

Full Length Research Paper

Effects of different levels of sodium chloride and photosynthetic photon flux density on some physiological traits in two wheat cultivars

H. Sadeghi^{1*} and M. J. Nazemosadat²

¹Assistant Professor, College of Agriculture, Shiraz University, Shiraz, Iran.

²Professor, College of Agriculture, Shiraz University, Shiraz, Iran

Accepted 12 January, 2011

In most southern provinces of Iran, soil salinity is a growing problem particularly in irrigated agricultural areas and has been found to reduce wheat yields. In order to investigate the effects of various levels of NaCl on some physiological characteristics of two wheat (*Triticum aestivum* L.) cultivars, an experiment was conducted with four replications in a controlled environment of the greenhouse during 2006 to 2007. Salinity levels were 0, 8 and 12 dS m⁻¹ and wheat cultivars include: Kavir (salt tolerant variety) and Shiraz (salt sensitive variety). The parameters which have been measured include : photosynthetic photon flux density (PPFD) incident on leaf surface, photosynthesis rate (A), sub - stomatal CO₂ (Ci), transpiration rate(E) and stomatal conductance of CO₂ (gs). These parameters were measured at stem elongation and flowering stages of wheat growth by infrared gas analyzer (IRGA). The results showed that PPFD was increased between 8 to 14 O'clock. At both stem elongation and anthesis stages photosynthesis rate, transpiration, stomatal conductance and intercellular CO₂ were consistently increased between 8 to 12 O'clock. However, the aforementioned traits were declined from 12 to 14 O'clock due to increasing of leaf temperature. Decrease in photosynthesis in saline conditions was attributed to excess in Na⁺ and K⁺ ions. Increasing of these ions in vacuole is leading to an increase in cytoplasm and declining photosynthesis and processes. A substantial reduction in photosynthesis, stomatal conductance and transpiration was detected of exposure to salt stress. In general photosynthesis rate, transpiration, stomatal conductance and Intercellular CO₂ of salt tolerant variety (Kavir) was found to be higher than the salt sensitive variety (Shiraz). Thus, it appeared that less adverse effect of salinity on Kavir cultivar may make it more suitable for growth in saline soils found in the investigation.

Key words: Wheat, sodium chloride, photosynthesis, transpiration and stomatal conductance.

INTRODUCTION

Salinity is a growing problem particularly in irrigated agricultural areas with rising water tables, poor water quality and/or deficient soil drainage. Soil salinity has been found to reduce wheat yields usually when values of electrical conductivity are above 6 deci siments per meter (dS/m) throughout the root zone. Salinity is known to inhibit photosynthesis in a number of plant species (Longstreth and Nobel, 1979; Flowers and Yeo, 1981; Yeo et al., 1985; Dionisio-Sese and Tobita, 2000).

Possible reasons for this include stomatal closure, feedback inhibition due to reduced sink activity, decreased efficiency of Rubisco, displacement of essential cations from the endo-membrane structure (leading to changes in permeability), and swelling and disorganization of the grana (Flowers and Yeo, 1981), or due to the direct effects of salt on stomatal conductance via a reduction in guard cell turgor and intercellular CO₂ partial pressure (Dionisio-Sese and Tobita, 2000). Salinity imposes both ionic and osmotic stresses on plants (Munns et al., 2006), and salt exclusion from photosynthetic tissues was considered an important mechanism associated with salt tolerance in monocots

*Corresponding author. E-mail: sadeghih@shirazu.ac.ir.

Table 1. Soil properties (0-30 cm) before plant sowing.

Year	OC (%)	pH	Sand (%)	Silt (%)	Clay (%)	Soil texture	EC (dSm ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Total N (%)
2005-06	0.83	7	7	66.7	26.3	Silty loam	0.05	16.5	476	0.08

(Yeo et al., 1990; Moradi et al., 2003; Davenport et al., 2005). Stomatal closure is often a rapid initial response to salt stress. There was a mean reduction in yield of wheat (12.6%) due to salt stress (Yildirim and Bahar, 2010).

In wheat, James et al. (2002) showed that a stress-induced reduction in stomatal conductance was seen when the leaf emerged, but after some time there was a further decline, probably caused by salt toxicity, as the concentrations of Na⁺ and Cl⁻ in the leaf tissue increase further. Dionisio-Sese and Tobita (2000) reported that the net photosynthetic rate, measured in terms of CO₂ assimilation of the youngest fully expanded leaf of four rice cultivars, declined with increasing level of salinity stress. They suggested that this might be due to a direct effect of salt on stomatal resistance via a reduction in guard cell turgor. Wheat is a moderately salt-tolerant crop (Maas and Hoffman, 1977). Most crops are relatively tolerant of salt stress during germination, active tillering and towards maturity, and are sensitive during the early growth and reproductive stages (Pearson and Bernstein, 1959; Zheng et al., 2001). Salinity tolerance at the early growth and reproductive stages is only weakly associated (Moradi et al., 2003), hence, the discovery of contributing traits at both stages is essential for developing resilient salt-tolerant cultivars. The physiological bases of salt tolerance during the early growth and anthesis stage are fairly established, key traits include salt exclusion, compartmentation of ions in structural and older tissues, vigorous growth and higher tissue tolerance (Yeo and Flowers, 1986; Yeo et al., 1990; Peng and Ismail, 2004), nonetheless, little is known about the important mechanisms associated with tolerance during reproduction.

