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ORIGINAL ARTICLE

Leaf Water Relationships and Canopy Temperature as Criteria to Distinguish Maize Hybrids under Drought Stress

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This research aimed at studying the physiologic traits of maize different hybrids and considering them as screening criteria to select the drought tolerant hybrids. The experiment was conducted using a randomized complete block design with three replications and in a split-plot arrangement. The treatments were as follows: Maize Hybrids (including SC400, ZP434, SC524, ZP599, BC66, SC704) and irrigation regimes (including optimum; 100% FC, moderate; 75% FC, and severe stress; 50% FC). Results showed that drought stress significantly affects most of the studied indices. These indices also had significant differences in the above mentioned hybrids. Indices of leaf relative water content and temperature of the canopy varied significantly under drought stress. So, they could be used as suitable criteria to measure the level of stress effect on the plant and also to lay out the irrigation schedule. Findings of the study suggest that blistering is the best growth stage to screen the hybrids and among the studied indices, the ELWL is the best item for screening.

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Key words: Maize, Drought stress, Physiologic traits

Due to its compatibility with various climatic conditions, maize was rapidly brought under cultivation world-wide. Regarding the cultivated area, it is recognized as the third cereal in the world. In addition to providing fodder for the livestock, it has a remarkable supply of energy. Thus, nowadays maize grain is considered as the most valuable cereal which plays an important role in fowl feed and egg production industries. Several live or dead stresses limit the growth and yield of the plants all over the world. Moisture stress is the most important factor in limiting the yield of the crops and due to the explosion of the population, its influence will be much more obvious in the next few decades. Therefore, many researchers have made themselves involved in studying the reaction of plants toward these environmental stresses. Physiologic traits have been among the studied subjects. Environmental factors, including water, affect the growth of plants through influencing the physiologic processes and plant inner conditions. Nike and colleagues (Naik *et al*., 1993.) reported that severe water stress extremely reduces the photosynthesis, interrupts the physiologic processes, and finally sears the leaves. Through crinkling, yellowing, and shedding their leaves, plants adjust themselves to these adverse conditions. Structural variations in the protoplasm besides the mechanical stress stimulation, due to cell water loss, are the main reasons behind damages resulting from water stress. As leaf water potential decreases, plant growth speed recedes due to breathing speed-up associated with plant temperature rise and photosynthesis rate reduction (Shaw, 1977). In South America, the effect of drought stress on leaves` photosynthesis rate and nitrogen and chlorophyll contents was studied on two maize genotypes. Results showed that drought stress reduced the chlorophyll content, stomal conductance and photosynthesis, but had no effect on nitrogen content (Sanchez *et al*., 1983). Blaum (1992) stated that drought resistance is not a simple and unique trait but rather it is a quantified complex one which has various aspects. Therefore, drought tolerance is a combination of morphologic, physiologic, and biochemical traits which is related to leaf relative water content (RWC), relative water loss (RWL), water use efficiency (WUE), prollyne accumulation, and excised leaf water retention (ELWR) (Manette *et al*., 1988.). Valentovič *et al*. (2006) studied two drought tolerant and vulnerable maize hybrids and found that RWC of both reduced under drought stress, but their difference was not significant. Also, drought stress reduced the LWL in both hybrids and there was a significant difference between the hybrids in terms of leaf water retention rate, so that LWL of the vulnerable hybrid was much more than that of the tolerant one. Osmoregulation is one the most important components of drought tolerance trait which is closely related to higher RWC and lower RWL. Clarke and Caig (1982) stated that reaped stems of drought tolerant wheat genotypes lose their moisture slower than vulnerable ones, so it can be concluded that shed leaf moisture content, drought tolerance, leaf moisture retention capacity, and yield are significantly interrelated. Wallace *et al*. (1972) observed that recognizing the morphologic and physiologic causes, yield difference and finding their genetic control is the most effective approach to improve the yield. Similarly, Manette *et al*. (1988) reported that drought resistant plants have special morphologic and physiologic traits which, under drought stress, enable them to store more water. In terms of RWC, they also observed a significant difference among wheat varieties. This research aimed at studying maize different morphologic and physiologic traits and taking new approaches to use these indices in screening tolerant hybrids and also perceiving the reason behind the tolerance of some hybrid toward drought stress.

