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Influence of Foliar Applied Thiourea on Flag Leaf Gas Exchange and Yield Parameters of Bread Wheat (*Triticum aestivum*) Cultivars under Salinity and Heat Stresses

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ABSTRACT

Salinity and heat stresses are highly damaging to plant growth and reduce productivity. In this study, we determined the effectiveness of foliar spray of thiourea in improving gas exchange properties of flag leaf and grain yield components and their possible interrelationships. Data showed that both of these stresses reduced net photosynthesis and increased substomatal CO_2 level, leading to lowered CO_2 assimilation by Rubisco. Among grain yield and its components, salinity and heat stress had no significant effect on the number of grains per spikelet, while number of grains per spike was reduced, leading to lowered grain yield and harvest index. Correlations among gas exchange parameters of flag leaf and yield and yield components were closer for S-24, which indicated its better ability to grow and respond to foliar applied thiourea under stressful conditions than MH-97. Data suggested that salinity stress was relatively more damaging to grain filling and final yield than heat, whereas foliar application of thiourea induced both salinity and heat tolerance by improving net photosynthesis and grain yield in wheat. Salinity tolerant (S-24) cultivars responded better to foliar application of thiourea in stress amelioration than the sensitive (MH-97). Results indicated that field use of thiourea is feasible for enhancing wheat yield under stressful conditions.

Key Words: Correlations; Foliar spray; Grain yield components; Stress tolerance; Wheat

INTRODUCTION

Soil salinity and high temperatures are major factors limiting the plant productivity. Commonly both these stresses alter morphological development, reduce leaf gas exchange, assimilate partitioning, activities of antioxidants and disruption of membrane functions (Zhu, 2003; Sairam & Tyagi, 2004; Taiz & Zeiger, 2006). Salinity of soils is increasing rapidly all over the world because of irrigation of crops with low quality water, thus leading to net accumulation of ions in the root zone (Misra *et al.,* 1997 & 2001; Pitman & Lauchli, 2002). Heat stress limits growth and yield of field crops by acting as a dehydrative force and aggravates the prevailing stress conditions (Wahid *et al.*, 2007; Gilani *et al*., 2008). Therefore, determination of responses and strategies to improve crop growth under changing climates is imperative. Available information indicates that heat stress combined with salinity prolongs the germination time and reduce the germinability of seeds primarily due to ionic-toxicity (Misra, 1993a; Wahid *et al.*, 1999). Existence of inter-specific differences in the timing of germination and seedling establishment under salinity, temperature or photoperiod has been reported in four halo-tolerant species (*Hutchinsia procumbens*, *Lythrum hyssopifolium*, *Parapholis incurva* & *Lasthenia glabrata* ssp. *Coulteri*) from salt marshes. In these species, salinity and moisture interacted to affect the germination (Noe & Zedler, 2000; Misra *et al.*, 2002).

Stress tolerance can be improved by various means; the most important of which include exogenous application of stress alleviating agents. These agents may be organic or inorganic in nature (Farooq *et al*., 2008). Exogenous use of stress signaling molecules such as hydrogen peroxide, ethylene, nitric oxide or inorganic sources has tremendous potential to improve stress tolerance (Zhu, 2003; Taiz & Zeiger, 2006; Wahid *et al.,* 2007). Thiourea is reported to relieve dormancy in salt stressed seeds (Gul & Weber, 1998), increase K^+ uptake by chickpea seed and reduce ABA biosynthesis under osmotic or heat stress (Aldasoro *et al.*, 1981). Foliar application of thiourea improved net photosynthesis and chlorophyll content in drought stressed clusterbean (*Cyamopsis tetragonoloba*) plants (Garg *et al*., 2006). Thiourea has also been reported to significantly improve growth, yield and water use efficiency of munghbean (*Phaseolus vulgaris*) seedlings (Misra *et al.*, 1997), pearl millet (Misra & Misra, 1991; Misra, 1993b) and clusterbean plants (Garg *et al.,* 2006) under arid and semi-arid conditions.

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Bread wheat (*Triticum aestivum* L.) is a major source of living for mankind and is grown all over the world. It has been ranked tolerant to salinity with a salt tolerance threshold (EC_e) value between 6 and 8.6 dS m^{-1} (Francois & Maas, 1999). Wheat is a winter crop and therefore sensitive to episodes of high temperature (Yang *et al*., 2002). In view of the increased global warming, high temperature may aggravate the salinity status of the field at crucial stages like germination and reproductive growth periods of wheat. This will lead to a reduced field stand under heat stress and declined final grain yield under salt stress.