Moradi and Ismail (2007) showed that rice (*Oryza sativa*) is sensitive during the seedling and reproductive stages to salinity, but little information about the effects of salinity during stem elongation and flowering stages of wheat growth is available. In this study, the effects of salinity on some physiological traits such as photosynthesis (A), sub-stomatal CO₂ (Ci), transpiration rate (E) and stomatal conductance of CO₂ (gs) were investigated during the stem elongation and the anthesis stages. The two new cultivars of wheat used in the present study, Shiraz and Kavir, that Kavir is an improved hybrid recommended for salinity areas in most southern provinces of Iran. However, the salt tolerance mechanisms of these cultivars have not been studied. The objective of this research was to quantify some physiological characteristics of the two wheat cultivars in relation to various concentrations of NaCl. The experiment was carried out in the Fars province, one of the main wheat-growing areas in southern Iran, with

more than 630,000 ha wheat grown as nearly continuous cropping.

MATERIALS AND METHODS

Site, treatment application and data collection

This experiment was conducted to evaluate the effects of sodium chloride with three levels of salinity: 0, 8 and 12 dS m⁻¹ (at 12 dS m⁻¹ due to NaCl excess, no plant grew) on two wheat cultivars (Kavir is a relatively salt tolerant genotype while Shiraz is relatively sensitive to salt). The desired salinity levels were developed by mixing the required amount of NaCl in soil before filling the pots (0 and 4.32 g/kg soil). The wheat crop was sown on 17 November 2006 and harvested on 29 April 2007. The experiment was carried out in a greenhouse at the college of agriculture, Shiraz University, Shiraz Iran (52°46'E, 29°50'N, altitude 1810 m asl), 12 km north of Shiraz, on a fine mixed, mesic typic Calcixerpets soil with air temperature in the range of about 25 to 30°C, and was conducted using as a factorial experiment arranged in a randomized complete block design with four replication. Soil properties, are shown in Table 1. Pre-germinated seeds were sown in 5 L perforated plastic pots filled with fertilized (50 N, 25 P and 25 K mg kg⁻¹) and were kept in concrete tanks filled with tap water according to Maas and Grieve (1990). The level of water was maintained at 3 cm below the soil surface for 2 days. Ten seeds of each of the two cultivars were sown in each pot, thinned to five seedlings. The pots were kept flooded thereafter for the duration of the experiment.

Gas exchange measurements

During the vegetative stage, gas exchange measurements were conducted on the youngest fully expanded leaf at stem elongation stage on two wheat cultivars. Net CO₂ assimilation, stomatal conductance, internal CO₂ concentration (Ci) and transpiration rate were assessed on intact leaves using the LiCor 6400 gas exchange system (Lincoln, NE, USA). During the reproductive stage the same parameters were measured at flowering (65 to 70 days after sowing) on flag leaves after 1 to 2 h of acclimation in a growth cabinet, under a light intensity of about 1000 μmol m⁻² s⁻¹, relative humidity of 70% and 29°C to ensure measurements under stable conditions.

Statistical analysis

Statistical analysis was performed for each parameter studied based on a randomized complete block design model with four replications. Means were separated by T-test at p ≤ 5 and p ≤ 1 (Table 2).

RESULTS AND DISCUSSION

The results showed that PPFD was increased between 8

Table 2. Means differences (T-test).

Traits	Kavir at anthesis stage	Shiraz at anthesis stage	Kavir at stem elongation stage	Shiraz at stem elongation stage	DA ⁺	DAC ⁺	DAS ⁺	DAB ⁺	DABC ⁺	DABS ⁺
PPFD	0.012 ^{ns}	0.002 ^{ns}	0.0160 ^{ns}	0.021 ^{ns}	-4.711 ^{**}	-3.00 [*]	-3.2 [*]	-5.16 ^{**}	-3.87 [*]	-3.00 [*]
T	-0.52 ^{ns}	-1.02 ^{ns}	-1.04 ^{ns}	-2.11 ^{ns}	-3.5 ^{**}	-2.11 ^{ns}	-5.91 ^{**}	-4.58 ^{**}	-7.07 ^{**}	-8.61 ^{**}
A	0.79 ^{ns}	3.594 [*]	0.479 ^{ns}	1.87 ^{ns}	1.21 ^{ns}	-1.89 ^{ns}	4.61 [*]	0.305 ^{ns}	-1.91 ^{ns}	* 3.91
E	1.18 ^{ns}	8.46 ^{**}	0.317 ^{ns}	7.44 ^{**}	2.33 ^{ns}	-7.00 ^{**}	11.4 ^{**}	2.11 ^{ns}	-3.4 [*]	8.19 ^{**}
Ci	0.359 ^{ns}	6.39 ^{**}	0.288 ^{ns}	6.42 ^{**}	2.43 [*]	-13.00 ^{**}	8.81 ^{**}	2.32 [*]	-10.7 ^{**}	7.01 ^{**}
gs	0.163 ^{ns}	3.802 ^{**}	0.301 ^{ns}	4.28 [*]	2.04 ^{ns}	-2.78 ^{ns}	4.625 [*]	1.98 ^{ns}	-5.00 [*]	4.19 [*]

