



Diurnal changes and effect of elevated CO₂ on gas exchange under individual and interactive salt and water stress in wheat (*Triticum aestivum*)

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ABSTRACT

To evaluate the individual and interactive effects of drought and salt stress on diurnal stomatal behaviour and gas exchange parameters, two salt tolerant (Kharchia 65, KRL 210) and two salt sensitive (HD 2851, HD 2009) wheat (*Triticum aestivum* L.) varieties were subjected to drought (25% and 50% water deficit) and salt (50 mM and 100 mM NaCl) stresses, imposed separately and in combination. Diurnal changes in photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rates (E) of wheat leaves were measured at 4 h intervals under field conditions, using an infrared open gas exchange system. Photosynthetic rate was also measured across a range of variable intercellular CO₂ concentrations (50 – 800 ppm). Kharchia 65 (salt tolerant) and KRL 210 (moderately salt tolerant) wheat varieties showed photosynthetic rate of 19.74 and 9.19 $\mu\text{mol}/\text{m}^2/\text{s}$, while HD 2009 and HD 2851 (salt sensitive) showed photosynthetic rate of -27.65 and -17.65 $\mu\text{mol}/\text{m}^2/\text{s}$ at 50 ppm intercellular CO₂ concentration which increased with increasing CO₂ concentration. Up to 200 ppm intercellular CO₂ concentration, these sensitive varieties showed negative P_n values. Diurnal photosynthetic activities of wheat varieties significantly differed under stressful environment but these differences could not be expressed in control conditions. P_n was low in the early morning, increasing with time and reached maximum ($\mu\text{mol}/\text{m}^2/\text{s}$) between 10:00 AM to 2:00 PM, thereafter, P_n decreased. Similar trends of increase and decrease were observed for g_s and E .

Key words: Diurnal changes, Elevated CO₂, Gas exchange attributes, Photosynthesis, Wheat varieties

World agriculture faces a challenging task to produce 70% more food for an additional 2.3 billion people by 2050 (FAO 2009). The lower agriculture crop productivity is mostly attributed to various abiotic stresses and is a major area of concern to cope with the increasing food requirements (Shankar and Venkateswarlu 2011). Irrigation water availability may prove to be a critical constraint to primary productivity under future scenarios of more arid climate due to global environmental change (Fischer *et al.* 2001). Increasing demand for water and the scarcity of water sources are creating pressure to improve water use productivity in agriculture (Feres and Soriano 2006). The need to improve our understanding of the dynamics of water use by plants is well recognized in order to develop efficient irrigation practices (Fernandez *et al.* 2008).

Among various abiotic stresses, lack of water resources and underground saline water are among the most important growth-restricting factors and decreased yield potential for plants species in arid and semi-arid regions of the world

(Levitt 1980). Globally more than 900 million ha of land, approx. 20% of the total agricultural land, are affected by salt, accounting for more than 6% of the world's total land area. Salt-affected soils (SAS) are widespread in irrigated arid and semi-arid regions of the world where irrigation is essential to increase agricultural production to satisfy food requirements. In India, SAS occupy an area of about 6.73 million ha of which saline and sodic soils constitute roughly 40% and 60 %, respectively (Sharma and Singh 2015). NaCl is the predominant salt causing salinization, and it is unsurprising that plants have evolved mechanisms to regulate its accumulation (Munns and Tester 2008). In agriculture, lack of precipitation, high rate of evapo-transpiration and unsustainable use of water resources cause drought and salinity problems in semi-arid and arid regions restricting agriculture productivity. Of these multitude abiotic stresses, salinity and drought are the most important ones adversely affecting plant growth and yield (Kumar *et al.* 2016b, 2017).

