

Investigation on the Physiological Basis of Grain Yield and Drought Resistance in Wheat: Leaf Photosynthetic Rate, Stomatal Conductance, and Non-stomatal Limitations

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ABSTRACT

Field and glasshouse studies were undertaken to relate leaf photosynthesis rate as well as stomatal and non-stomatal behaviors to drought resistance and yield performance of wheat. Based on a two-year field trial, 11 wheat cultivars were divided into three groups of susceptible, intermediate and resistant, using stress susceptibility index (SSI) and then were further examined in a glasshouse experiment. Water stress decreased leaf photosynthesis rate (P_n), stomatal conductance (g_s) transpiration rate (T_r) and mesophyll conductance (MC), and increased sub-stomatal CO_2 concentration (C_i). Cultivars differed in their response to water stress. In general, resistant cultivars showed a higher P_n and g_s and leaf water content under both moisture conditions comparing to susceptible ones. A greater reduction in g_s and T_r and a smaller reduction in P_n under stress condition led to a remarkably higher photosynthetic water use efficiency of the resistant cultivars. A greater decrease in MC and a greater increase in C_i due to water stress of the susceptible cultivars exhibited the enhanced sensitivity of the photosynthetic apparatus to water stress.

Key Words: Wheat; Drought resistance; Photosynthesis; Stomatal conductance; Mesophyll conductance

INTRODUCTION

Understanding of physiological mechanisms that enable plants to adapt to water deficit and maintain growth and productivity during stress period could help in screening and selection of tolerant genotypes and using this trait in breeding programs (Zaharieva *et al.*, 2001). Among the physiological processes, photosynthesis (P_n) is the basic determinant of plant growth and productivity and the ability to maintain the rate of carbon assimilation under environmental stress is fundamental importance to plant production (Lawlor, 1995).

Association between wheat grain yield and P_n has been reported (Loomis & Amthor, 1999; Reynolds *et al.*, 2000). Water stress causes reduction in the rates of P_n and transpiration (T_r) in many crop species (Condon *et al.*, 2002; Guo Li *et al.*, 2000). The reduction in P_n can be attributed to stomatal and non-stomatal factors (Del Blanco *et al.*, 2000; Ahmadi, 1998). The importance of stomatal closure in regulating P_n under water stress condition can be recognized by the findings showing parallel reduction in P_n and stomatal conductance (g_s) (Del Blanco *et al.*, 2000; Koc *et al.*, 2003). Although stomatal closure limits CO_2 flux to the site of its fixation in chloroplast, it could contribute to the maintenance of leaf turgor and thus improve drought tolerance of plant by limiting water transpirational rate. Frederick *et al.* (1989) reported that cultivars with greater stability in yield performance over a range of moisture conditions showed greater drought-induced increase in

stomatal resistance.

Some investigators, on the other hand, reported that MC was the dominant factor in the expression of high or low P_n (Barutcular *et al.*, 2000). There is extensive experimental evidence that water stress has direct effect on chloroplast biochemistry (non-stomatal factors) including decrease in photosystem I and II activities, the inhibition of Calvin cycle, and reduction in photophosphorylation activity (Lawlor, 1995). There are numerous reports in which C_i has remained unchanged, or even increased in leaves of stressed plants in spite of a decline in g_s . It has been concluded that mesophyll capacity to assimilate CO_2 was affected by water stress. In many cases non-stomatal inhibition of P_n has been estimated based on C_i (Ahmadi, 1998).

Genotypic variation for photosynthesis rate, stomatal and non-stomatal parameters under water stress conditions have been reported (Koc *et al.*, 2003; Stiller *et al.*, 2005). This variation indicates potential for genetic advancement through selection programs. The extent to which these physiological parameters can contribute to grain production as well as drought resistance in wheat cultivars has not been well investigated. The present work was conducted to investigate leaf gas exchange parameters (P_n , g_s , T_r) and water status as well as the relative importance of stomatal and non-stomatal factors controlling photosynthesis rate in 11 wheat cultivars differing in potential yield and drought resistance.

MATERIALS AND METHODS

Field experiments. The field experiments were conducted at Dry land Research Stations of Gerizeh (35°16' N, 47°1' E, elevation 1373m) in Kurdistan Province (Iran) in 2001 and 2002. Long-term mean annual rainfall was 440 mm and the soil texture was clay-loam (27% Clay, 36% silt and 37% sand) with 0.82% organic matter and a pH of 7.4. Absorbable P and K were 7 and 356 mg kg⁻¹ respectively.