Photosynthesis (A), sub - stomatal CO₂ (Ci), transpiration rate (E) and stomatal conductance of CO₂ (gs), photosynthetic photon flux density (PPFD), T: leaf temperature. DA⁺: differences between two cultivars at anthesis stage; DAC⁺: differences between two cultivars at anthesis stage in controls; DAS⁺: differences between two cultivars in saline conditions; DAB⁺: Differences between two cultivars stem elongation; DABC⁺: differences between two cultivars stem elongation in controls; DABS⁺: differences between two cultivars stem elongation in saline conditions; *, ** significant at P < 0.05 and 0.01, respectively, ns, non-significant.

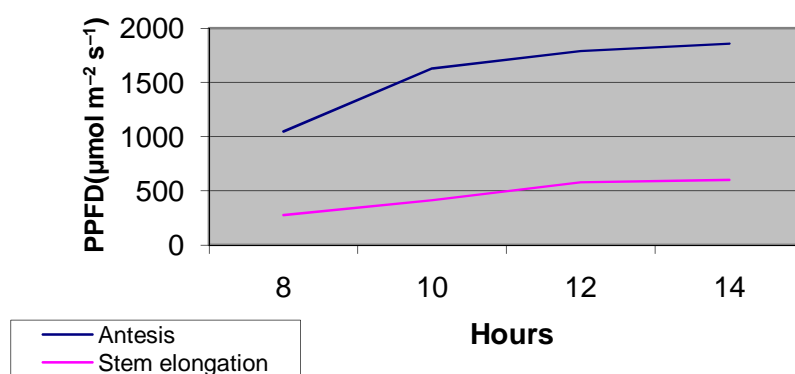


Figure 1. Photosynthetic photon flux density (PPFD) at the anthesis and stem elongation stages of wheat growth.

to 14 O'clock at both stem elongation and anthesis stages. PPFD at the anthesis stage was significantly higher than the stem elongation stages of the two wheat cultivars which are attributed principally to growth season differences (Figure 1). James et al. (2002) showed that the efficiency of PSII photochemistry in Line 455 (relatively salt tolerant genotype) was unaffected throughout. However, in Wollaroi (relatively sensitive to salt), the potential and actual quantum yield of PSII photochemistry began to decline as the leaf aged and the thermal energy dissipation of excess light energy (NPQ) increased and growth of Wollaroi was less than Line 455. Photosynthesis rate from 8 to 12 O'clock increased at both stem elongation and anthesis stages due to increasing PPFD and then decreased from 12 to 14 O'clock due to increasing temperature, transpiration and stomatal resistance. Photosynthesis rate at anthesis stage was significantly higher than the stem elongation stage of the two wheat growth which is attributed principally to increasing PPFD. Gas exchange attributes were significantly reduced by salt stress. Photosynthesis

rate, of salt tolerant variety (Kavir) was found to be higher than salt sensitive variety (Shiraz) (Figure 2). The salt-tolerant cultivar (Kavir) seem to have better control over their stomata and maintained lower transpiration rates in the short term due to salt stress, probably to limit damaging effects of the influx of large quantities of salt and to allow further acclimation. James et al. (2002) observed a large decrease in stomatal conductance of two contrasting wheat genotypes under salinity, which was not associated with poor water relations, but presumably due to 'root signals' as the leaf turgor of both genotypes did not change under stress. The results showed that transpiration from 8 to 12 O'clock increased at both stem elongation and anthesis stages and then decreased from 12 to 14 O'clock. Transpiration rate at the anthesis stage was significantly higher than the stem elongation stage of the two wheat cultivars which are attributed principally to increasing radiation use efficiency and dry matter assimilation at the anthesis stage (Figure 3).

The observed decrease reduction in transpiration rate might be among the important adaptive mechanisms of