MATERIALS AND METHODS

This experiment was conducted in the summer 2010 at the agricultural station of agriculture collage, Shiraz University. It was located in Bajgah and its geographical specifications were as follows: elevation= 1810 m, longitude= 46′ 52″, latitude= 29′

50". This experiment was conducted using a randomized complete block design with three replications and in a split-plot arrangement. The treatments were as follows: Maize Hybrids (Table 1) and irrigation regimes (including optimum; 100% FC, moderate; 75% FC, and severe stress; 50% FC). The irrigated water amounts are presented in Table 2. The morphologic traits were measured in three different growth stages: stem elongation, tassel emergence, and blistering stage. Samples were collected from the topmost leaves and each time 3 to 5 leaves were collected. Canopy temperature was measured by an infrared thermometer (Kyoritsu 5500). The physiologic traits relevant to drought stress were calculated by inserting the values in the following equations:

$$RWC = \frac{WF-WD}{WT-WD}$$

Barrs (1968)

$$ELWR = 1-\left(\frac{WF-W3}{WF}\right)$$

Clarke and Caig (1982)

$$ELWL = \frac{WF-W3}{WF-WD}$$

Manette *et al*. (1988)

$$LWL = \frac{WF-W1}{WF}$$

Xing *et al*. (2004)

In which, WF is leaf fresh weight, WD is leaf dry weight (by leaving the leaves in the oven with 80˚ C for 24 hours), WT is turgidity weight (by immersing the leaves in distilled water for 18 to 20 hours), W1, W2, and W3 are respectively the weight of the leaf after 2, 4, and 6 hours of being shed from the plant (and placing them inside the incubator in 25˚ C). After testing the hypotheses and conforming data normality and variances uniformity, data were analyzed using SAS and MINITAB software. Duncan method was used to compare means of the treatments.

RESULTS

Leaf Relative Water Content (RWC)

RWC is a suitable index to show leaf water level as a physiologic result of cell water shortage (Barrs 1968). Results indicated that drought stress has a significant effect on this index (Table 3). It suggests that drought stress reduces leaf water potential (Lak *et al*., 2006). Indeed, in the first stage, moderate stress, unlike the severe one, has not a significant effect which means that in stem elongation stage, the plant is not vulnerable to moderate stress (Emam, 2006). In the first stage, the highest RWC belonged to vulnerable SC704 hybrid, but in the second and third stage, it was observed respectively in vulnerable SC524 and ZP434 hybrids. Those were the most succulent hybrids in the farm. In the first two stages, no obvious trend (in terms of drought resistance) was observed among the hybrids and leaf relative moisture content did not decreased due to drought stress. But, in the third stage, tolerant SC524 and SC400 hybrids showed the lowest RWC decrease. Some researchers such as Bayer and Pearson (Bayer and Pherson, 1975) also stated that due to the more ability of tolerant varieties in controlling the stomas closure and therefore, losing less water under drought condition, their RWC decrease is less than that of vulnerable varieties. By using this index in the stem elongation stage, resistant hybrids can be screened. RWC reduction in tassel emergence stage was more severe than stem elongation stage which indicated the intensification of the drought in this stage (Manette *et al*., 1988). Similarly, it was observed that in the second stage, moderate stress led to severe RWC decrease which could be a consequent of plant vulnerability toward drought stress, especially in tassel emergence stage (Bismillah *et al.,* 2001). In the blistering stage, RWC was higher than the second stage which could be relevant to plant acclimation to stressful condition and its intensification over time (Campos *et al*., 2002). Ashkani *et al*. (2007) also confirmed the effect of blistering stage vulnerability and asserted that, through increasing the stomatic resistance, plants avoid more water loss.

Leaf Water Loss (LWL)

In all three stages, moderate and severe drought stresses have no significant effect on LWL, but the hybrids were significantly different in the first and third stages (Table 4). In stem elongation stage, the lowest LWL reduction belonged to the tolerant SC400 and the highest belonged to the vulnerable SC704 hybrid. Similarly, under severe drought stress, the tolerant SC524 and SC400 had the lowest and the vulnerable BC666 hybrid had the highest LWL decrease. This item could be used to distinguish the tolerant and vulnerable hybrids (Boyer 1986). In the second stage, LWL variations did not show a significant trend, but under severe stressful conditions, LWL increased which was not expectable (Table 4). It has been reported that it might be the result of this stage vulnerability toward drought stress and cell loss (Bayer and person, 1975). Also, in the blistering stage, the vulnerable hybrids, compared to the tolerant ones, showed a higher LWL reduction under moderate stressful condition. This item could be considered for distinguishing the resistant hybrids. According to LWL formula, drought vulnerable hybrids should have higher LWL, because the structure of their leaf is so that it loses much more water. In this study, it was observed that generally the highest LWL decrease belonged to the vulnerable SC704 hybrid. As was mentioned before, this hybrid has the highest RWC which highlights its efficient performance under normal condition and its vulnerability and severe yield reduction under stressful condition.