Eleven cultivars including Sardari, Sabalan, Roushan Back cross, Alvand, Mahdavi, Agosta-Sefid, Toos, Cross-Shahi, Azar2, Glinson and M-75-7 which are commonly grown under rain-fed or irrigation conditions were chosen for this study. A factorial experiment with a split plot design and four replications was employed. Two moisture regimes – non-irrigated (rain-fed) and irrigated conditions - were applied to the main plots. Irrigated plots received irrigation at the planting, tillering, jointing, flowering and grain filling stages. The non-irrigated plots received no water apart from rainfall. Total precipitation in 2001 and 2002 were 236 and 360 mm respectively and mean temperature during growing seasons ranged between 13 to 28 °C. In all the experiments, sowing was carried out in November. In each plot, 6 rows of each cultivar 20-cm apart and 6-m long were sown. Ammonium phosphate and zinc sulphate at the rate of 100 and 25 Kg ha⁻¹ respectively were applied at sowing. Urea (100 Kg ha⁻¹) was added at jointing stage.

The grain yields were estimated by harvesting a 4.2 m² area in the center of each plot at crop maturity. The harvested materials were dried and threshed to obtain the yield of each cultivar. Stress susceptibility index (SSI) was used to differentiate resistant and susceptible cultivars (Fischer & Maurer, 1987) as follow:

$$SSI = (1 - (Y_s / Y_p)) / (1 - (\bar{Y}_s / \bar{Y}_p))$$

Where \bar{y}_s and \bar{y}_p are the mean grain yields of all cultivars under stress and non stress, and Y_s and Y_p are grain yield of each cultivars under stress and non-stress conditions, respectively.

Glasshouse experiment. Eleven cultivars of wheat, described above, were grown in pots (15 x 25 cm) containing 2.4 kg mixture of clay, sand and manure (2:3:2) in a greenhouse. A factorial experiment based on completely randomized design with three replications, were employed. Three pots were allocated to each plot. Plants were vernalised at three-leaf stage by transferring pots outside of greenhouse from 21st of Jan to 28th Feb. Emerged seedlings were thinned to three plants per pot and watered daily until anthesis when stress treatment began by withholding water for five days. Soil moisture content at FC and at the end of stress period in stressed pots were 20% and 7% respectively. All measurements were made at 11 a.m. on flag leaves (FL) after five days of withholding water in both

stressed and non-stressed plants.

Simultaneous measurements for P_n , g_s , T_r and C_i were made on intact FL in an open system using a portable infrared gas analyzer equipped with Parkinson narrow leaf chamber (LCA4, ADC, Hoddeson, U.K.). Measurements were made from the middle part of FL at PAR of 1000 to 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Mesophyll conductance was calculated by dividing P_n by C_i (Fischer *et al.*, 1998). Photosynthetic water use efficiency (PWUE) was calculated by dividing P_n by g_s . Leaf relative water content (RWC) was determined based on method of Barrs and Weatherley (1962). Leaf water potential was determined using Pressure Chamber technique. Data were subjected to ANOVA using MSTATC program and means were compared using the LSD value.

RESULTS AND DISCUSSION

Data revealed that SSI values for Alvand (with highest SSI), M-75-7 and Mahdavi were significantly higher than those for Sardari (with the lowest value), Agosta Sefid, Azar2 and Sabalan (Table I). It has been proposed that genotypes with an SSI value of less than unit are drought resistant (Fischer & Maurer, 1987). Thus the three former can be considered as susceptible cultivars and the four latter as drought resistant ones. Susceptible cultivars produced greater yield under irrigated condition, however, this advantage disappeared under stress condition (Table I). The yield reduction of the resistant and susceptible cultivars under stress condition were 55% and 72% respectively (Fig. 1).

The main effects of moisture and cultivar were highly significant for all the measured traits. The interaction between cultivar by moisture was also significant for all traits, except C_i . A dramatic decline was observed in leaf RWC and water potential (Table IIA) (Siddique, *et al.*, 2000; Halder & Burrage 2003; Basu *et al.*, 2004). Detrimental effect of cell dehydration on physiological and biochemical reactions and consequently growth and productivity is well documented (Lawlor, 1995). Thus, an ability to maintain high water potential or RWC under stress conditions could be an adaptive feature. In the present study, resistant cultivars were able to maintain greater RWC (62%) compared to susceptible ones (58%). The stability of photosynthetic components could be attributed by maintenance of positive leaf turgor under stress as a result of osmotic adjustment (Basu, *et al.*, 2004). In general, resistant cultivars performed higher P_n , g_s and T_r than susceptible ones under either conditions (Fig.1&2). As observed for grain yield, water stress caused a significant reduction in P_n (85% across the cultivars). Similar results have been reported by other investigators (Siddique *et al.*, 2000, Stiller, *et al.*, 2005; Ratnayaka & Kincaid, 2005). This appears to be a possible physiological mechanism by which drought can affect growth and productivity of crops such as wheat (Lawlor, 1995).

