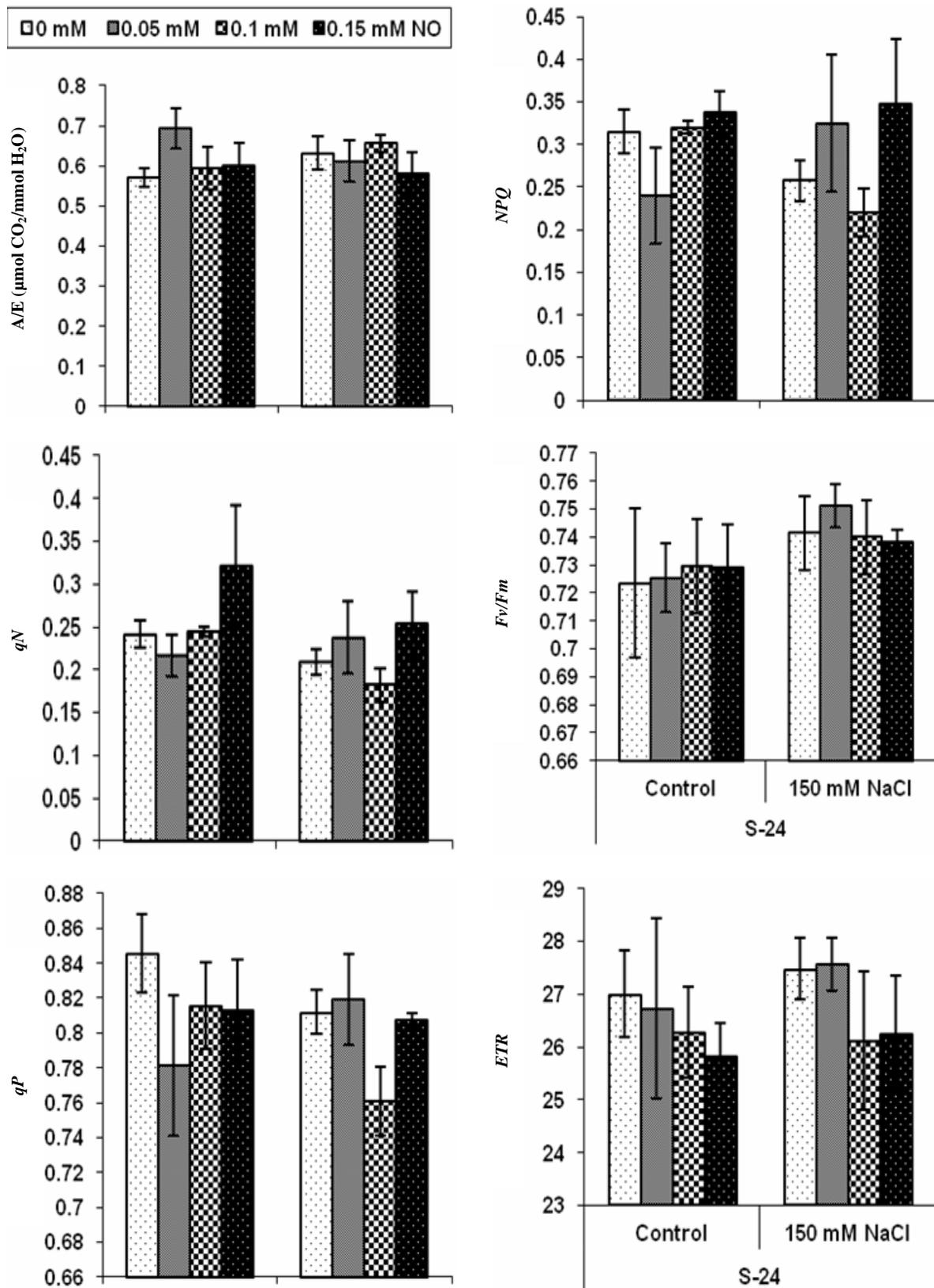


Fig. 2. C_i/C_a and chlorophyll fluorescence attributes of wheat (*Triticum aestivum* L.) plants, foliar-sprayed with nitric oxide under salt stressed and non-stressed conditions.

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(Received for publication 1 September 2012)