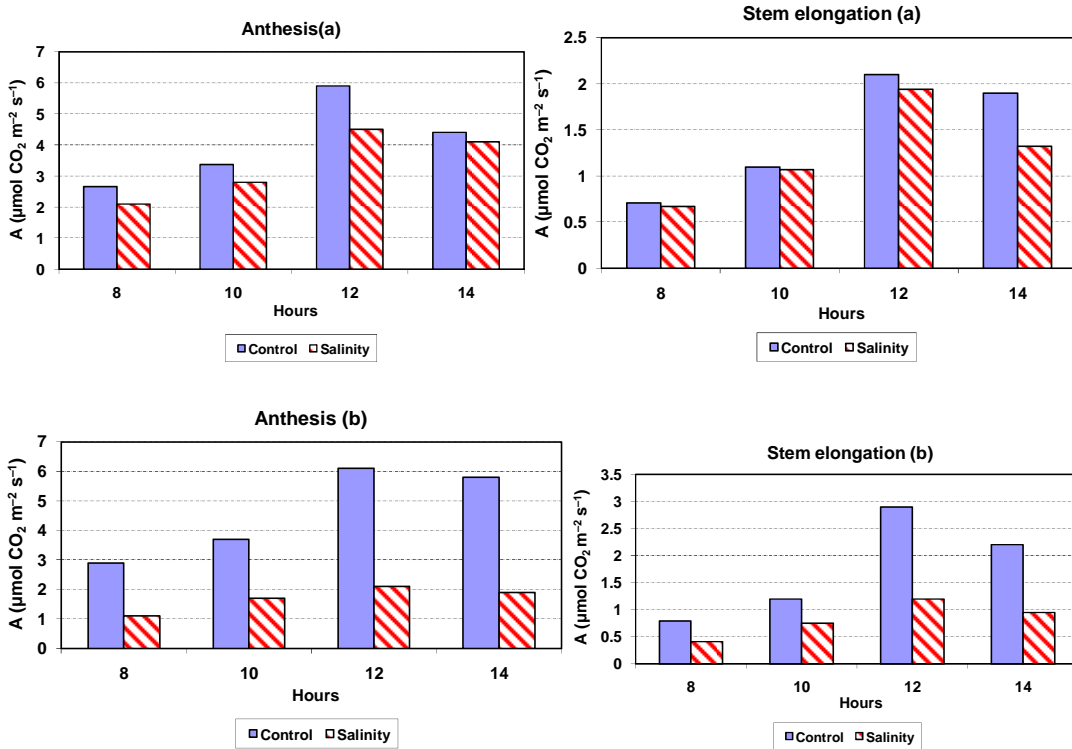


Figure 2. Photosynthesis rate of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages.

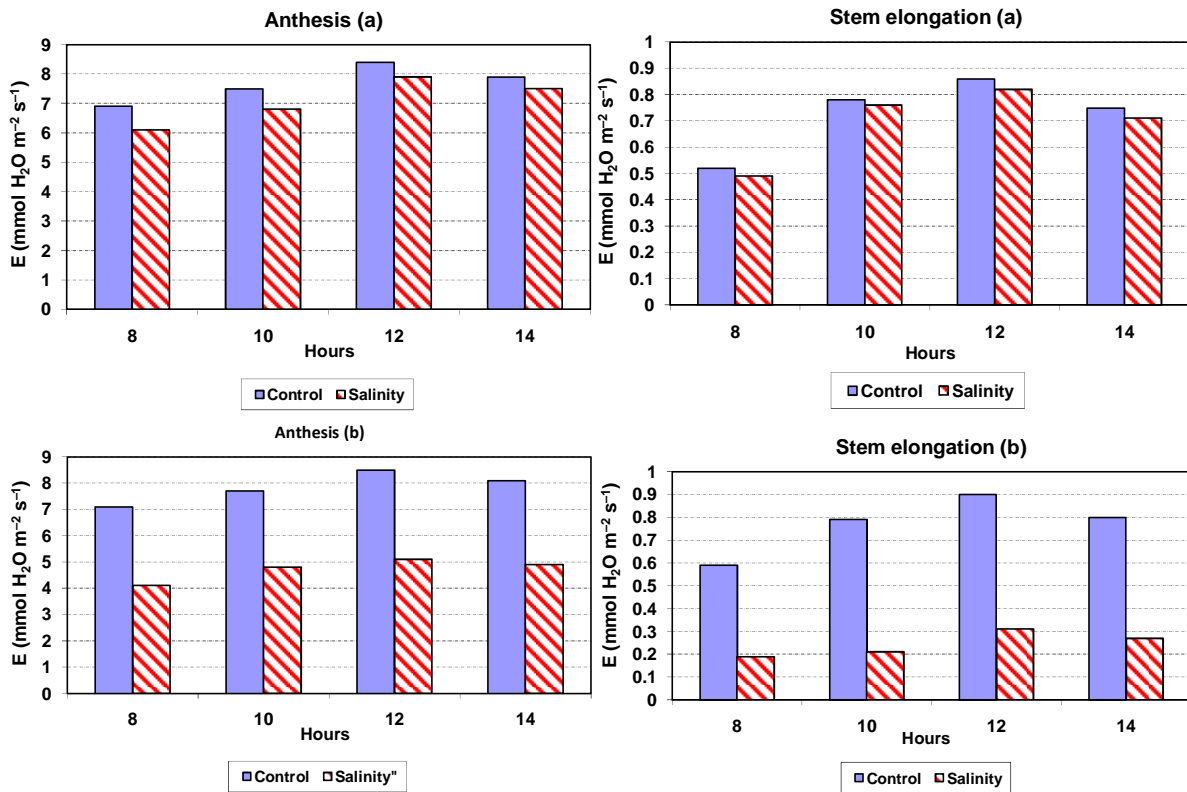


Figure 3. Transpiration rate(E) of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages.