Table I. Grain yield (kg ha⁻¹) and stress susceptibility index (SSI) of eleven wheat cultivars grown under irrigated and non-irrigated field condition in 2001 and 2002.

	2001		2002		SSI
	Irrigated	Non-irrigated	Irrigated	Non-irrigated	
Alvand	4220±241	1030±88	4720±312	2030±219	1.16±0.03A
M-75-7	4370±242	1275±112	4720±218	2540±236	1.09±0.05AB
Mahdavi	4940±149	1187±114	4900±218	2810±245	1.09±0.06AB
Toos	5400±275	942±57	3790±243	2650±108	1.03±0.02BC
Cross-Shahi	3850±138	1105±58	4890±188	3040±179	1.02±0.02BC
Glinson	4340±277	1142±84	4690±247	2840±147	1.00±0.04BC
Roushan-Back cross	4860±243	1205±84	4190±285	2570±103	1.00±0.02BC
Sabalan	4240±224	1705±113	3720±207	2330±183	0.97±0.02CD
Azar 2	4320±93	1652±108	3520±289	1900±152	0.88±0.02D
Agosta Sefid	3900±117	1385±128	3040±283	2100±88	0.87±0.02D
Sardari	3730±126	1462±110	2730±186	2260±150	0.68±0.04E
Mean	4340	1224	4080	2460	1.00
LSD (5%)	610	391	630	540	0.06

Means followed by the same letters in SSI column are not significantly different at the probability level of 0.05 according to Duncan's Multiple Range Test. Means are followed by standard errors. LSD: least significant difference at 5% probability

Table II. Leaf water potential, ψ , (Mp), relative water content, RWC, (%), photosynthesis rate, P_n , ($\mu\text{mol CO}_2 \text{ s}^{-2} \text{ s}^{-1}$) stomatal conductance, g_s , ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), (A), transpiration rate, Tr, ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), photosynthetic water use efficiency, PWUE, ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$), internal CO_2 concentration, C_i , (ppm) and mesophyl conductance ($\text{mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) (B), of irrigated and non-irrigated wheat cultivars in glasshouse condition. Means are followed by standard errors.

	irrigated				Non-irrigated			
	ψ	RWC	P_n	g_s	ψ	RWC	P_n	g_s
Alvand	-1.6±0.06	90.1±1.3	12.0±1.6	178±14	-2.29±0.13	62.0±2.2	1.83±0.28	17.1±3.7
M-75-7	-1.75±0.10	91.4±0.9	8.2±0.8	109±16	-2.56±0.14	56.3±2.9	1.44±0.17	10.9±1.1
Mahdavi	-1.98±0.11	91.3±1.3	9.5±1.0	90±26	-2.64±0.09	55.9±2.1	0.64±0.28	19.1±1.1
Toos	-1.78±0.06	91.3±1.3	15.0±1.9	167±22	-2.53±0.07	55.4±3.0	2.49±0.11	12.6±0.7
Cross-Shahi	-1.79±0.09	94.8±1.2	17.3±2.0	205±11	-2.74±0.11	67.8±1.7	2.16±0.13	10.0±0.9
Glinson	-1.91±0.09	91.9±1.0	12.6±0.9	157±14	-2.78±0.06	59.9±2.8	0.91±0.30	19.8±3.5
Roushan-Back cross	-1.47±0.07	91.4±0.8	11.4±1.1	178±25	-2.57±0.11	74.2±3.9	2.47±0.23	19.3±3.7
Sabalan	-1.79±0.07	89.6±0.5	7.4±0.6	96±18	-2.52±0.08	63.4±4.6	2.15±0.09	16.6±1.5
Azar 2	-1.97±0.011	90.2±1.1	11.0±1.3	219±21	-2.36±0.10	62.5±2.4	1.98±0.18	19.3±1.2
Agosta Sefid	-1.71±0.07	91.9±0.7	13.5±1.7	174±26	-2.62±0.09	62.4±2.2	2.54±0.21	18.1±3.0
Sardari	-1.73±0.08	89.0±0.8	20.4±1.6	270±8	-2.24±0.10	59.1±0.9	2.44±0.18	21.7±2.0