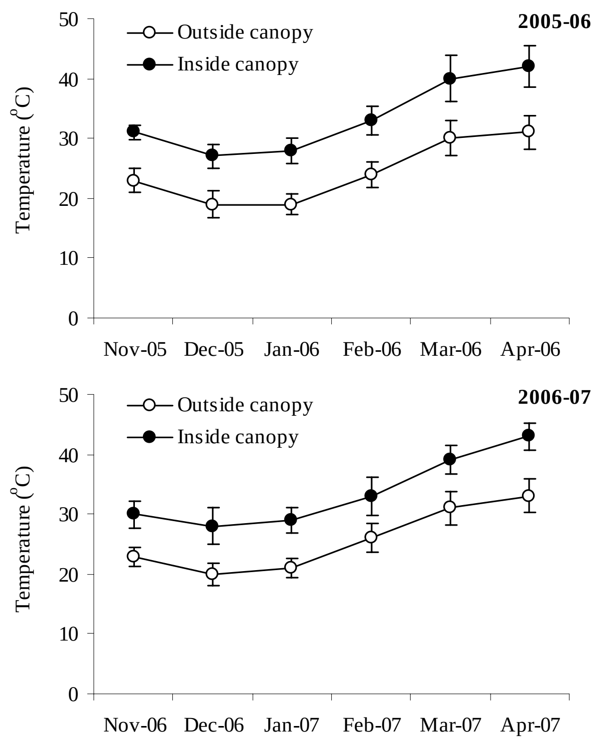
Grain yield and yield components are ultimate yardsticks to assess the stress tolerance in cereals, which are greatly influenced by photosynthetic activity of flag leaf (Misra & Misra, 1991). Both salinity and heat stresses substantially affect the economic yield and its components in plants. Reduction in these attributes is primarily related to changes in photosynthetic capacity of the proximal or subtending leaf in various plant species and flag leaf in cereals (Wahid & Rasul, 2005). In the present study, we tested the possible effect of foliar spray of thiourea in improving growth and yield of two wheat cultivars under the salt and heat stress conditions.

MATERIALS AND METHODS

The experiments were performed in the net-house of the Department of Botany, University of Agriculture, Faisalabad, Pakistan during November-April of the years 2005-06 and 2006-07. From previous studies we noted that of the two bread wheat (*Triticum aestivum* L.) cultivars (S-24 & MH-97) used in this research; MH-97 was heat tolerant but salt sensitive, while and S-24 was salt tolerant but heat sensitive (Anjum, 2008). Seeds of both these cultivars were directly sown in earthen pots (45 cm high with 25 cm diameter from the neck & 18 cm from bottom, with a hole in the bottom for drainage of the solution) containing 10 kg of thoroughly water washed river sand and then they were kept in the net-house. After germination, three uniform size seedlings were left in each pot. Plants were grown up to pre-anthesis stage under normal net-house conditions.

At pre-anthesis stage, one half of the pots of both the cultivars were shifted to open door plexiglass fitted canopies, which had temperature 7–10^{o}C higher than ambient (Fig. 1). In second half of the pots 120 mm salinity level was developed in three days using solution of sodium chloride (99.1% pure) gradually @ 40 mm per pot per day. Aqueous solution of thiourea (10 mm) was foliar sprayed mechanically. Following were the experimental treatments in triplicate: (a) control (salinity, heat stress or thiourea not applied); (b) water control (plants spreayed with water,; salinity, heat or thiourea not applied); (c) foliar spray of thiourea alone; (d) salinity alone; (e) salinity with foliar spray of thiourea; (f) heat stress alone; (g) heat stress with foliar spray of thiourea; (h) salinity and heat stresses

Fig. 1. Changes in temperature (recorded at 12 noon) inside and outside the canopy during the wheat growing seasons



combined; (i) salinity and heat stresses combined and thiourea spray. Design of the experiments was completely randomized with three replications. In the absence of any remarkable difference in the control (a) and water-sprayed and control (b) plants, the latter treatment was excluded from the statistical analysis. Half-strength nutrient solution (Hoagland & Arnon, 1950) in case of heat stress and that salinized (120 mm NaCl) in case of salinity treated plants was replaced once in a week throughout the growth periods. Pots outside the canopy were protected from rainfall by covering with transparent plastic sheet. Photosynthetically active radiations on the leaf surface ranged between 855 to 975 µmol m^{-2} s^{-1} (measured with Infra Red Gas Analyzer, LCA-4, Analytical Development Company, Hoddesdon, England), while relative humidity was ~40±4% inside and outside the canopy.