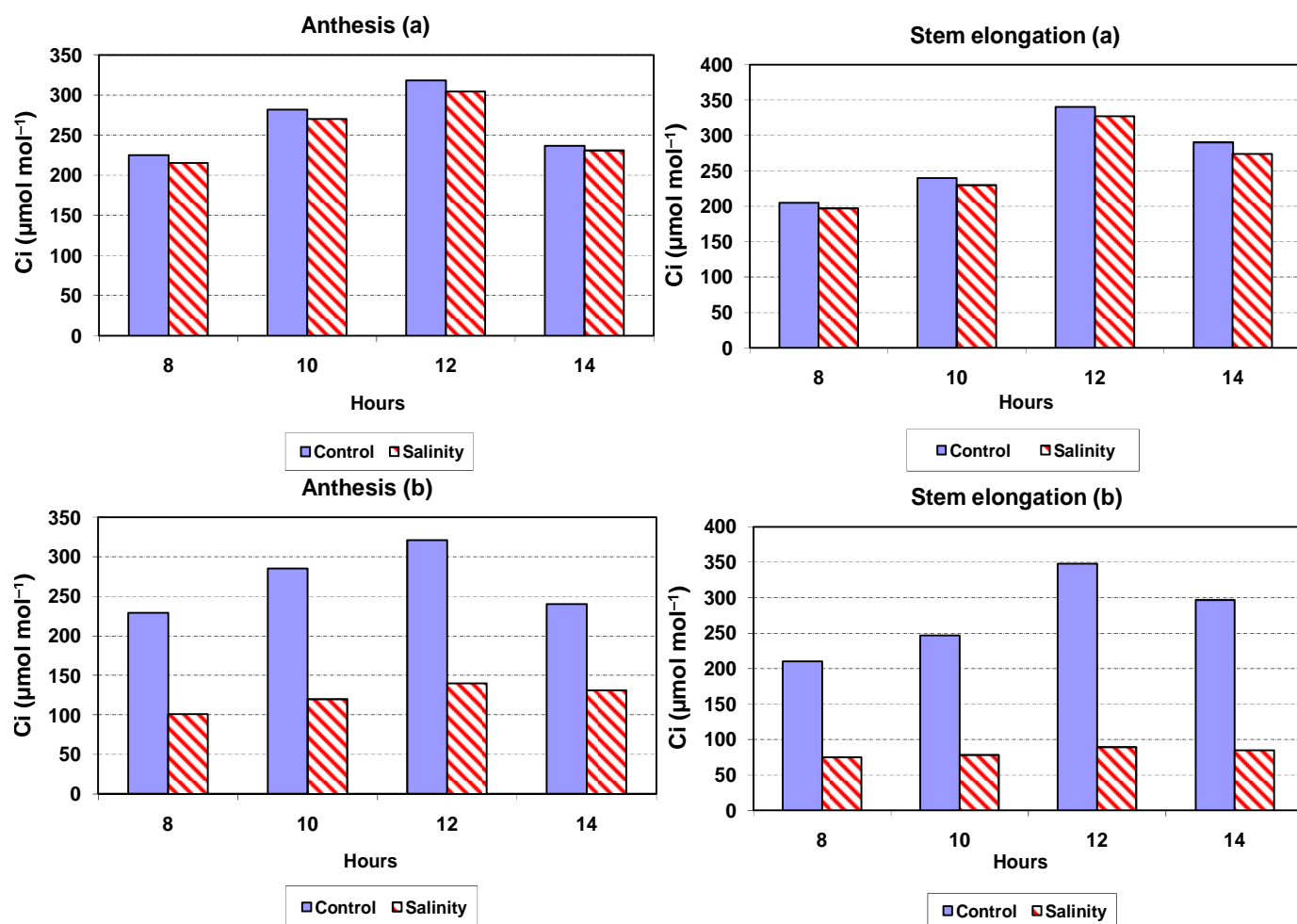


Figure 4. Sub-stomatal CO₂ (C_i) of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages.

salinity tolerance in wheat (Flowers and Yeo, 1981; Robinson, 1988). Interestingly, the most tolerant cultivar (Kavir) had the highest transpiration rate in the salt treatment than in controls. The salt concentration in leaves of the tolerant cultivars was lower than that of the intolerant cultivars (Sadeghi, 2008), and this could be attributed to the initial period of acclimation following exposure to salt stress, when a reduction in transpiration will also result in reduced salt uptake, as most of this uptake in wheat is known to occur passively, through the transpiration stream (Yeo and Flowers, 1986). However, processes occurring during this early acclimation period await further investigation. Mechanisms underlying the longer term adaptive responses observed in the tolerant cultivar are not clear, but probably involve processes that resulted in better control of uptake and/or translocation of toxic salts to the shoot. Recently, Chen and Gallie (2004) reported that the AsA redox state controls the rate of transpiration and stomatal conductance. They observed that a higher AsA redox state substantially increases the

total open stomatal area of a leaf and consequently results in greater stomatal conductance and a higher transpiration rate. Zheng et al. (2001) reported that the transpiration rate generally tends to decline with increasing rhizospheric salinity and this could be attributed to lower water potential in roots and the transport of abscisic acid (ABA) from root to shoot to induce stomatal closure. Excessive amounts of salt entering the transpiration stream will eventually injure cells in the transpiring leaves and this may further reduce growth.