Wheat (*Triticum aestivum* L.) is one of the main cereal crops in the world together with maize and rice and is significant staple food for majority of the world population (Boyer 1982). Rice-wheat cropping system accounts for about one-fourth of total food grain production of South-East Asia and about 31% of the total food grain production of India (Prasad 2005). In most of developing

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countries, wheat is mainly grown in rainfed lands without supplementary irrigation. About 37% of land area in these countries consists of semiarid environments in which available moisture constitutes a primary constraint to wheat production (Abdul *et al.* 2001). Photosynthesis, as the primary process by which plants use light energy to drive the synthesis of organic compounds, is pivotal for plant growth. The reduction in crop production observed in various plant species exposed to abiotic stresses is linked to the decline in photosynthesis (Chaves *et al.* 2009, Kumar *et al.* 2018). Diurnal trend in gas exchange is often recognized as one of the best indicators in reflecting the ability of plants to maintain their photosynthetic apparatus to readily respond to environmental conditions.

MATERIALS AND METHODS

Experimental details

Present study was performed in porcelain pots in net house of Research Institute, Karnal, in the winter (*Rabi*) season of 2014-15. The maximum temperature range of 17.1–34.1°C and minimum of 6.7–16.3°C was observed during crop season. Annual rainfall ranges between 700 and 800 mm. To study the diurnal response of wheat under salinity and drought (water deficit; WD) stresses, an experiment was conducted in randomized complete block design in 20 kg capacity porcelain pots filled with soil with 5 replications. The net house was covered with a high quality polythene sheet to avoid the entry of rain water and maintain the desired salinity and water deficit stress in the pots as per treatments. Surface sterilized seeds of Kharchia 65, KRL 210, HD 2851 and HD 2009 were sown. Plants were supplied with Hoagland nutrient solution before imposition of stress. Osmotic stress was imposed 3 weeks after sowing by application of 50 and 100 mM concentration of sodium chloride (NaCl) solution in water and drought stress by withholding irrigation water. Drought stress was created by gradually decreasing the water supply to the pots. Drought treatments were imposed by 100% saturation of soil in pots first and then withholding irrigation supply till the depletion of water to 25 and 50% in soil. Diurnal responses of gas exchange parameter, i.e. photosynthetic rate (Pn), transpiration (E), and stomatal conductance (gS) were measured with an infrared open gas exchange system at 15 days after imposition of osmotic and drought stress treatments. All the data were subjected to variance analysis using the SAS (Version 9.3, SAS Institute Inc., Cary, NC, USA). Least significant difference test was applied at 5% probability level to compare the mean differences.

Treatments

Water deficit: 20 kg capacity porcelain pots were filled with 16 kg soil (field capacity 28% v/v) at bulk density of 1.45 g/cc which have porosity approximately 40 per cent. Initially 6.5 l water (up to field capacity) was given in the pots at weekly interval and evaporation was noted through pan. During the entire study period pan evaporation was

2-3 mm/day, i.e. 21 mm/week. On this basis, 25 and 50% water deficit treatments were induced.

Salt treatment: Based on the water requirement of the pots, 50 mM and 100 mM NaCl concentrations were given to pots at regular weekly intervals. At harvest, 50 mM and 100 mM NaCl concentration pots had mean salinity level about 4.2 dS/m and 9.1 dS/m, respectively.

RESULTS AND DISCUSSION

Photosynthetic rates were measured across a range of variable intercellular CO₂ concentrations (50–800 ppm) in four wheat varieties. The correlation between photosynthetic rate and intercellular CO₂ concentration for Kharchia 65 was significant with R² value of 0.857. Photosynthetic rates increased with increasing intercellular CO₂ concentrations in all the four wheat varieties.