Flag leaf gas exchange parameters including net rate of photosynthesis (FL-Pn), transpiration rate (FL-E), stomatal conductance (FL-gs) and substomatal CO_2 concentration (FL-Ci) were determined 15 days after the treatment application using Infra-Red Gas Analyzer (IRGA, Model LCA-4). The set of conditions for these determinations were: molar air flow per unit leaf 335 mmol m^{-1} s^{-1}, atmospheric pressure 99.6 kPa, PAR on leaf surface ranged from 855 to 975 µmol m^{-2} s^{-1}, CO_2 concentration 357 µmol mol^{-1} and ambient temperature was 27-29^{o}C. Photosynthetic water use efficiency was measured as Pn:E ratio, while stomatal limitation to CO_2 assimilation was calculated as FL-Pn:Ci ratio.

The watering of plants was halted when senescence (yellowing) of flag leaf started. Fully dried plants were harvested to determine yield and yield components. The spikes were removed and number of spikelets was counted from each spike. Grains were extracted from each spikelet to determine their number per spikelet and summed up to present number of grains per spike. The weight of 100 extracted grains (HGW) was determined from each treatment of both the cultivars. Total grain yield per plant (GRY) was determined after extracting the grains from all the spikes of a plant. Aboveground parts were determined for dry straw yield per plant (STY). Both GRY and STY were used determine harvest index (HI; %) as: (GRY/STY) × 100.

The data for both the years was averaged to determine comparative responses of cultivars in terms of changes in salinity and heat stress tolerance separately with or without thiourea spray. Data were statistically analyzed using COSTAT computer software (COHORT, Monterey, California) to find meaningful differences in the cultivars and various treatments. Correlations coefficients of flag leaf gas exchange parameters and grain yield and yield components were derived to determine the possible influence of foliar applied thiourea in enhancing salinity tolerance under salinity and heat stresses.

RESULTS

Gas exchange parameters of flag leaf. FL-Pn differed significantly in the cultivars ($P<0.05$), treatments ($P<0.01$) and interaction ($P<0.01$) of both these factors. The FL-Pn value was greater in controls, which declined in all the stress treatments, but it was found to be lowest under salinity followed by combined salinity and heat stress in S-24, while the reduction in this attribute was similar under salinity as well as heat stress in MH-97. Foliar application of thiourea improved FL-Pn values in both the cultivars; MH-97 showed a greater improvement under salinity and S-24 under heat stress (Fig. 2). For FL-E, although cultivars differed non-significantly ($P>0.05$), a significant ($P<0.01$) difference among the treatments and a significant interaction of these factors was evident. The FL-E was not much different in both the cultivars under control (no stress treatment), increased a little ($P<0.05$) in S-24 but did not change in MH-97 with thiourea application. Although damaging to both the cultivars, salinity induced decline in the FL-E, while foliar applied thiourea improved it. The impact of salinity and thiourea was relatively greater in MH-97 than S-24. Likewise, foliar spray of thiourea increased FL-E in both the cultivars under heat stress or combined effect of salinity and heat stresses (Fig. 2).

