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Measuring leaf temperature and stomatal conductance to evaluate leaf water content in barley cultivars under terminal drought stress

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Abstract

Drought stress is the main abiotic stress that limits crop production especially cereals that cope with water limitation in reproductive stage in semi arid areas. In this study, stomatal conductance and leaf temperature were consider as the precise and non destructive methods to evaluate water status in 3 barley cultivars under stress (withholding irrigation from anthesis until the end of growth season), and non stress (normal) conditions. The experiment was conducted in factorial based on CRD with three replications in greenhouse in Iran. Results indicated drought stress conditions significantly reduced leaf water content, stomatal conductance, cell membrane stability, number of tiller per plant, grain yield and harvest index. On the other hand, drought stress increased leaf temperature and hollow percent of grains in all cultivars ($P<0.01$). A significant correlation was observed between grain yield and stomatal conductance ($r=+0.94$), water content ($r=+0.95$), leaf temperature ($r=-0.66$), cell membrane stability ($r=+0.62$), number of tiller per plant ($r=+0.65$), number of grain per spike ($r=+0.51$) and harvest index ($r=+0.96$) in 20 days after anthesis. According to significant correlation between stomatal conductance and leaf temperature with other traits, using the infrared thermal image and prometer as the reliable methods to study water status in drought stress condition instead of other destructive methods are recommended.

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Introduction

Drought stress is considered one of the major environmental factors, limiting the crop productivity worldwide (González *et al.,* 2008). Among different types of drought stress, terminal drought is the most important stress in areas with Mediterranean climates like in Iran (Bannayan *et al.*, 2010). Iran is located in an arid and semi-arid part of the earth (FAO, 2013), where rainfall decreases as soil evaporation increases in spring when cereals crops enters the grain-filling period (Ehdaie *et al.*, 2006). So grain yield of small-grained cereals such as barley (*Hordeum vulgare* L.) which is cultivated in these regions (Tambussi *et al.*, 2005), can be largely influenced by terminal drought stress (Francia *et al.*, 2011).

Plants implement a variety of different mechanisms to withstand drought stress (Sicher *et al.*, 2012). Stomata closure is the well-known first responsive event of plants to water deficiency (Lisar *et al.*, 2012), and this negatively impacts rates of CO_2 assimilation (Sicher *et al.*, 2012). Water deficit by closing stomata, causes increasing leaf temperature, considering the major determinant of leaf temperature is the rate of evaporation or transpiration from the leaf (Jones *et al.*, 2009).

Rising temperature causes damage to cell membranes, since membranes play an important role in different cell activities, the maintenance of cell membrane integrity during drought stress conditions, is a key factor in drought resistance (Chaves and Oliveira, 2004; Torres-Franklin *et al.*, 2007; Labuschagne *et al.*, 2008). The increased solute leakage, as an indication of decreased cell membrane stability (CMS), has long been used but the relationship between CMS and crop yield may vary from plant to plant and it needs to study individual crops before using it as an important physiological selection criterion (Wahid *et al.*, 2007).

Some measurement such as leaf temperature using thermal infrared imaging is primarily used to study plant water relations, and specifically stomatal conductance, especially under laboratory conditions (Raskin and Ladyman 1988; Merlot *et al.*, 2002), because leaf temperature is dependent on environmental factors such as air temperature, humidity, wind speed (Jones *et al.*, 2009). Thermal imaging appears to have potential advantages over the use of porometer or gas-exchange measurement when screening for stomatal responses in phenotyping studies. It has been previously used successfully for monitoring differences in stomatal responses to drought in crops such as wheat and rice (Reynolds *et al.*, 1998, Garrity and O'Toole 1995).

Studies have shown that canopy temperature (CT) is correlated with many physiological processes such as stomatal conductance, transpiration rate, plant water status, water use, leaf area index and crop yield (Lopes and Reynolds, 2010). Both grain yield and spikelet fertility were significantly correlated with canopy temperature in rice cultivars, and high drought-avoidance rice cultivars remain coolest under stress (Garrity and O'Toole 1995). Genotypes with cooler canopy temperatures can be used to indicate a better hydration status (Pietragalla, 2012).

This paper outlines the application of rapid and non destructive instrument such as infrared thermal imaging camera and prometer to study water content in barley cultivars under terminal drought stress, with particular emphasis on the relations between stomatal conductance, leaf temperature and grain yield in greenhouse condition.