	Non-stressed				Water stressed			
	Tr	PWUE	C_i	MC	Tr	PWUE	C_i	MC
Alvand	7.23±1.1	63.8±9.9	170±12	70±10	1.42±0.25	74.2±27	272±11	6.8±1.2
M-75-7	4.69±0.61	77.3±10	199±23	41±8	0.9±0.06	129.5±31	249±6	5.8±1.0
Mahdavi	4.23±0.62	136.6±15	181±24	52±12	1.14±0.03	35.3±16	337±22	1.9±0.7
Toos	6.0±0.25	89.2±7.4	155±17	96±7	1.06±0.02	200.0±11	256±12	9.7±0.5
Cross-Shahi	5.35±0.54	84.4±6.5	191±11	91±14	0.79±0.09	231.2±18	261±16	8.3±0.8
Glinson	7.32±0.94	81.4±8.4	195±9	64±6	1.13±0.08	84.8±23	317±34	2.9±1.1
Roushan-Back cross	6.61±0.87	66.2±8.7	146±9	78±6	1.33±0.09	173.2±19	249±26	10±1.9
Sabalan	5.34±0.57	76.8±7.2	219±16	34±5	1.28±0.12	146.5±11	228±14	9.4±5
Azar 2	8.07±0.22	50.2±10.	218±19	50±9	1.24±0.07	102.5±24	302±18	6.6±1.2
Agosta Sefid	6.36±1.13	81.8±9.4	173±10	78±11	1.4±0.06	211.3±32	236±16	10.8±1.4
Sardari	7.16±0.53	75.2±6.3	200±6	102±8	1.32±0.13	118.6±17	265±12	9.2±1.1

Decline in leaf P_n under water stress was accompanied by decline in leaf RWC (Table IIA). Siddique *et.al.* (2000) reported that the higher leaf water potential and relative water content of wheat cultivars were associated with a higher photosynthetic rate. Leaf dehydration can lead to turgor loss of guard cells causing passive stomatal closure, which in turn, would reduce g_s and consequently supply of CO_2 to the fixation site. A remarkable decline in g_s (89%) and P_n (85%) due to water stress implies to the importance

of stomatal limitation to P_n under water stress in the examined cultivars (Table IIA). Although reduction in g_s under water stress limits P_n , it may, on the other hand, reduce transpirational water loss which can be beneficial for plant under limited moisture supply. Compared to the susceptible ones, resistant cultivars manifested a greater reduction in g_s and Tr, but a smaller reduction in P_n under stress condition (Fig.1 & 2). The interesting consequence of such responses was a remarkable increase (100%) in

photosynthetic water use efficiency (PWUE) of the resistant and a 15% decrease in PWUE of the susceptible cultivars under stress condition (Fig. 2). Condon *et al.* (2002) explained that, the ratio of CO₂ assimilation rate to transpiration rate at the stomata may be one means of achieving greater yield per unit rainfall in dryland area. It has been hypothesized that any improvement in components of water use efficiency (WUE) would be expected to partially reduce the adverse effects of water stress. (Stiller *et al.*, 2005).

Despite decline in g_s, water stress increased C_i of cultivars an average of 45% (Table IIB) implying an inability of photosynthesis machinery to utilize internal CO₂ (Luo, 1991). A greater amount of water stress-induced increase in C_i was observed in susceptible cultivar compared to resistant ones (Fig. 2). This indicated a greater sensitivity of photosynthesis apparatus of susceptible cultivars to water stress. Mesophyll conductance (MC), proposed by Fischer *et al.* (1998) is another indicator of non-stomatal factors involved in CO₂ assimilation. Mesophyll conductance was generally greater in resistant cultivars (Fig. 2). Water stress reduced the MC of leaves up to 89%, the reduction being greater in susceptible cultivars than the resistant ones (Table IIB). This ability to maintain high carbon gain appears to confer stress tolerance in crops (Ratnayaka & Kincaid, 2005). Greater decline in g_s and a smaller decline in MC were observed for resistant cultivars compared to susceptible ones. Therefore, it can be concluded that under water stress condition P_n of resistant

Fig. 1. Grain yield (g.m⁻²), leaf relative water content, RWC, (%), photosynthesis rate (μmol CO₂ m⁻²s⁻¹) and stomatal conductance (mmol H₂O m⁻² s⁻¹) of resistant and susceptible wheat cultivars under stress and non-stress conditions in glasshouse experiment. Vertical bars represents standard error.