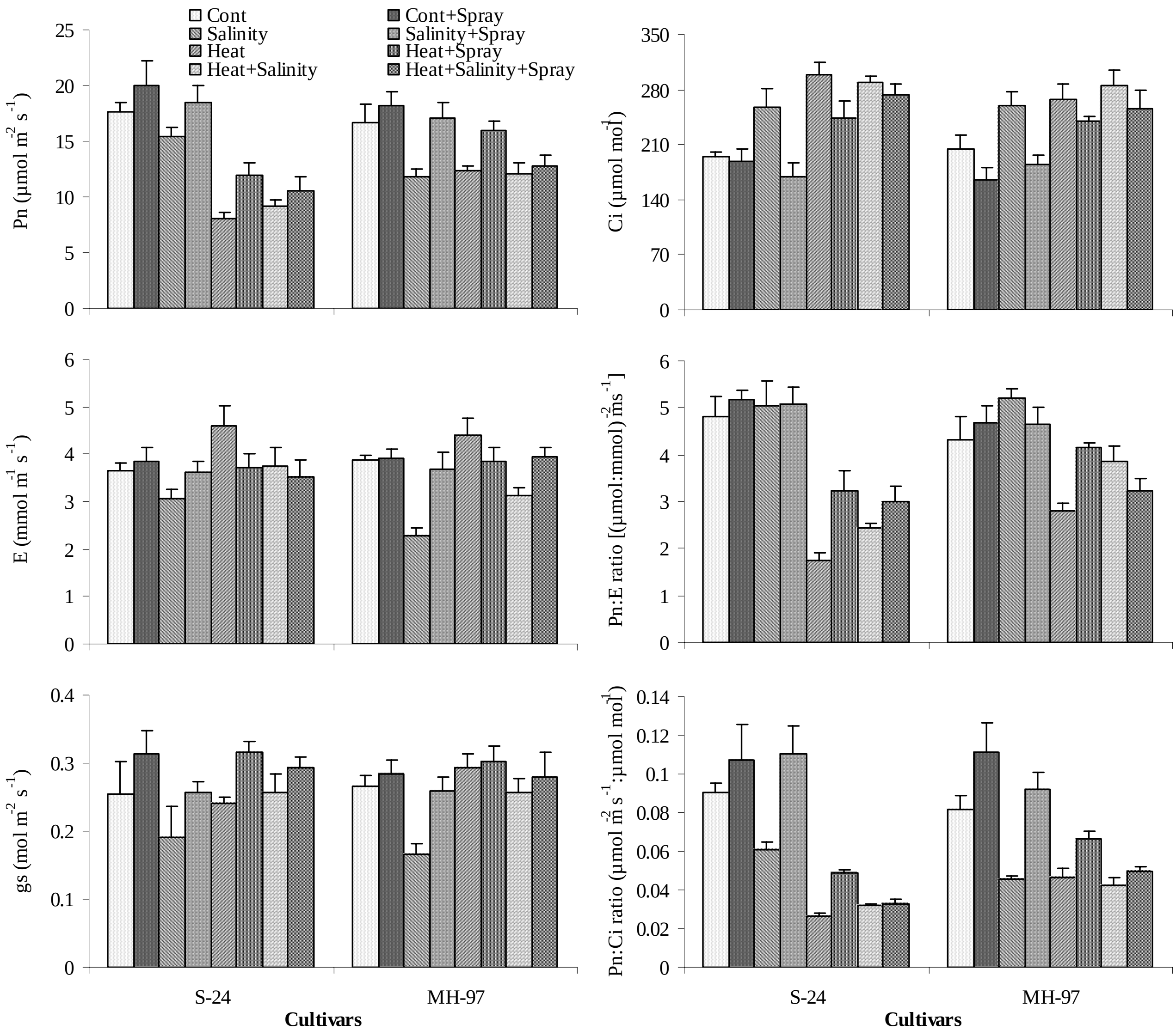
Data for the FL-gs exhibited no-significant ($P>0.05$) difference in the cultivars, but a significant ($P<0.01$) difference among treatments was evident and there was no interaction ($P>0.05$) of both these factors. The FL-gs decreased in both the cultivars under salinity, but increased under heat stress and combined effect of salinity and heat stress. The foliar application of thiourea enhanced FL-gs under control and all stress treatments (Fig. 2). Data for the FL-Ci indicated significant ($P<0.05$) differences in the cultivars and various treatments ($P<0.01$) with a significant ($P<0.01$) interaction of both these factors. Under control, foliar spray of thiourea reduced FL-Ci slightly ($P<0.05$) in S-24 but greatly in MH-97. Foliar applied thiourea was the most effective in reducing the FL-Ci in salinity treated plants, followed by heat stressed and those stressed with combined effect salt and heat stresses (Fig. 2).

Data for FL-Pn:E revealed significant ($P<0.01$) difference in the cultivars, treatments and their interaction ($P<0.01$). Under control condition, the FL-Pn:E was greater in S-24 than MH-97, while the foliar spray of thiourea improved it almost equally in both the cultivars. Applied salinity had no effect on FL-Pn:E, while heat stress alone or combined with salinity stress affected on this attribute more severely in S-24. Foliar applied thiourea partially alleviated this stress effect in S-24. In MH-97, this ratio increased under salinity but declined both under salinity and heat stress (Fig. 2).