Excised Leaf Water Loss (ELWL)

ELWL is a useful index to screen the varieties capable of growing under drought stressful conditions (Manette *et al*., 1988). Drought vulnerable varieties have a higher ELWL and compared to these conditions, their ELWL is higher under normal conditions (Clarke and Caig 1982). Drought stress decreased the ELWL (Table 5). The level of this decrease was not significant in the first two growth stages, but under moderate and severe drought stressful conditions, vulnerable varieties showed a higher decrease than tolerant ones (Wang and Clarke, 1993). In the blistering stage, the type of the variety and the level of drought stress significantly affected this trait. In this stage and compared to tolerant hybrids, the level of ELWL decrease from normal to moderate drought stress was more in the vulnerable ones. So, this index can also be used to distinguish the tolerant hybrids.

Excised Leaf Water Retention

ELWR was also another drought resistance index studied in this research and the results are presented in table 6. Naturally, the value of this index in resistant varieties is higher than vulnerable ones. Likewise, its value rises under drought stressful conditions. Like ELWL, in the first two growth stages, this index did not show significant variations in different hybrids. In tassel emergence stage, both the stress and variety have a significant effect which highlights the importance of this growth stage. In the moderate stress treatment and in the resistant varieties, the rise of ELWR in this growth period was almost higher which could be due to higher stomatic resistance of these varieties which prevent water perspiration (Wang and Clarke, 1993).

Temperature of the Canopy

Measuring the temperature of the plant green cover is an effective criterion for discovering water stress situation, laying out the irrigation schedule, and predicting the yield of the plant (Karimizadeh and Mohammadi, 2011). The effect of water shortage stress on temperature of the crown was significant at a 1% significance level (Table 7). Similarly, Shaw (1977) stated that maize yield loss under drought stressful conditions is due to the rise in photosynthesis and canopy temperature. As the drought stress rises, the temperature of plant cover crown increases which has different effects on different hybrids and it seems that in some hybrids, it decreases the area of the leaf, so more light penetration rises the temperature. Also, drought stress can increase the temperature of the canopy through stomatic closure and perspiration decrease. In stem elongation and tassel emergence stages no significant difference was observed, but in the blistering stage, the highest and the lowest temperatures belonged to SC400 (under severe stressful condition) and SC524 (under normal condition), respectively (Table 7). Compared to vulnerable hybrids, temperature of the canopy is higher in tolerant hybrids. Due to higher stomatic resistance, tolerant varieties have a lower perspiration which, even under normal conditions, leads to an increase in canopy temperature (Idso *et al*., 1981). Therefore, in this experiment and under severe stressful condition, the most tolerant hybrid (SC400) has the highest canopy temperature. According to the significance of the difference among the treatments in the blistering stage, it seems that the difference between tolerant and vulnerable hybrids is resulting from better stoma control and consequently lower perspiration in blistering stage.

Table 1. Specifications of the studied hybrids

Drought tolerance	Seed Yield	Maturity	Origin	Hybrid
Most Tolerant	12	Premature	Iran	SC400
Vulnerable	15	Premature	Yugoslavia	ZP434
Tolerant	12-10	Untimely	Hungary	SC524
Relatively Vulnerable	12	Untimely	Yugoslavia	ZP599
Most Vulnerable	12-10	Post mature	Croatia	BC666
Relatively Vulnerable	12-10	Post mature	Iran	SC704

Table 2. The amount of irrigated water

Month	Precipitation (mm)	Irrigated Water (mm)		
		Normal	Moderate	Severe
July	0	290.2	258.8	227.35
August	0	244	183	122
September	0	177	132.75	88.5
October	0	44.8	35.1	23.4
Total	0	758	609.65	461.25

Table 3. The mean value of relative leaf water in different stages and under normal and drought stress in maize varieties