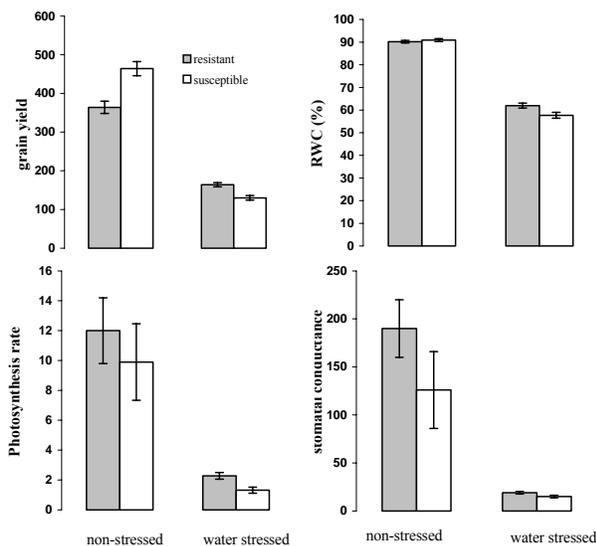
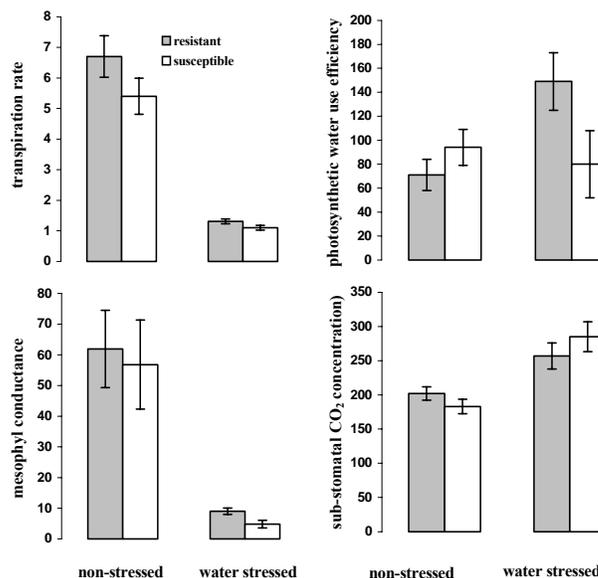


Fig. 2. Leaf transpiration rate (mmol H₂O m⁻² s⁻¹), photosynthetic water use efficiency (μmol CO₂ mol⁻¹ H₂O), internal CO₂ concentration (ppm) and mesophyll conductance (mmol CO₂ m⁻² s⁻¹) of resistant and susceptible wheat cultivars under stress and non-stress conditions in glasshouse experiment. Vertical bars represents standard error.



cultivars is primarily limited by stomatal rather than non-stomatal factors (Basu *et al.*, 2004). This kind of limitation could be an advantage to conserve water under limited water supply. In absolute terms, however, Tr and g_s was generally higher in resistant than susceptible cultivars. Stomatal closure under water stress and high irradiance rate may cause photo oxidative damage to chloroplast (Smirnoff 1995), increased leaf temperature (Siddique *et al.*, 2000; Halder & Burrage, 2003) and reduced uptake of water and nutrient by root as a result of reduced Tr (Verona & Calcagno, 1991). It has been hypothesized that genotype which keep their stomata open under stress condition while maintaining adequate leaf RWC can be considered as suitable for dry region (Blum *et al.*, 1981). In the present study, resistant cultivars had higher values of Tr and RWC indicating their greater ability to water uptake from the soil compared to susceptible ones.

CONCLUSION

Photosynthetic rate is reduced by stomatal, non-stomatal and leaf water status parameters. Stomatal and non-stomatal inhibition to P_n under stress condition may vary in susceptible and resistant cultivars. High Leaf P_n, RWC and MC appear to be involved in drought resistance. A smaller stress-induced reduction in P_n and a greater stress-induced reduction in g_s leading to increased PWUE could be an adaptive response in resistant cultivars. However, in term of

absolute values, higher T_r and g_s are associated with better performance of resistant cultivars under stress condition.

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