FL-Pn:Ci ratio showed non-significant ($P>0.05$) difference in the cultivars, but had a significant ($P<0.01$) difference among treatments and an interaction ($P<0.01$) of both these factors. Under control, this ratio was relatively higher in S-24 than MH-97, which was improved ($P<0.01$) in both the cultivars with foliar applied thiourea. Likewise, foliar applied thiourea markedly reduced this attribute in both the cultivars under salinity, heat stress or their combined effect (Fig. 2).

Grain yield and yield components. There was a significant ($P>0.01$) difference between the cultivars and interaction of cultivars with treatments for number of grains per spikelet. Under control condition, numbers of grains per spikelet was greater in S-24 as compared to MH-97 Foliar spray of thiourea showed no significant effect on the control plants of either cultivar. Applied salinity declined this number in S-24 only. Foliar applied thiourea showed no significant effect on either cultivar under salinity stress. Although non-significantly ($P>0.05$), heat stress was relatively less damaging to this attribute in S-24, but enhanced it in MH-97, while foliar spray of thiourea declined it in S-24 and increased in MH-97. Salinity in combination with heat stress reduced this number in both the cultivars, while foliar applied thiourea decline it in S-24 but did not change in MH-97 (Fig. 3). For number of grains per spike, there was a significant difference in the cultivars ($P<0.05$), treatments ($P<0.01$) with an interaction ($P<0.01$) of these factors. This number was higher in S-24 than MH-97 under control condition, but did not change appreciably with foliar spray of thioutea in both. Applied salinity decreased this number in S-24 but showed no significant change in MH-97. Foliar application of thiourea effectively improved it in both the cultivars. Heat stress alone and its combination with salinity declined this attribute, which

Fig. 2. Changes in the gas exchange parameters of flag leaf of two bread wheat cultivars under salinity and heat stresses, and the effect of foliar applied thiourea