The results showed that C_i from 8 to 12 O'clock increased at both stem elongation and anthesis stages and then decreased from 12 to 14 O'clock. There were no significant differences between the anthesis and stem elongation stages of the two wheat cultivars (Figure 4). The same results were also reported by Moradi and Ismail (2007). However, C_i of both genotypes was higher in the salt treatment than in controls. The results showed that stomatal conductance (g_s) from 8 to 12 O'clock

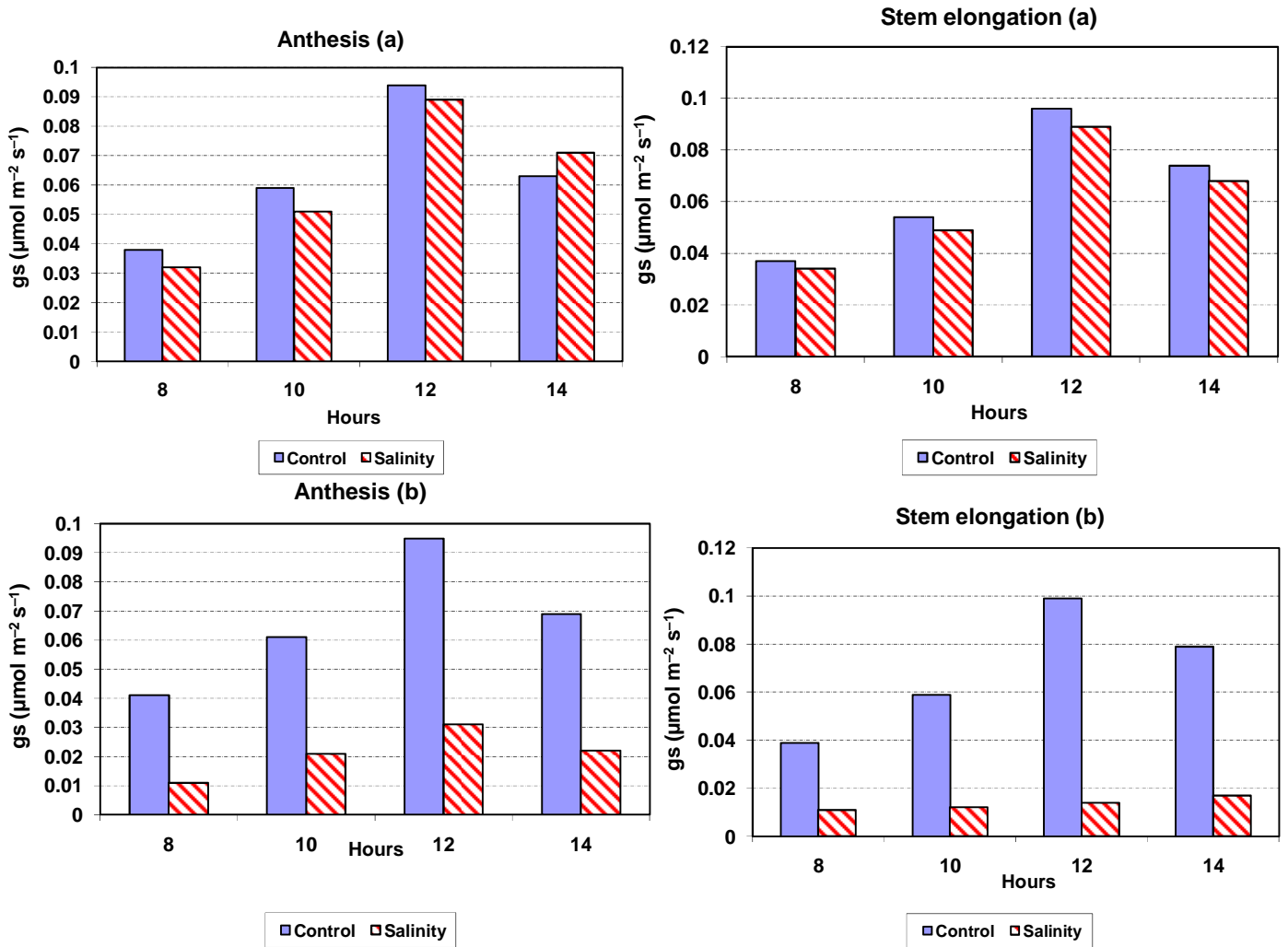


Figure 5. Stomatal conductance of CO₂ (gs) of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages.

increased at both stem elongation and anthesis stages and then decreased from 12 to 14 O'clock. Stomatal conductance (g_s) stem elongation at the stages was higher than anthesis at the stages of the two wheat cultivars (Figure 5). In wheat, James et al. (2002) observed that reduction in g_s occurred under salt stress before an apparent decline in leaf water potential, and argued that chemical signals are likely to cause the decrease in g_s . The preliminary data on early stomatal response to salinity also suggested the involvement of root–shoot communication in this initial acclimation stage of the tolerant lines, where stomatal conductance decreased substantially before any noticeable change in leaf water potential.

The results showed that leaf temperature was increased between 8 to 14 O'clock at both stem elongation and anthesis stages. Leaf temperature at the anthesis stage was significantly higher than the stem elongation stage of the two wheat cultivars, which is

attributed principally to growth season differences (Figure 6). The highest correlation ($R^2 = 0.9588$) between photosynthetic photon flux density (PPFD) and photosynthesis rate was obtained at stem elongation stage in Kavir cultivars at control conditions. The lowest correlation ($R^2 = 0.6306$) between photosynthetic photon flux density (PPFD) and photosynthesis rate was obtained at anthesis stage in Kavir cultivars at control conditions (Figure 7). In general, there is a strong correlation between photosynthetic photon flux density (PPFD) and photosynthesis rate especially at stem elongation stage in controls compared to salt treatment (Figure 7).