Materials and methods

Plant material and growth conditions

Three barley (*Hordeum vulgare* L.) cultivars; Yousof, Fajr 30 and Morocco, were chosen for this study. For each cultivar, 400 seeds were allowed to germinate on filter paper moistened with distilled water for 24 hours in a growth chamber at 25/20 C° (day/night) under 10/ 14 hours (light/ dark). The germinating seeds were then transferred to pots (8 seeds per pot) containing a mixture of field soil, sand and fertilizer (2:1:1) and maintained in greenhouse condition at Agricultural Biotechnology Research Institute of Iran

(ABRII) in Karaj in 2011- 2012. For each cultivar 48 pots were considered and totally 144 pots used in this experiment. The experiment was conducted in factorial based on completely randomized design (CRD) with two treatments (well-watered and drought-stressed) and three replications.

Plants were grown under well-watered condition until anthesis. When the cultivars were at the anthesis stage, drought stress was started by withholding water but the control pots were irrigated daily (at 70% AWC) until maturity stage. When the soil moisture in drought stress pots reached to 30% AWC (about 10 days after anthesis) and 10% AWC (about 20 days after anthesis), measurements in control and treated plants were done.

Measurments

Leaf water content, stomatal conductance, leaf temperature and cell membrane stability

Leaf Water Content (LWC) was calculated using the following formula:

LWC = (FW-DW/FW) × 100..................(1).

Where FW is fresh leaf weight and DW is dry leaf weight.

Stomatal conductance of 8 flag leaves in each replication was measured by Porometer (AP4, Delta-T Devices) early in the morning.

In order to measure leaf temperature, an infrared thermal camera (IVN 770-P) was used in midday (measurement time was between 12 and 1 p.m.). The pictures were evaluated by special software of the camera (Fig. 1).

The cell membrane stability (CMS) was determined by measuring the electrical conductivity following the method of Monneveux and Ribaut (2011). As shown in the following formula:

%CMS= 1- (E1/E2) × 100.................. (2).

Where E1 is the electrical conductivity after 24 h in room temperature, and E2 is the electrical conductivity of autoclaved solutions (15 min in 120 °C and after cooling the liquid).

Grain yield and Harvest Index

When the plants were mature, spikes from 10 plants in each replication, cut, weighed and threshed to obtain the grain yield of each cultivar. The number of tiller per plant was measured. Also by counting the number of grain in spike the hollow percent were calculated. The straw was cut to the ground to calculate the total biomass of the crop. The spike, grain and straw were oven-dried (48 h at 72 C°) before weighing. Harvest Index (HI) was calculated according to the formula:

HI= (grain yield/ biological yield) × 100.......... (3).

Statistical analysis

All data were subjected to analysis of variance using the GLM procedure of SAS (Statistical Analysis System ver 9.1) and comparisons were made using Duncan's multiple range tests at $P < 0.05$.

Result and discussion

Results of data analysis showed that drought stress had a significant effect on leaf water content, stomatal conductance and leaf temperature, also cultivars differed significantly in these traits ($p<0.01$) (Table 1). By increasing water stress, leaf water content and stomatal conductance reduced and the leaf temperature increased significantly in all cultivars (Fig. 2 a, b, c, d, e, f). The highest leaf temperature obtained from Fajr 30, also the lowest stomata conductance obtained from this cultivar in severe drought stress condition (10% AWC) (Fig. 2 b, d).

Drought stress had a significant effect on hollow percent of grains and cultivars differed significantly in this trait ($p<0.01$) (Table 1). The highest hollow percent obtain from Fajr 30 (Fig. 2 i). According to high correlation between leaf temperature and hollow percent ($r=+ 0.70$) at 20 days after anthesis in both control and drought stress condition (Table 4), it seems that increasing leaf temperature as a result of stomatal closure in drought stress condition, is the most effective factor to increase the hollow percent of the grains. Also Yousof had cooler leaves in severe drought stress condition (Fig. 2 d), the lowest hollow percent of the grains (9.1%) and the lowest yield reduction (67.23%) when compared to other cultivars (Fig. 2 i, j). The results are similar to Lopes and Reynolds (2010), which was reported a positive correlation between cool canopy temperature and yield under drought stress.

Table 1. Analysis of variance for stomatal conductance, leaf temperature, cell membrane stability and water content of three barley cultivars under terminal drought stress conditions (30% and 10% AWC) and well-watered condition in greenhouse.

S.O.V	df	MS							
		Stomatal conductance (30%)	Stomatal conductance (10%)	Leaf temperature (30%)	Leaf temperature (10%)	Cell membrane stability (30%)	Cell membrane stability (10%)	Water content (30%)	Water content (10%)
water treatment	1	74440.1**	54237.55**	6.46**	42.78**	1189.0**	5567.7**	360.03**	1901.5*
cultivar	2	5349.9**	899.96**	3.15**	12.34**	65.04*	1374.0**	36.20**	185.84**
water treatment × cultivar	2	4297.7**	1890.70**	0.527**	3.73**	5.397^{ns}	590.36**	15.83**	32.07*
Error	12	21.84	59.72	0.0059	0.0075	14.85	14.76	1.46	7.05
CV (%)		4.9	11.2	0.28	0.29	5.2	6.9	1.7	4.1

* and **: Significant at 5% and 1% probability levels, respectively and ns: Non-significant.