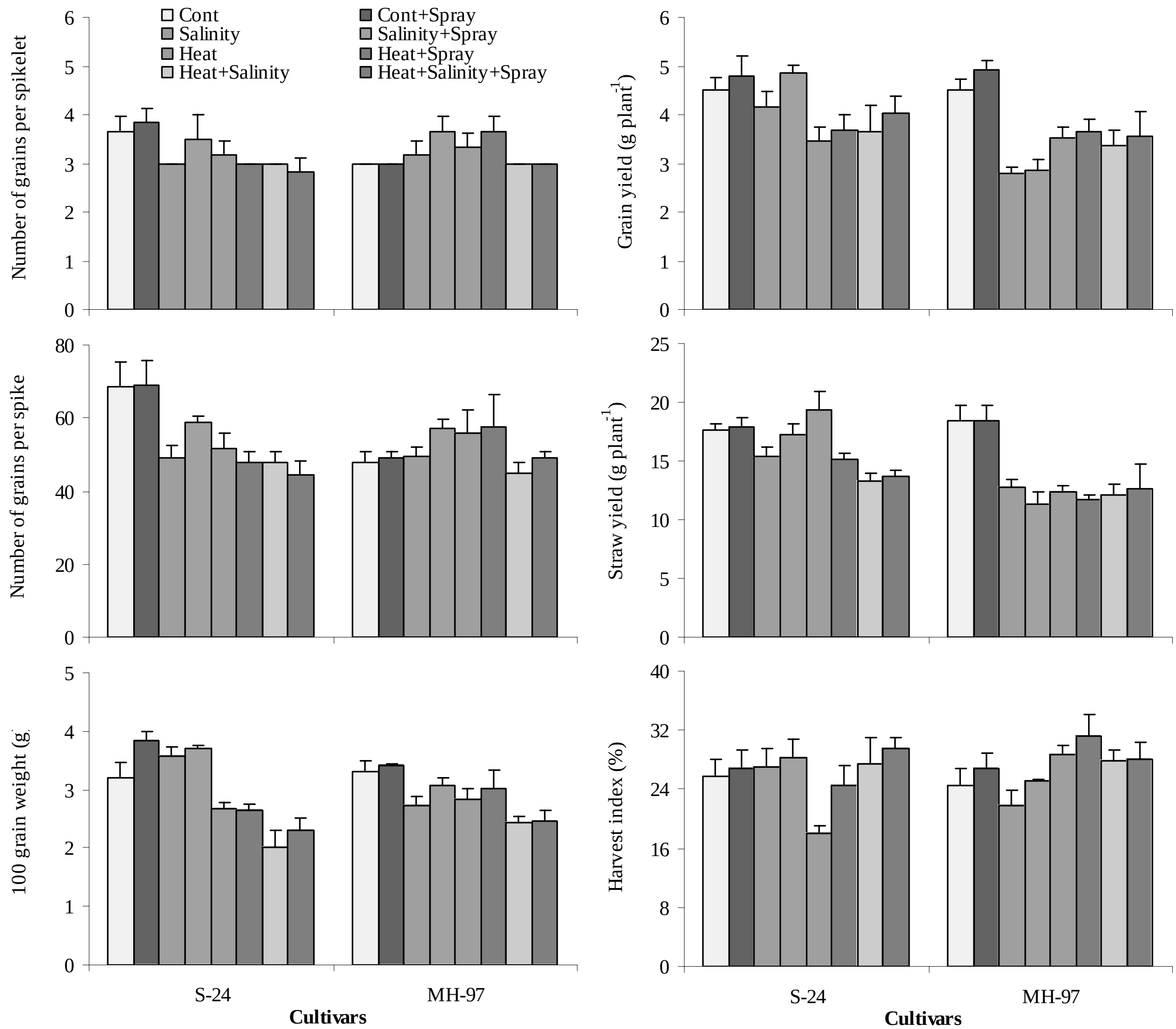


declined further with foliar spray of thiourea in S-24. Contrarily in MH-97, heat stress increased while salinity combined with heat decreased this parameter, which was improved with foliar applied thiourea (Fig. 3).

Regarding 100 grain weight (HGW), although cultivars indicated no significant (P>0.05) difference, the treatments differed significantly (P>0.01), with an interaction (P<0.01) of these factors. Under control, HGW increased substantially in S-24 only with foliar spray of thiourea. Salinity stress increased this attribute, though non-significantly, in S-24 but reduced it in MH-97, while foliar spray of thiourea improved it in the latter variety. Heat stress alone was more damaging to S-24 than MH-97; foliar spray of thiourea increased HGW in both cultivars, although this increase was greater in MH-97. Salinity combined with heat stress was highly damaging to HGW in both the varieties, which improved (P>0.05) with thiourea spray in S-24 but not in MH- 97 (Fig. 3). Data for grain yield (GRY) indicated significant (P<0.01) difference in cultivars, treatments with interaction of these factors. Although cultivars indicated similar GRY under control, it increased greatly (P<0.05) in MH-97 than S-24 with foliar spray of thiourea. Applied salinity was damaging to GRY lowly (P>0.05) in S-24 but highly (P<0.05) in MH-97, while foliar spray of thiourea improved it in S-24 but did not change in MH-97. The GRY was *at par* under heat stress or its combination with salinity; while thiourea spray showed significant effect under these stress conditions (Fig. 3).

Straw yield per plant (STY) revealed significant (P<0.01) difference in the cultivars, treatments and interaction (P<0.01) of these factors. Thiourea showed no significant effect on SY of both the cultivars under control

Fig. 3. Changes in the yield and yield components of wheat cultivars under salinity and heat stresses singly and in combination, and the influence of foliar applied thiourea



conditions. Applied salinity produced a greater decline in STY of MH-97 than S-24, while thiourea spray improved it in S-24 only with no significant (P>0.05) effect on MH-97. Heat stress increased the SY highly in S-24 but lowly in MH-97. Thiourea showed a significant decrease in STY only in S-24 under heat stress alone. Salinity combined with heat stress decreased SY and thiourea spray showed no great effect on both the cultivars (Fig. 3).

Harvest index (HI) did not differ in the cultivars (P>0.05), but treatments indicated significant (P<0.01) difference with an interaction (P<0.01) of cultivars and treatments. The HI was similar in both the cultivars under control and foliar applied thiourea showed no significant effect on HI in either in MH-97 or S-24. Salinity alone or in combination with heat stress showed no significant difference in HI of both the cultivars with or without thiourea treatments. However, heat stress was highly damaging to HI of S-24 contrary to that of an improvement in MH-97. Foliar spray of thiourea improved HI in both the cultivars under heat stress (Fig. 3).

