Research Information

Excised leaf water loss in wheat (*Triticum aestivum* L.) as affected by short periods of heat and water-deficit treatment followed by recovery

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Abstract

Drought and high temperature are especially considered as key stress factors with high potential impact on crop yield. Two contrasting wheat (*Triticum aestivum* L.) cultivars WH730 (high temperature tolerant) and UP2565 (high temperature sensitive) were tested for differential response to short periods of combined and individually applied high temperature (HT) and drought (D) stress as well as revival to examine differences for excised leaf water loss as drought and heat tolerance character. Assessment of water loss from excised leaves has shown promise for characterizing drought resistance and thermo-tolerance in wheat genotypes. The effects of high temperature and drought were additive. High temperature increased the degree of water stress and the combined effects of drought and high temperature were more severe than those of each individual treatment.

Key words: drought, tolerance, excised leaf water loss, wheat, high temperature

Abbreviations: ELWL: Excised leaf water loss; HT: high temperature; D: drought; HT + D: high temperature + drought; DAA: days after anthesis; DAS: days after sowing; PWP: permanent wilting point; RH: relative humidity

Introduction

Bread wheat (*Triticum aestivum* L.), due to its wide adaptability it can be grown under diverse agro-ecological conditions ranging from temperate to subtropical climates. Thus, considerable climatic differences in temperature and relative humidity exist in these areas and wheat crop experiences wide seasonal variations which causes large annual fluctuations in the yield (Munjal and Dhanda, 2005). Under field conditions wheat plants are often simultaneously exposed to soil drying and high temperature stress. These two stress factors could create water deficit in plant tissues, which, in turn, may affect the yield. On a global basis, high temperature in conjugation with coincident drought poses the most important environmental constraint to plant survival and to crop productivity. Drought is often accompanied by relatively high temperatures, which increases the evapotranspiration, reduces photosynthetic capacity of plants consequently reducing crop yields (Reynolds and Ortiz, 2010). Production of plants tolerant to high temperature and drought stress is of immense significance in the light of global warming and climate change. Genetic improvement of wheat for drought/heat

resistance requires a search for possible physiological components of stress resistance and the exploration of their genetic variation (Passioura, 2010; Sinclair, 2011). Synchronization of growth duration with the expected or the predicted seasonal soil moisture supply is an important aspect of plant breeding for water-limited environments (Blum, 2009). A large number of plant water relation parameters have been identified for use in breeding programmes (Zaman-Allah et al., 2011). Assessment of water loss from excised leaves (ELWL) has shown promise for characterizing drought resistance and thermotolerance in wheat genotypes (Clarke and Richards, 1988; Clarke et al., 1989; McCaig and Ramagosa, 1991; Mir et al., 2012). This trait is moderately heritable and can be easily determined in large population (Dhanda and Sethi, 1998; Kumar and Sharma, 2007). Following excision, stomata close and after 20 to 30 min the rate of water loss enters a linear phase that lasts for several hours (McCaig and Romagosa, 1991). During this phase the water is lost from incompletely closed stomata. This trait also influences the recovery of plant from stress and consequently affects yield and yield stability. If water retention capacity of wheat genotypes is increased, the yield of rainfed wheat could be increased or at least stabilized. This parameter can also be easily determined, and is hence applicable for use in large populations. Since identification of germplasm having drought/heat tolerance is of paramount importance to develop new stress tolerant cultivars, the systematic characterization of differences in physiological responses to stress among elite lines may lead to a better understanding of underlying mechanisms.

Materials and Methods
a) Plant materials and growing conditions:
Two contrasting wheat (*Triticum aestivum* L.) cultivars WH730 (thermo-tolerant) and UP2565 (thermo-sensitive) were tested for differential response to high temperature and drought tolerance for excised leaf weight loss. Plants were raised in earthen pots (30 cm in diameter) lined with polythene bags and each containing 5 kg of dune sand (*Typic torripsamments*) [93.3% sand + 3.0% silt + 3.7% clay, saturation capacity 25 %, pH 8.2, ECe_2 0.8 dS m^{-1} at 25°C, 10.3 mg (N) kg^{-1}, 2.5 mg (P) kg^{-1}, 180 mg (K) kg^{-1}] under natural conditions of a screen house. After thinning four healthy plants were maintained in each bag. The experiment was three factors complete randomized design (CRD). CD was calculated at 5% level of significance.