The tolerant wheat variety (Kharchia 65) showed photosynthetic rate of 19.74 µmol/m²/s at 50 ppm intercellular CO₂ concentration and increased with increasing CO₂ concentration to a maximum (34.74 µmol/m²/s) at 700 ppm intercellular CO₂ concentration (Fig 1A). Similar increasing trend was observed for moderately salt tolerant variety KRL 210, i.e. 9.19 µmol/m²/s at 50 ppm intercellular CO₂ concentration and 36.0 µmol/m²/s at 700 ppm (Fig 1C) whereas the two sensitive wheat varieties showed a different trend at variable CO₂ concentrations (Fig 1B and 1D). At 50 ppm intercellular CO₂ concentration, HD 2009 showed photosynthetic rate of -27.65 µmol/m²/s which showed progressive increase with increase in CO₂ concentration. Up to 200 ppm intercellular CO₂ concentration, it showed negative photosynthetic rate, i.e. -1.72 µmol/m²/s, whereas the rate increased with increasing CO₂ concentrations and reached to their maximum (26.97 µmol/m²/s) at 700 ppm intercellular CO₂ concentration (Fig 1D). The association between photosynthetic rate and intercellular CO₂ concentration is significant with R² value of 0.898. Similar trend was shown by variety HD 2851 (Fig 1B). Short-term CO₂ enrichment stimulates the rate of photosynthesis (Makino and Mae 1999). This is a common response in C₃ plants that continued to respond photo-synthetically to ongoing increases in atmospheric carbon dioxide and leaf photosynthetic rate measured at the ambient CO₂ concentration was significantly increased at elevated concentration relative to ambient CO₂ concentration (Campbell *et al.* 1988, Ziska and Bunce 1997).

The wheat varieties showed diurnal patterns in leaf gas-exchange with peak rates of photosynthesis around midday, and at night. Diurnal changes in net photosynthetic rate (Pn), stomatal conductance (gS) and transpiration rate (E) were measured in differentially treated (drought and salt) fully expanded young leaves of wheat varieties. Unstressed plants of all the varieties showed mostly higher Pn throughout the daytime. Pn was low in the early morning, increasing with time and reaching a maximum (33-37 µmol/m²/s) between 10:00 AM to 02:00 PM and decreased thereafter (Fig 2). The midday depression of photosynthesis is a common phenomenon for many C₃