Correlation coefficients. The fact that stress-induced changes in flag leaf have great impact on the final yield and yield components, parallels were drawn of gas exchange attributes of flag leaf with yield and yield components for both the cultivars (Table I). Except STY and HI, all yield components were positively correlated to FL-Pn in S-24, while only HGW was correlated with Fl-Pn in MH-97. FL-gs, showing no correlation with any of the yield and yield components in S-24, but was positively related to HI in MH-97. FL-Ci was negatively related to all yield components except straw yield and HI in S-24, but negatively to HGW in MH-97. FL-Pn:E ratio was positively

Table I. Correlations coefficients (r) of some flag leaf parameters with yield and yield components in two bread wheat cultivars under individual or combined effects of salinity and heat stresses with or without thiourea application (n = 8)

Flag leaf gas exchange characteristics	*Yield and yield components*	*S-24*	*MH-97*
Net rate of photosynthesis	Number of grains per spike	0.797*	0.263ns
	Number of grains per spikelet	0.801*	0.236ns
	100 grain weight	0.899**	0.893**
	Grain yield per plant	0.952**	0.593ns
	Straw yield per plant	0.369ns	0.564ns
	Harvest index	0.422ns	0.029ns
Transpiration rate	Number of grains per spike	0.133ns	0.383ns
	Number of grains per spikelet	0.186ns	0.146ns
	100 grain weight	–0.247ns	0.325ns
	Grain yield per plant	–0.385ns	0.509ns
	Straw yield per plant	0.585ns	0.193ns
	Harvest index	–0.811*	0.630ns
Stomatal conductance	Number of grains per spike	0.155ns	0.342ns
	Number of grains per spikelet	0.187ns	0.201ns
	100 grain weight	–0.153ns	0.238ns
	Grain yield per plant	0.090ns	0.508ns
	Straw yield per plant	–0.097ns	0.088ns
	Harvest index	0.140ns	0.845**
Substomatal CO_2 concentration	Number of grains per spike	–0.788*	–0.194ns
	Number of grains per spikelet	–0.810*	–0.129ns
	100 grain weight	–0.788*	–0.872**
	Grain yield per plant	–0.926**	–0.519ns
	Straw yield per plant	–0.385ns	–0.604ns
	Harvest index	–0.385ns	0.221ns
Photosynthetic water use efficiency	Number of grains per spike	0.644ns	–0.096ns
	Number of grains per spikelet	0.633ns	0.081ns
	100 grain weight	0.879**	0.438ns
	Grain yield per plant	0.911**	–0.048ns
	Straw yield per plant	0.213ns	0.268ns
	Harvest index	0.526ns	–0.654ns
Stomatal limitation to CO_2 assimilation	Spike length	0.716*	0.174ns
	Number of grains per spike	0.836**	0.166ns
	Number of grains per spikelet	0.863**	0.116ns
	100 grain weight	0.870**	0.878**
	Grain yield per plant	0.948**	0.587ns
	Straw yield per plant	0.455ns	0.617ns
	Harvest index	0.344ns	–0.113ns

Significant at *, P<0.05; and **, P<0.01.ns, non-significant

related to HGW and GRY in S-24 only. FL-Pn:Ci ratio was positively related to all the yield components, except STY and HI in S-24, while only with HGW in MH-97 (Table I).

DISCUSSION

Being located proximally and having major contribution, stress related changes in the flag leaf are critically important to final grain yield and productivity (Misra & Misra, 1991; Wahid & Rasul, 2005). These changes are related to available area for light harvesting and gas exchange and ultimately photoassimilate production and assimilation (Dubey, 2005; Wahid *et al.*, 2007). In this study determinations made for gas exchange parameters indicated that applied salinity and heat stress individually or in combination reduced FL-Pn, while enhanced FL-Ci. Both FL-E and FL-gs were reduced under salinity but enhanced under heat stress or salinity and heat stresses combined effect. Photosynthetic water use efficiency of flag leaf (expressed as Pn:E ratio) and stomatal limitations to CO_2 uptake (given as Pn:Ci ratio) were increased under salinity but severely declined under heat stress in S-24, while declined almost similarly under all stresses in MH-97. Foliar applied thiourea was effective in either promoting these attributes under control condition or alleviating the adverse effects of these stresses on most of these attributes (Fig. 2). This indicated that foliar applied thiourea improves the CO_2 uptake by stomata and its utilization by Rubisco, which carries great implications for final grain yield. Earlier reports show that exogenous use of thiourea enhanced the germination, growth and photosynthetic attributes of various plants species under stressful condition (Gul & Weber, 1998; Burman *et al.*, 2004; Garg *et al.*, 2006). The present findings suggest specific role of foliar applied thiourea in enhancing the flag leaf photosynthesis and its effectiveness was more explicit under salinity stress.