Table 2. Analysis of variance for hollow percent, No. tiller, No. grain, Harvest Index and grain yield of three barley cultivars under terminal drought conditions (30% and 10% AWC) and well-watered condition in greenhouse.

S.O.V	df	MS				
		Hollow percent	No. tiller	No. grain	Harvest Index	Grain yield
water treatment	2	140.30**	48.71**	44.04*	712.35**	1.69**
cultivar	2	1162.7**	17.39**	883.34**	23.09^{ns}	0.17**
water treatment × cultivar	4	166.94**	1.64**	21.55^{ns}	113.08**	0.013*
Error	18	1.67	0.097	8.56	10.04	0.004
CV		7.2	8.4	8.9	8.8	8.9

* and **: Significant at 5% and 1% probability levels, respectively and ns: Non-significant.

Drought stress had a significant effect on cell membrane stability, number of tiller per plant, number of grain per spike, grain yield and harvest index, also cultivars significantly varied in cell membrane stability, number of tiller per plant, number of grain per spike and grain yield (Table 1 and Table 2). Cell membrane stability under severe drought stress condition (10% AWC) in all cultivars was significantly reduced compared to the control condition (Fig. 2 g). The most reduction in cell membrane stability obtained from Morocco (61.5%).

Drought stress by reducing water content and leaf stomatal conductance, declined the leaf evaporation, since the major determinant of leaf temperature is the rate transpiration from the leaf (Jones *et al*., 2009), so leaf temperature increased. Rising leaf temperature caused the destruction of cell membranes and cell membrane stability reduced significantly. Also Wahid *et al.* (2007) reported high temperature alter permeability of membranes so the integrity and functions of membranes are sensitive to heat stress. Cell membrane damage caused by drought does not allow the plant to continue normal metabolic processes, (Labuschagne *et al.*, 2008) and hence the plant growth period reduced. On the other hand stress by reducing the number of tillers per plant (Fig. 2 h) actually reduces the plant's production capacity and finally grain yield reduced (Fig. 2 j). The results of stress will be intensified in severe drought stress condition, so that the lowest grain yield obtained in 10% AWC in Morocco (Fig. 2 j). By increasing drought stress severity, yield reduction increased in all cultivars and the most reduction in grain yield (80.6%) obtained from Morocco in 10% AWC.

Table 3. Pearson correlation coefficients between traits of barley cultivars in well-watered and 30% AWC conditions.

	Stomatal conductance	Leaf temperature	Cell membrane stability	Water content	Hollow percent	No. tiller	No. grain	Harvest Index	Grain yield
Stomatal conductance	1								
Leaf temperature	-0.80**	1							
Cell membrane stability	0.77**	-0.43ns	1						
Water content	0.76**	-0.39ns	0.91**	1					
Hollow percent	-0.08ns	0.07ns	-0.24ns	-0.34ns	1				
No. tiller	0.76**	-0.92**	-0.57*	0.50*	-0.05ns	1			
No. grain	0.01ns	0.49*	0.29ns	0.31ns	0.36ns	-0.39ns	1		
Harvest Index	0.65**	-0.48*	0.59**	0.48*	-0.41ns	0.49*	-0.10ns	1	
Grain Yield	0.70**	-0.32ns	0.92**	0.91**	-0.41ns	0.47*	0.33ns	0.67**	1

* and ** Significant at 5% and 1% probability levels, respectively and ns: Non-significant.

Table 4. Pearson correlation coefficients between traits of barley cultivars in well-watered and 10% AWC conditions.

	Stomatal conductance	Leaf temperature	Cell membrane stability	Water content	Hollow percent	No. tiller	No. grain	Harvest Index	Grain yield
Stomatal conductance	1								
Leaf temperature	-0.62**	1							
Cell membrane stability	0.72**	-0.49*	1						
Water content	0.86**	0.58*	0.61**	1					
Hollow percent	-0.40ns	0.70**	0.09ns	-0.26ns	1				
No. tiller	0.69**	-0.74**	0.82**	0.58*	-0.16ns	1			
No. grain	0.38ns	0.21ns	0.10ns	0.59**	0.17ns	-0.17ns	1		
Harvest Index	0.89**	-0.63**	0.61**	-0.96**	-0.39ns	0.55*	0.56*	1	
Grain Yield	0.94**	-0.66**	0.62**	0.95**	-0.40ns	0.65**	0.51*	0.96**	1

* and ** Significant at 5% and 1% probability levels, respectively and ns: Non-significant.