Conclusion

During the anthesis stage, photosynthesis, stomatal conductance and transpiration, measured on the flag leaf

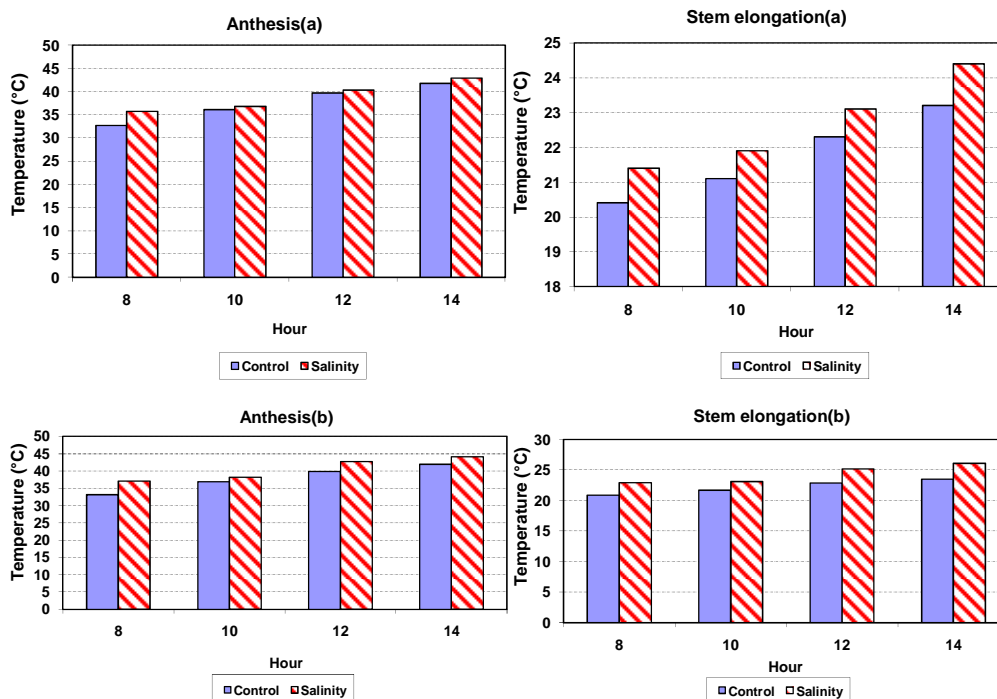


Figure 6. Leaf temperature of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages.

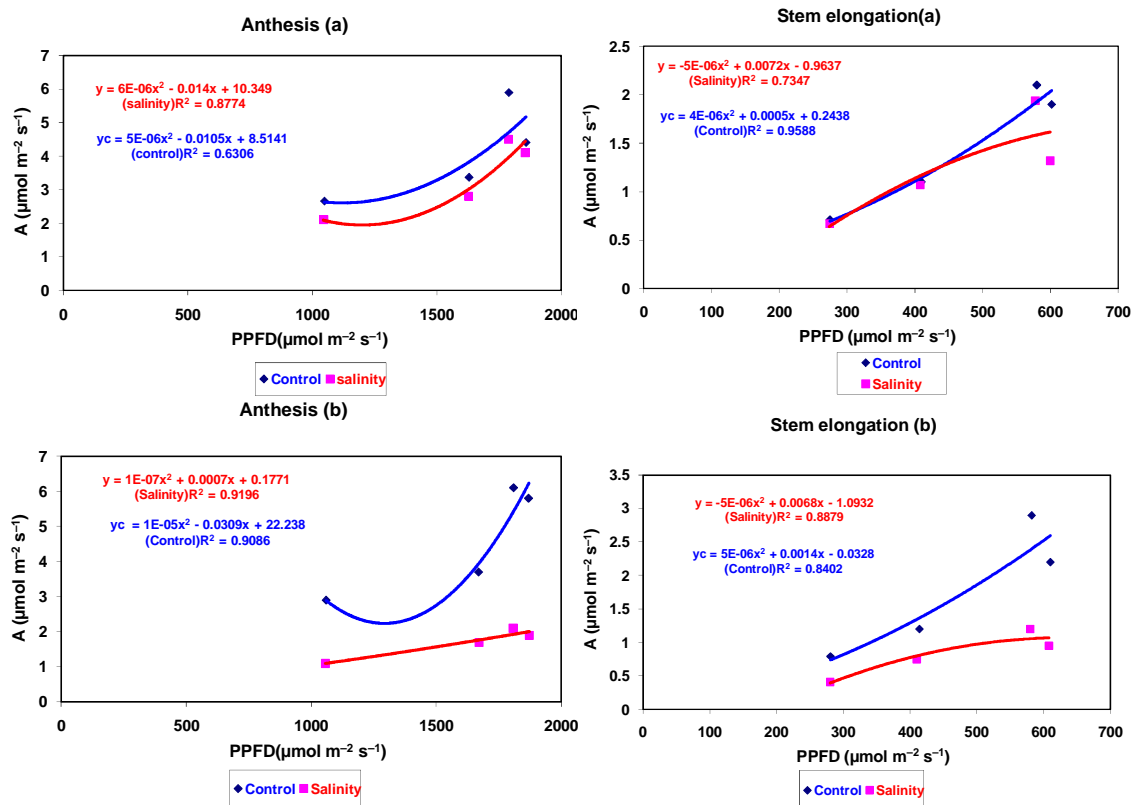


Figure 7. Correlation between photosynthetic photon flux density (PPFD) and photosynthesis rate of Kavir(a) and Shiraz(b) cultivars at anthesis and stem elongation stages. *, **, significant at $P < 0.05$, and 0.01 , respectively.