Incidence of stress at reproductive growth periods is very critical to final plant productivity (Misra, 1991; Misra & Misra, 1991; Francoise & Maas, 1999). Diminution in final economic yield might be due to changed pattern of reproductive growth, which mainly entails earlier completion of life cycle or allocation of resources to vegetative growth rather than to grain/seed filling (Misra, 1995; Wahid *et al*., 2004). Yield and yield components determined in this study revealed that number of grains per spikelet and spike, although showed reduction due to individual and combined effects of salinity and heat stress in both the cultivars, did not differ greatly among these treatments, whilst foliar applied thiourea showed stress mitigation effect. Applied salinity lowly affected the HGW and GRY in S-24 but highly in MH-97, while reverse of this trend was noted in both the cultivars under heat stress. Salinity alone or combined with heat stress was more damaging to MH-97 for these characters (Fig. 3). Foliar applied thiourea indicated low to moderate improvement in this attribute of both the cultivars. STY was greater in S-24 under all stresses than MH-97, while foliar applied thiourea improved this attribute in both the cultivars, except under heat stress in S-24. Heat stress was more damaging to HI in S-24 but salinity to MH-97; which improved in both cultivars with thiourea spray (Fig. 3). These findings revealed that effects of salinity and heat stresses singly or in combination were more damaging to grain filling, which supposedly were related to hampered assimilate production and their partitioning towards grain filling (Natr & Lawler, 2005; Wahid *et al.*, 2007). Improvement in yield and yield components with foliar applied thiourea in this study is consistent with the earlier reports for other crop species (Burman *et al.*, 2004; Garg *et al.*, 2006).

Exposure to stress has the potential to reduce the growth and productivity of crops by altering the growth patterns, which may be consistent with various growth and physiological attributes. During reproductive growth, photosynthesis of flag leaf or that proximal leaf to the developing grain plays a crucial role in fruit growth and its ultimate yield (Misra, 1995; Wahid & Rasul, 2005). In this study, correlations of flag leaf gas exchange parameters with yield and yield components revealed that FL-Pn and FL-Pn:Ci ratio were positively, while FL-Ci was negatively correlated with grain yield components. Correlation of STY and HI in S-24 (salinity tolerant but heat sensitive) and no correlations were noted in MH-97 (a salinity sensitive and heat tolerant), except a few related to grain yield and harvest index. However, parameters like FL-E and FL-gs hardly indicated any correlation (Table I). These findings suggested that S-24 had a better ability to growth and respond to foliar applied thiourea under stressful conditions than MH-97. This is important considering the fact that consistency in the partitioning of resources is important to stress tolerance. These changes further witnessed that salinity stress, due to its multiple nature of effects such as ion-toxicity, induced nutrient deficiency, physiological water deficit etc. (Misra *et al.*, 2001; Pitman & Lauchi, 2002; Zhu, 2003; Epstein & Bloom, 2005), is more damaging to grain filling and final yield than heat stress in wheat, while foliar applied thiourea has the ability to induce both salinity and heat tolerance and displaying greater yield in wheat.

In conclusion, although salinity and heat stresses were damaging gas exchange properties of flag leaf and yield and its components of both the cultivars, foliar applied thiourea was effective in enhancing all these attributes greatly in S-24 (salinity tolerant) than MH-97 (salinity sensitive). These effects of thiourea were linked to improved net CO_2 uptake by Rubisco. Close correlations of flag leaf gas exchange parameters with most of the yield and yield components of S-24 suggested that foliar applied thiourea was more effective in improving wheat growth under salinity than under heat stress. Further in depth studies on the biochemical basis of the observed effects of thiourea are imperative.

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