b) High temperature and water deficit treatments:
i. Control [Field capacity (20-22%) and ambient temperature (100-260C) during growth of crop]
ii. High temperature stress (by shifting the pots to polyhouse for one week with maximum temperature 5-80C > than ambient)
iii. Drought [Drought was imposed by withholding water supply till permanent wilting point (PWP, gravimetric soil moisture 6-7%) was attained.]. The plants were re-irrigated (600 ml water per pot to attain field capacity) after PWP.
iv. High temperature along with drought [Combined stress was given to plants by shifting pots to polyhouse for one week and simultaneously drought conditions were maintained by withholding water supply].
  Temperature, relative humidity (RH) and soil moisture (gravimetrically) were recorded during treatment period under screen house and polyhouse (Fig. 1 and Table 1).

c) Treatment imposition stages:
The plants of both varieties of wheat viz. WH730 and UP2565 were exposed to high temperature, drought and the combination of both stresses at the following stages:
i) Booting stage (60-65 DAS)
ii) Post anthesis stage (90-95 DAS)
iii) (i) + (ii)

d) Sampling:
The plants were observed for temporary wilting in the evening and only those plants which did not recover during the night were measured on the following day. The excised flag leaves were placed in polythene bags and transported to the laboratory as quickly as possible in order to minimise water losses due to evaporation. The plants were sampled at the termination of stress and one week after the revival period.

e) Excised leaf weight loss (mg h^{-1}):
Three flag leaves of each variety per treatment were excised from the plant and their fresh weight was immediately recorded. These leaves were then kept in an incubator at 28°C at 50% relative humidity and their weights were recorded after every hour to determine the loss in weight per hour. The weight loss (mg h^{-1}) from the excised leaves in the form of water vapours was calculated for each genotype as:

Rate of ELWL (1st h) = Initial weight excised leaf weight after 1st h
Rate of ELWL (2nd h) = Excised leaf weight after 1st h – excised leaf weight after 2ndh
And so on up to five h.
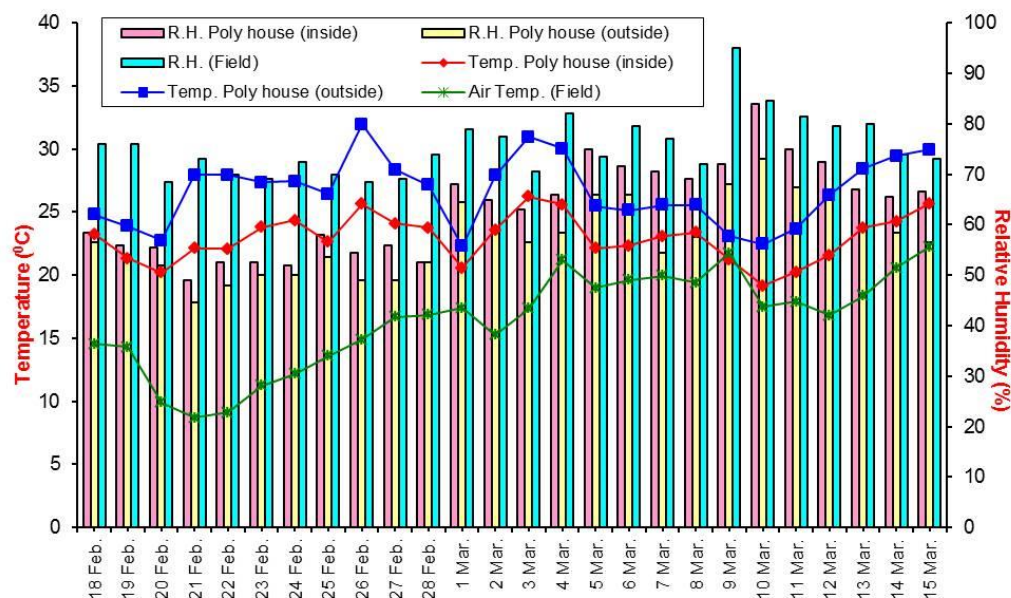
Fig. 1. Daily temperature and relative humidity conditions in polyhouse and field during the imposition of individually applied high temperature (HT), drought (D) and combined (HT+D) stress on wheat genotypes.