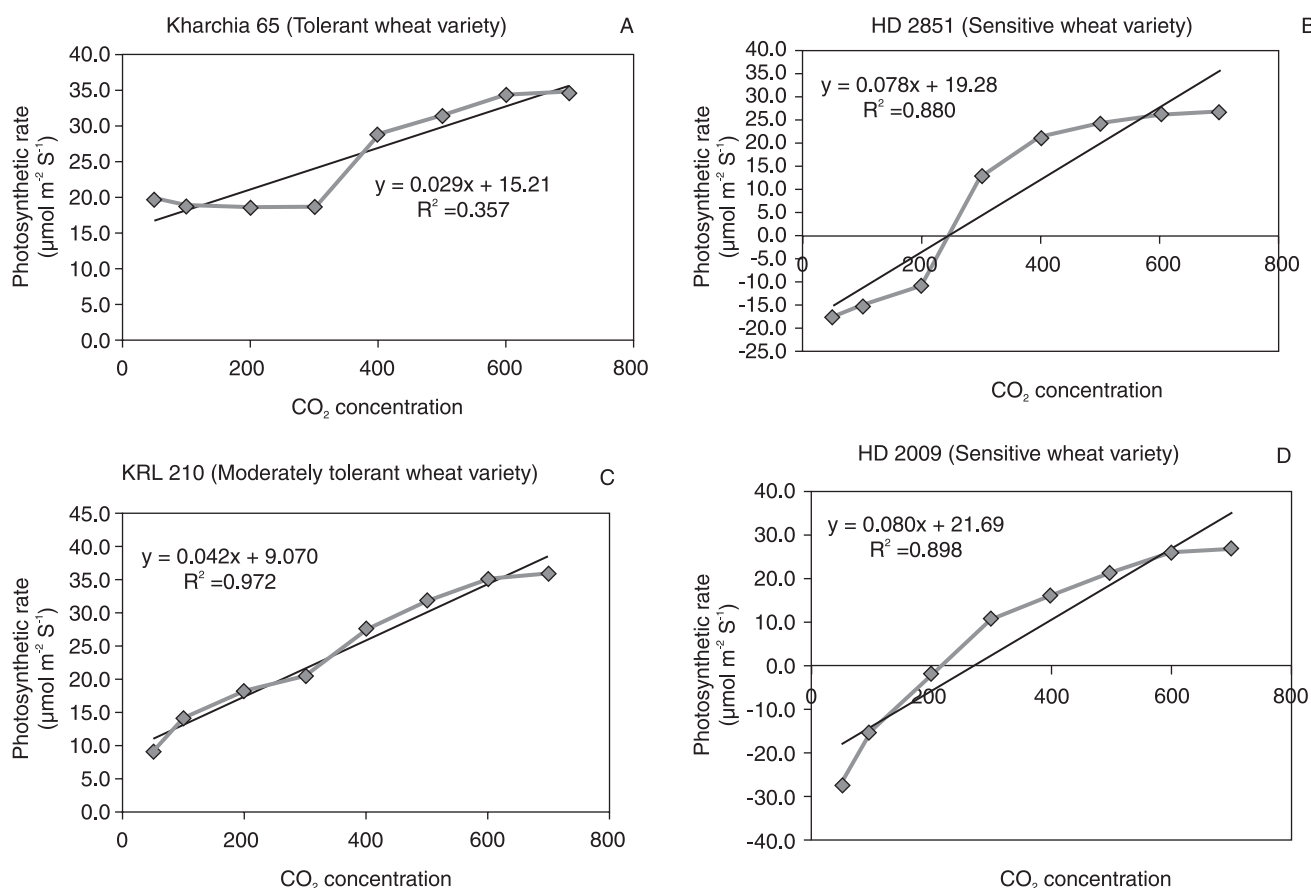


Fig 1 Effect of variable intercellular CO_2 concentration on photosynthetic rate in wheat; (A) Kharchia 65, (B) HD 2851, (C) KRL 210 and (D) HD 2009.

plants. Similar trend of increase and decrease was observed for g_s (stomatal conductance, Fig 3) and E (Transpiration rate, Fig 4). Water-deficit caused significant decreases in P_n , g_s and E regardless of time of day. Under common field conditions, the assimilatory apparatus are exposed to variable intensities of light, temperature and humidity that may result in a typical midday depression of CO_2 assimilation or photoinhibition of photosynthesis (Hirasawa and Hsiao 1999). The midday depression in P_n could be attributed to the decreased photosynthetic activity of mesophyll cells, rather than the closure of stomata (Yang and Chen 2015). In general, the gas exchange pattern was controlled by g_s . Maximum g_s occurred in the early morning before dawn, this is also the time with the greatest P_n and E for the 24 h period. As the early morning progresses into midday g_s decreases; however, g_s never equals to zero and as a result CO_2 and water are lost from the stem to the atmosphere. After sunset, g_s increases along with increasing P_n and E until they reach maximum values before dawn. In Kharchia 65, maximum P_n of $37.52 \mu\text{mol/m}^2/\text{s}$ was recorded between 10 AM to 2 PM, whereas the minimum P_n ($13.63 \mu\text{mol/m}^2/\text{s}$) was recorded between 6 PM to 10 PM, respectively (Fig 2A), and the corresponding values of maximum and minimum g_s were 0.667 and $0.385 \text{ mmol/m}^2/\text{s}$ (Fig 3A), and similarly the corresponding values of maximum E was $3.96 \text{ mmol/m}^2/\text{s}$ at 10 AM to 2 PM and minimum E was $1.07 \text{ mmol/m}^2/\text{s}$ at 2 AM to 6 AM under control conditions

(Fig 4A). However, these gas exchanges attribute decreased with increasing stress intensity. The similar diurnal changes of P_n and g_s suggest that both processes are dependent, with the high photosynthetic performance in the morning being supported by high stomatal aperture (Ribeiro *et al.* 2009).