at flowering, also decreased under saline conditions, with greater effects on stomatal conductance and transpiration, whereas C_i did not show a significant reduction (Figures 2, 3 and 4), and is similar to that observed at the stem elongation stage. Kavir again maintained the highest photosynthetic CO_2 fixation rate under salt stress and this is associated with higher g_s (Figure 5). The lack of parallel effects of salinity on C_i again suggested a direct effect of salinity on carbon assimilation, apart from its effect on g_s . Salt accumulation in the mesophyll cells may inhibit carbon assimilation, resulting in an increase in internal CO_2 concentration, with eventual reduction in stomatal conductance (Maxwell and Johnson, 2000), particularly in sensitive cultivars such as Shiraz. In general, photosynthesis rate, transpiration, stomatal conductance and intercellular CO_2 of salt tolerant variety (Kavir) was found to be higher than the salt sensitive variety (Shiraz). Thus, it appeared that less adverse effect of salinity on Kavir cultivar may make it more suitable for growth in saline soils found in the investigation.

REFERENCES

- Chen Z, Gallie DR (2004). The ascorbic acid redox state controls guard cell signaling and stomata movement. *Plant Cell*, 16: 1143-1162.
- Cuin TA, Shabala S (2005). Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant and Cell Physiology*, 46: 1924-1933.
- Davenport R, James RA, Zakrisson-Plogander A (2005). Tester M, Munns R Control of sodium transport in durum wheat. *Plant Physiol.*, 137: 807-818.
- Dionisio-Sese ML, Tobita S (2000). Effects of salinity on sodium content and photosynthetic responses of rice seedlings differing in salt tolerance. *J. Plant Physiol.*, 157: 54-58.
- Flowers TJ, Yeo AR (1981). Variability in the resistance of sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *New Phytologist*, 88: 363-373.
- James RA, Rivelli AR, Munns R, von Caemmerer S (2002). Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. *Funct. Plant Biol.*, 29: 1393-1403.
- Longstreth DJ, Nobel PS (1979). Salinity effects on leaf anatomy. *Plant Physiol.*, 63: 700-703.
- Maas EV, Hoffman GJ (1977). Crop salt tolerance – current assessment. *Journal of the Irrigation and Drainage Div. Am. Soc. Civ. Eng.*, 103: 115-134.
- Maas EV, Grieve CM (1990). Spike and leaf development in salt-stressed wheat. *Crop Sci.*, 30: 1309-1313.
- Maxwell K, Johnson GN (2000). Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.*, 51: 659-668.
- Moradi F, Ismail AM, Gregorio GB, Egdane JA (2003). Salinity tolerance of rice during reproductive development and association with tolerance at the seedling stage. *Indian J. Plant Physiol.*, 8: 276-287.
- Moradi F, Ismail AM (2007). Responses of Photosynthesis, Chlorophyll Fluorescence and ROS-Scavenging Systems to Salt Stress During Seedling and Reproductive Stages in Rice. *Ann. Bot.*, 99(6): 1161-1173.
- Munns R, James RA, Läuchli A (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57: 1025-1043.
- Pearson GA, Bernstein L (1959). Salinity effects at several growth stages of rice. *Agron. J.*, 51: 654-657.
- Peng S, Ismail AM (2004). Physiological basis of yield and environmental adaptation in rice. In: *Physiology and biochemistry integration for plant breeding—Nguyen HT, Blum A, eds. (2004) New York: Marcel Dekker, Inc., pp. 83-140.*
- Robinson JM (1988). Does O_2 photoreduction occur within chloroplast *in vivo*. *Physiol. Plant.*, 72: 666-680.
- Sadeghi H, Emam Y (2006). Effect of different sodium chloride levels on morphological characteristics, chemical composition and yield Components of two bread wheat cultivars. *Biaban. J.*, 10: 267-279 (In Farsi with English abstract).
- Yeo AR, Capon SJM, Flowers TJ (1985). The effect of salinity upon photosynthesis in rice (*Oryza sativa* L.): gas exchange by individual leaves in relation to their salt content. *J. Exp. Bot.*, 36: 1240-1248.
- Yeo AR, Yeo ME, Flowers SA, Flowers TJ (1990). Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical Appl. Genet.* 79: 377-384.
- Yildirim M, Bahar B (2010). Responses of some wheat genotypes and their F2 progenies to salinity and heat stress. *Sci. Res. Essays*, 5(13): 1734-1741.
- Zheng L, Shannon MC, Lesch SM (2001). Timing of salinity stress affecting rice growth and yield components. *Agric. Water Manage.*, 48: 191-206.