Table 1. Average gravimetric soil moisture percentage in sand at the termination of individually applied high temperature (HT), drought (D) and combined (HT+D) stress treatments in var. UP2565 and WH730 at different stress imposition stages.

| Treatment imposition stage	Treatments	Soil moisture (%) in UP2565	Soil moisture (%) in WH730
Booting stage	Control (C)	21.60 (1.42)	21.68 (1.35)
	High temperature (HT)	20.42 (1.97)	21.46 (1.71)
	Drought (D)	6.05 (1.02)	6.15 (1.12)
	High temperature + Drought (HT+D)	5.95 (0.92)	5.48 (1.41)
Post-anthesis stage	Control (C)	20.68 (1.49)	21.79 (1.37)
	High temperature (HT)	21.02 (1.62)	20.00 (1.48)
	Drought (D)	6.17 (1.12)	6.02 (1.22)
	High temperature + Drought (HT+D)	5.70 (1.69)	5.24 (1.04)
Booting + Post-anthesis stage	Control (C)	22.51 (1.88)	21.60 (1.82)
	High temperature (HT)	20.13 (1.99)	21.54 (1.68)
	Drought (D)	6.02 (1.52)	6.45 (1.42)
	High temperature + Drought (HT+D)	5.75 (1.42)	5.42 (1.51)

Values are means with S.E. in parenthesis.

Results and Discussion

ELWL in var. UP2565: Fig. 2 represents the rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment imposed at booting stage in var. UP2565. Maximum loss in water vapours from the excised flag leaf was observed during the 1st h of excision which was very fast and then slowly declined up to five h. The rate of weight loss was different because of differential rate of stomatal closure during different treatments. Interactive HT+D stress resulted in minimum (25.90 mg) ELWL. After revival, rate of ELWL during 1st h was less than the stress treatments, however maximum reduction was observed during 1st h of excision. Slope was low as compared to termination of stress treatments. Interactive HT+D stress resulted in maximum loss of water vapours during all the five h of observation in comparison to HT and drought revived plants. At post-anthesis stage (Fig. 3) the loss was very rapid, except interactive HT+D stress treatment. Out of all the stress treatments, maximum rate of ELWL during first h was recorded in HT stress (75.5 mg) while HT+D resulted in minimum loss (22.1 mg). After revival, rate of ELWL due to drought stress was higher than that of other stress treatment. Magnitude of ELWL (booting+post-anthesis stage) was same to post-anthesis stage (Fig. 4). Highest rate of ELWL was observed in HT stress (67.45 mg) while lowest in interactive HT+D stress (33.56 mg) during 1st h of observation. After revival, reversal of this situation was observed where interactive HT+D stress resulted in maximum rate of ELWL (47.7 mg) and HT stress led to minimum ELWL (28.25 mg) when compared to control (54.4 mg) during 1st h of observation. This indicates differential stomatal conductance during different stress treatments.

ELWL in var. WH730: Overall magnitude of ELWL was less at booting stage (Fig. 5). Rate of ELWL was lower than var. UP2565. Similar to results observed in UP2565 (Fig. 2) rate of ELWL in WH730 declined progressively during 2nd to 5th h of observation indicating slow stomatal closure. Out of all the three stress treatments, interactive HT+D stress resulted in maximum decline during all the five h of observation in comparison to HT and drought revived plants. At post-anthesis stage (Fig. 6) maximum rate of ELWL was recorded due to HT stress (55.4 mg) in comparison to control, however the same was less when compared to var. UP2565 (Fig. 3). ELWL of plants relieved from drought stress was higher (43.7 mg) during all the five h of observation in comparison to other two stresses;
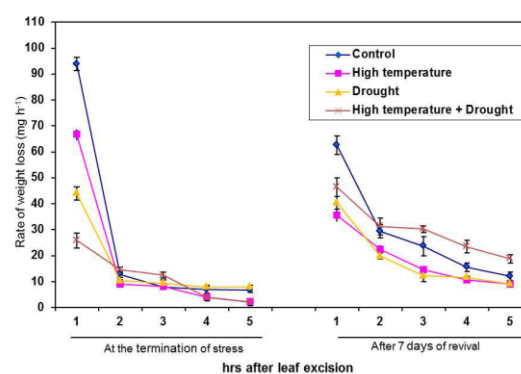
Fig. 2. Rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment given at booting stage in var. UP2565.
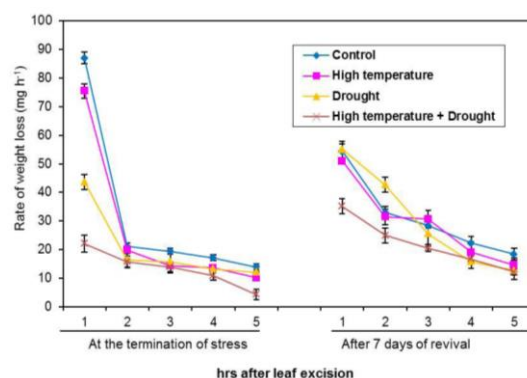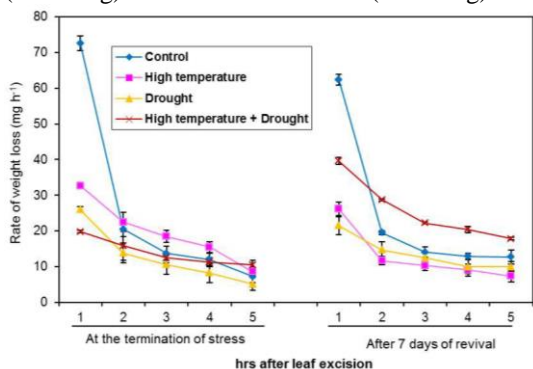