Similar trend was observed in KRL 210 and maximum P_n of 33.72 and $33.77 \mu\text{mol/m}^2/\text{s}$ was recorded between 10 AM to 2 PM and 2 PM to 6 PM (Fig 2B), respectively. The highest rate of stomatal conductance (g_s) was recorded between 2 PM to 6 PM and the minimum rate between 6 PM to 10 PM (Fig 3B), whereas maximum transpiration of $3.95 \text{ mmol/m}^2/\text{s}$ occurred between 10 AM to 2 PM and minimum $1.31 \text{ mmol/m}^2/\text{s}$ between 2 AM to 6 AM (Fig 4B). Exposure of salt stress and water-deficit stress (osmotically stressed seedlings) caused sharp reduction in P_n , g_s and E values during diurnal observations (Fig 2B, 3B and 4B). Plants regulate their diurnal water status at a favourable level by the control of stomatal aperture. Stomatal closure helps to maintain high leaf water content and thereby a higher leaf water potential, which leads to a reduction in photosynthetic activity. Stomatal closure reduces CO_2 entry into leaves which reduces the intercellular CO_2 concentration and lowers C fixation. This causes an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, and leads to increased susceptibility to photo-damage (He *et al.* 1995, Flagella *et al.* 1998). Similar results were observed for P_n , g_s and