Significant reduction in harvest index in drought stress conditions in this study (Fig. 2 k) is probably because of more reduction in grain yield than biological yield. It seems that in drought stress conditions because of stomatal closure and early senescence (Nayyar and Walia 2004; González *et al.*, 2008; Daneshmand *et al.*, 2011), plants do not have enough time to transfer all photosynthetic assimilates to reproductive organ.

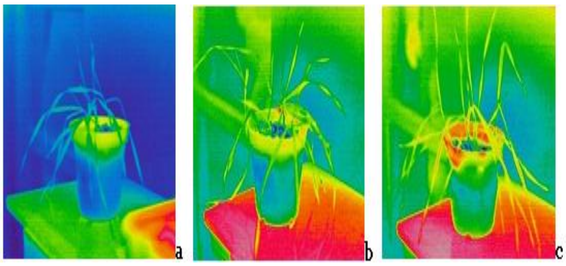
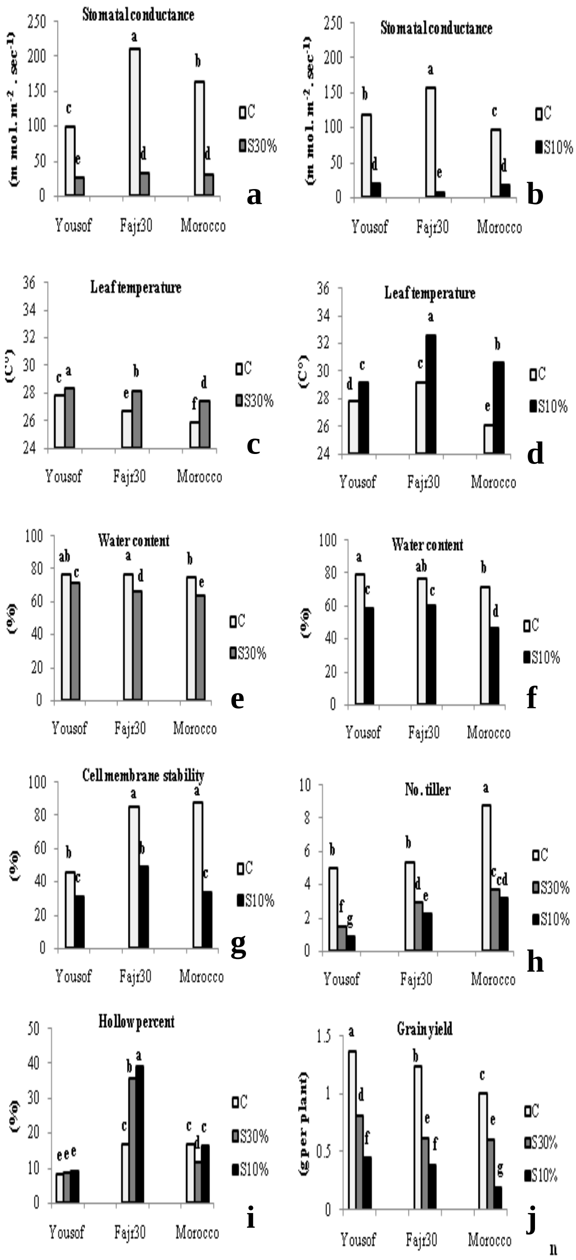


Fig. 1. Sample pictures of infrared camera in different drought stress levels; a (control), b (30% AWC), c (10% AWC).



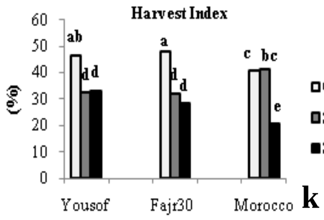


Fig. 2. Comparisons the traits of barley cultivars in control (C), moderate drought stress (S 30%) and severe drought stress (S 10%) conditions in greenhouse.

The highest correlation between grain yield and water content (r= +0.95), stomatal conductance (r= +0.94) and leaf temperature (r= -0.66) obtained at 20 days after anthesis (Table 4). Present study emphasizes that leaf water content is the most important factor on grain yield. Also according to high significant correlation between cell membrane stability and stomatal conductance (r= +0.72) and leaf temperature (r= -0.49) at 20 days after anthesis, it seems that by measuring these two traits that are highly correlated with the cell membrane stability, the amount of damage to membrane can be estimated. Also according to high significant correlation between cell membrane stability and grain yield at 10 and 20 days after anthesis (Table 3, Table 4) we can estimate yield reduction percentage by measuring stomatal conductance and leaf temperature in different drought stress levels.

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