Fig. 3. Rate of excised flag leaf weight loss at the termination and after 7 days of revival of stress treatment given at post-anthesis stage in var. UP2565.
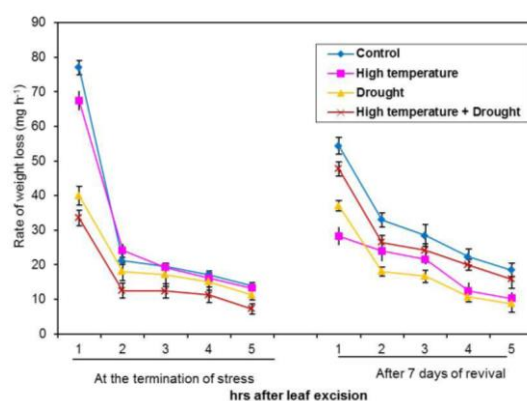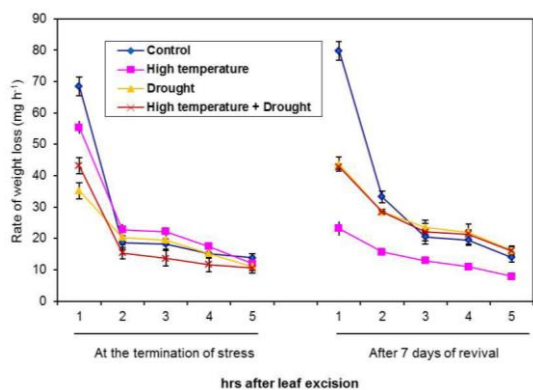

Fig. 4. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting + post-anthesis stage in var. UP2565.

however it was still lower in comparison to var. UP2565. Results from Fig. 7 reveal rate of ELWL when stress was imposed at booting +
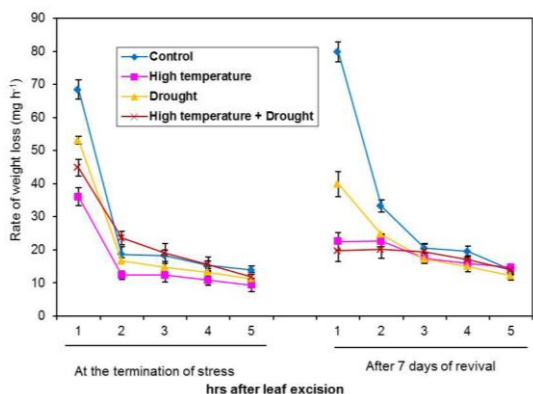
post-anthesis stage. ELWL was high in drought (63.15 mg) and interactive HT+D (64.85 mg) than

Fig. 5. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting stage in var. WH730.

Fig. 6. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at post-anthesis stage in var. WH730.

Fig. 7. Rate of ELWL at the termination and after 7 days of revival of stress treatment given at booting + post-anthesis stage in var. WH730.

HT (36.05 mg) during 1st h of observation however, the same was less than that observed in UP2565 (Fig. 4). After revival, the ELWL of

drought relieved plants was higher (39.9 mg) in comparison to other two stress treatments.

The results show higher excised leaf water loss in UP2565 compared to WH730. This indicated that closing of stomata was not as rapid in UP2565 and continued to lose more water through transpiration than WH730. The result of stress treatments were more acute at post anthesis stage relative to other two stages for plants sampled after stress as well as revival. Relative to stress termination, decline in ELWL was noted after revival at each hour of observation (Fig. 5-7) thus suggesting persisting effects of stress treatments after one week of revival period.

Conclusion

ELWL may more closely reflect the balance between water supply to the leaf and transpiration rate. This improves the ability of the plant to recover from stress and consequently the grain yield and its stability. Genotypes indicating low excised leaf-water loss under drought or heat stress have better capability to maintain water balance in their leaves seems to be attributable to stress tolerance indicating considerable scope for selection under stress conditions. This parameter can be easily determined and is hence applicable for use in large populations.

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