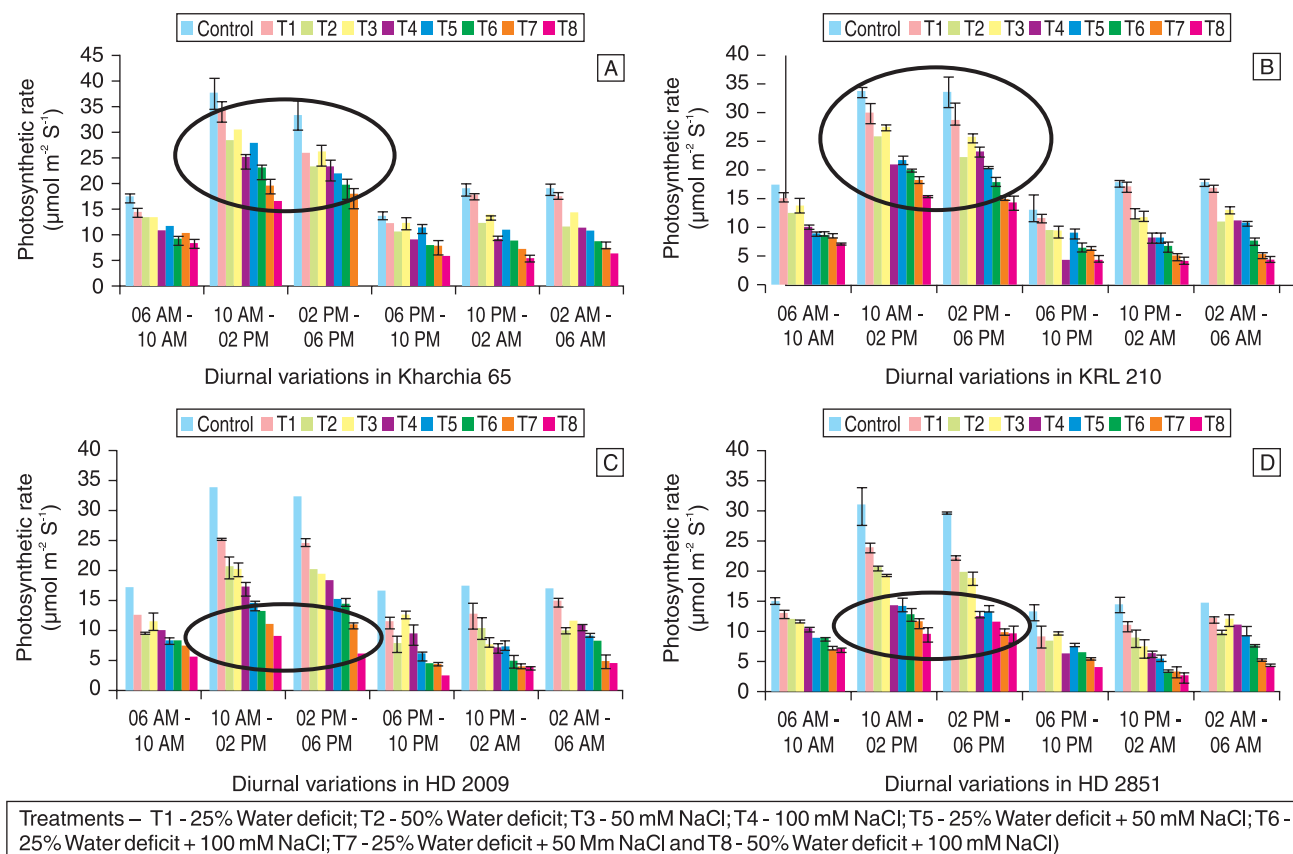


Fig 2 Effect of individual and interactive salinity and drought stresses on diurnal change in photosynthetic rate (Pn) in wheat.

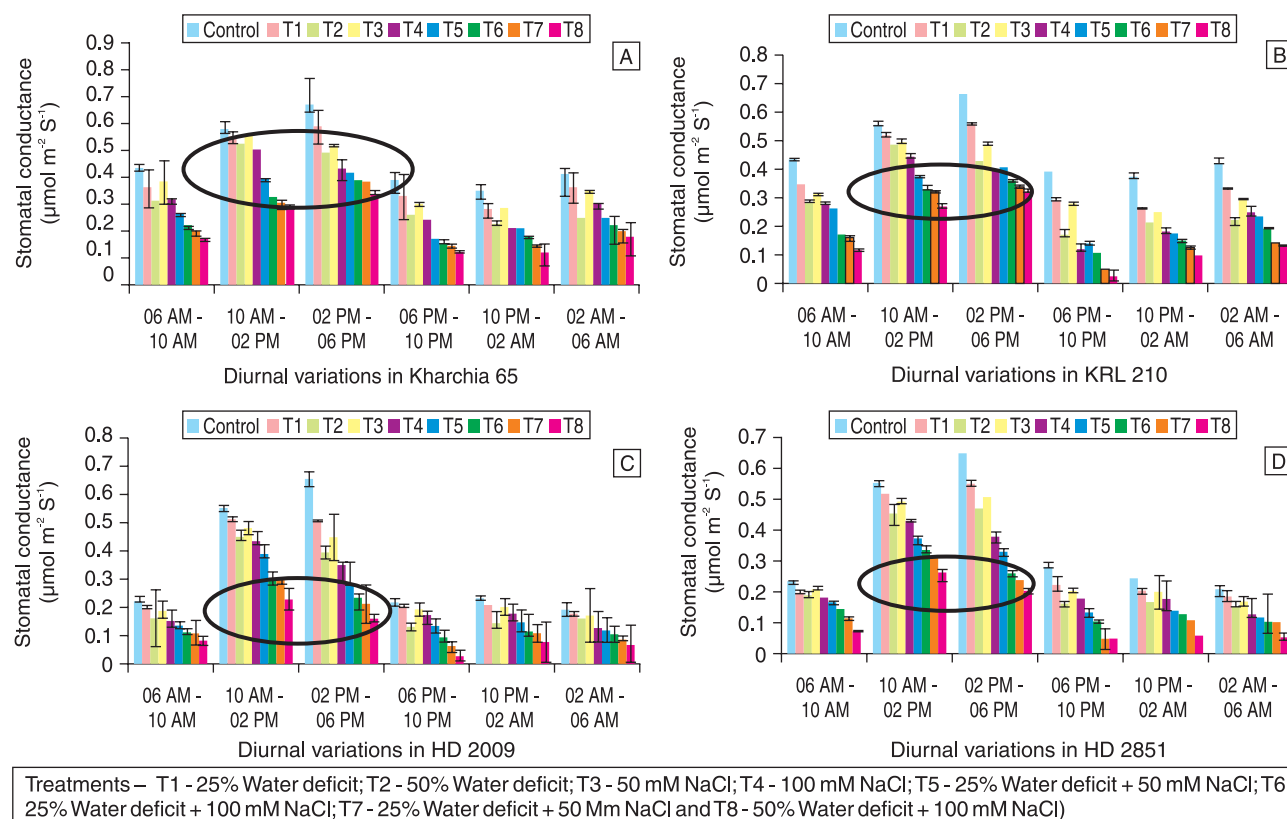


Fig 3 Effect of individual and interactive salinity and drought stresses on diurnal change in stomatal conductance (gS) in wheat.

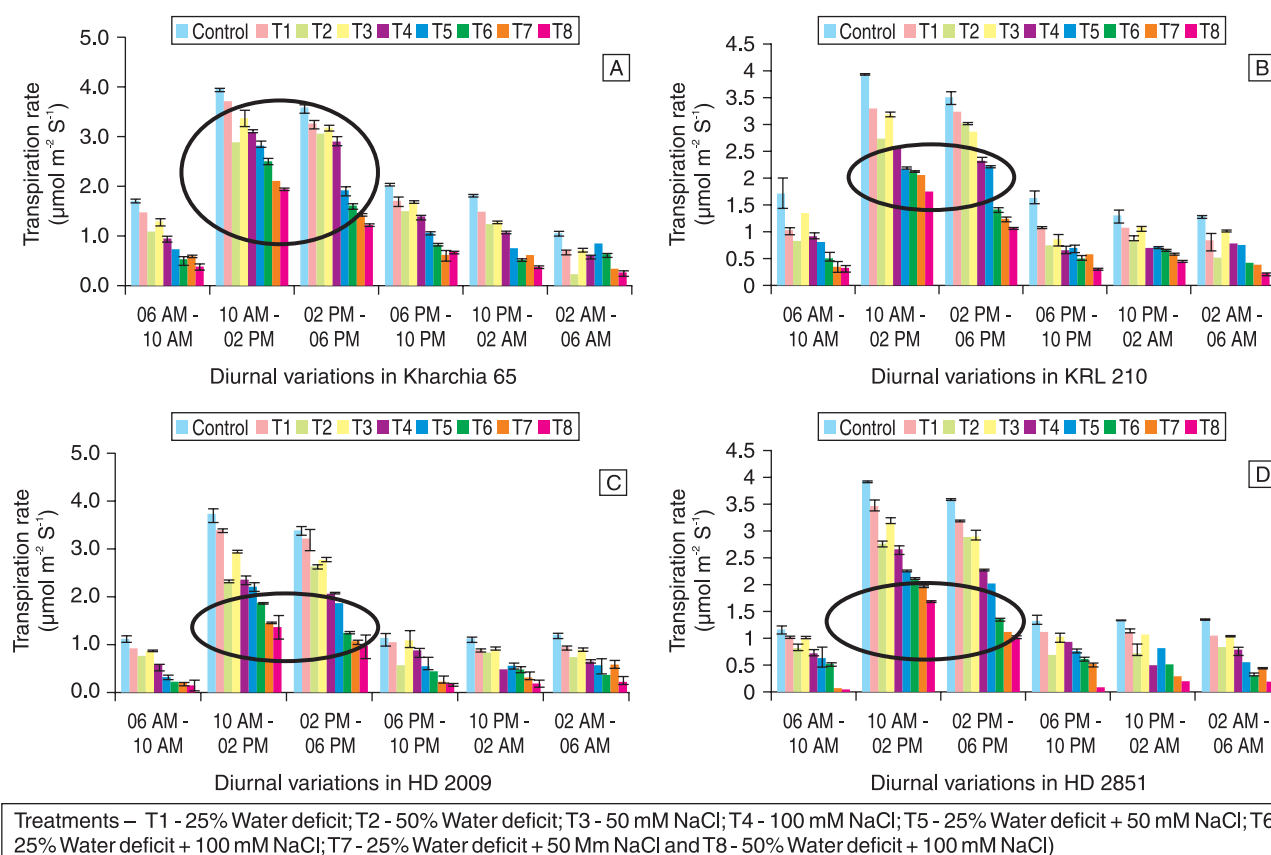


Fig 4 Effect of individual and interactive salinity and drought stresses on diurnal change in transpiration rate (E) in wheat.

E in HD 2009 (Fig 2C, 3C and 4C). The high correlation between P_n and g_s suggests that photosynthesis depends largely on g_s (Gong *et al.* 2005, Hattori *et al.* 2005). Similar trend of diurnal courses of P_n and g_s between well-watered and water-stressed plants, pointing that both P_n and g_s reached the highest values in the early morning (after 9 AM) regardless of plant water status. Such reductions in the net photosynthesis (P_n), transpiration rate (E) and stomatal conductance (g_s) may attribute to a reduced efficiency of ribulose-1, 5-bisphosphate (RuBP) carboxylase (Kumar *et al.* 2016a) to a reduction in RuBP regeneration capacity, or to the sensitivity of PS2 to NaCl (Ball and Anderson 1986, Riveli *et al.* 2002). Salt-induced photosynthesis inhibition was coincided with a strong decrease in the transpiration rate (E).

Among the gas exchange characteristics, photosynthetic activities of wheat varieties significantly differed but these differences could not be expressed in control conditions. Maximal stress of 50% WD + 100 mM NaCl in HD 2851, induced reduction in photosynthetic rate and the maximum rate of $14.4 \mu\text{mol}/\text{m}^2/\text{s}$ was noted between 10 AM to 2 PM and minimum rate ($2.39 \mu\text{mol}/\text{m}^2/\text{s}$) between 6 PM to 10 PM (Fig 2D). Monitoring gas exchange in plants is a common approach, with stomatal conductance (g_s) reported as one of the most sensitive indicator of stress. Maximum stomatal conductance of $0.648 \text{ mmol}/\text{m}^2/\text{s}$ was observed between 2 PM to 6 PM, whereas minimum stomatal rate ($0.211 \text{ mmol}/\text{m}^2/\text{s}$) was noted between 2 AM to 6 AM

under control conditions (Fig 3D). Our results revealed that diurnally gas exchange parameters declined as stress conditions prevailed, i.e. reduced photosynthesis, minimum transpiration, high stomatal resistance and minimum internal CO_2 concentration. Perturbation in different gas exchange attributes could be associated with decreased utilization efficiency of light, photo-inhibition of photosystem (Chedila *et al.* 2007) or might be due to increased reactive oxygen species (ROS) production, which lead to decreases in plant photosynthetic capacity. There is a strong link between photosynthesis and stomatal conductance, stomatal closure reduced photosynthetic activity and transpiration rate could be considered as adaptive mechanisms to cope with excessive salt, rather than merely a negative consequence of it (Flanagan and Jefferies 1988). Stomatal closure and the resulting CO_2 deficit in the chloroplasts is the main cause of decreased photosynthesis under mild and moderate stresses (Flexas and Medrano 2002). Although reduced stomatal conductance imposed by high salinity restricts CO_2 diffusion, it might elevated the CO_2 partial pressure across the stomata that is utilized by leaves to maintain a consistently moderate rate of photosynthesis throughout the day, thus avoiding CO_2 starvation and photo-inhibition.

Transpiration increased rapidly following sunup, presumably because of stomatal opening, transpiration (E) was maximum ($3.89 \text{ mmol}/\text{m}^2/\text{s}$) between 10 AM to 2 PM and minimum ($1.16 \text{ mmol}/\text{m}^2/\text{s}$) between 6 AM to 10 AM under control condition (Fig 4D). Stress condition of salt

and drought alone and in combination caused reduction in E and found minimum at 50 % WD + 100 mM NaCl. The corresponding maximum value (1.68 mmol/m²/s) was recorded between 10 AM to 2 PM and minimum (0.026 mmol/m²/s) between 6 AM to 10 AM (Fig 4D). The variation of photosynthetic rate is dependent on environmental conditions. Depression in photosynthetic rate at midday was mostly attributed to stomatal limitation since the reduction in photosynthetic rate was followed by the significant reduction in stomatal conductance.

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