		Stem Elongation				Tassel Emergence				Blistering		
Hybrid		Normal	Moderate	Severe		Normal	Moderate	Severe		Normal	Moderate	Severe
SC704	a	0.82	0.78	0.77	a	0.75	0.67	0.66	a-b	0.80	0.80	0.79
BC666	a-b	0.78	0.78	0.72	a	0.77	0.65	0.63	b	0.81	0.77	0.77
ZP599	b	0.77	0.73	0.72	a	0.75	0.61	0.59	a-b	0.82	0.77	0.76
SC524	a-b	0.82	0.81	0.73	a	0.73	0.63	0.62	a-b	0.80	0.78	0.78
ZP434	a-b	0.79	0.76	0.75	a	0.76	0.66	0.63	a	0.85	0.81	0.78
SC400	a-b	0.78	0.76	0.70	a	0.72	0.64	0.62	a-b	0.81	0.80	0.79
Total Mean		0.79	0.77	0.73		0.75	0.64	0.63		0.81	0.79	0.78
Difference between Varieties			0.19				0.22				0.14	
difference between Irrigation Levels			0.03				0.006				0.02	
Interaction between Variety and Irrigation			0.90				0.97				0.81	

common letters in each column indicate no significant difference (according to results of Duncan test)
*values beneath the table indicate the significance levels (P) of the studied traits

Table 4. The mean value of leaf water loss in different stages and under normal and drought stress in maize varieties

		Stem Elongation				Tassel Emergence				Blistering		
Hybrid		Normal	Moderate	Severe		Normal	Moderate	Severe		Normal	Moderate	Severe
SC704	a	0.061	0.047	0.056	a	0.039	0.029	0.031	c-d	0.025	0.024	0.018
BC666	b	0.040	0.039	0.015	a	0.034	0.017	0.021	d	0.020	0.015	0.020
ZP599	b	0.043	0.035	0.026	a	0.031	0.024	0.029	a-b	0.038	0.027	0.025
SC524	b	0.032	0.038	0.031	a	0.043	0.018	0.027	c-d	0.026	0.023	0.020
ZP434	b	0.046	0.042	0.036	a	0.045	0.023	0.024	b-c	0.028	0.26	0.025
SC400	b	0.034	0.037	0.034	a	0.036	0.022	0.031	a	0.043	0.035	0.023
Total Mean		0.042	0.040	0.033		0.038	0.022	0.027		0.030	0.025	0.022
Difference between Varieties			0.00				0.36				0.00	
difference between Irrigation Levels			0.46				0.06				0.16	
Interaction between Variety and Irrigation			0.51				0.68				0.27	

common letters in each column indicate no significant difference (according to results of Duncan test)
*values beneath the table indicate the significant levels (P) of the studied traits

Table 5. The mean value of ELWL in different stages and under normal and drought stress in maize varieties

		Stem Elongation				Tassel Emergence				Blistering		
Hybrid		Normal	Moderate	Severe		Normal	Moderate	Severe		Normal	Moderate	Severe
SC704	a	0.18	0.17	0.16	a	0.12	0.11	0.11	a-b	0.13	0.12	0.10
BC666	b	0.15	0.15	0.09	a	0.11	0.07	0.07	c	0.11	0.07	0.07
ZP599	bb	0.14	0.13	0.11	a	0.11	0.09	0.09	d	0.13	0.10	0.09
SC524	a-b	0.16	0.16	0.12	a	0.12	0.10	0.10	b-c	0.11	0.10	0.09
ZP434	a-b	0.15	0.15	0.14	a	0.13	0.11	0.09	b	0.11	0.11	0.10
SC400	a-b	0.15	0.14	0.13	a	0.12	0.11	0.10	a	0.16	0.14	0.09
Totall Mean		0.16	0.15	0.13		0.12	0.10	0.09		0.13	0.11	0.09
Difference between Varieties			0.08				0.37				0.00	
difference between Irrigation Levels			0.31				0.30				0.04	
Interaction between Variety and Irrigation			0.94				0.99				0.19	

common letters in each column indicate no significant difference (according to results of Duncan test)
*values beneath the table indicate the significane levels (P) of the studied traits

Table 6. The mean value of ELWR in different stages and under normal and drought stress in maize varieties

	Stem Elongation				Tassel Emergence				Blistering			
Hybrid		Normal	Moderate	Severe		Normal	Moderate	Severe		Normal	Moderate	Severe
SC704	a	0.85	0.88	0.89	a	0.90	0.92		a	0.93	0.93	0.94
BC666	a	0.88	0.88	0.92	a	0.91	0.93		a	0.91	0.93	0.93
ZP599	a	0.86	0.87	0.89	a	0.91	0.93	0.93	a	0.90	0.92	0.94
SC524	a	0.85	0.86	0.91	a	0.89		0.91	a	0.91	0.92	0.93
ZP434	a	0.86	0.88	0.88	a	0.90		0.93	a-b	0.91	0.91	0.92
SC400	a	0.86	0.87	0.90	a	0.91		0.94	b	0.87	0.89	0.93
Totall Mean		0.86	0.87	0.90		0.903		0.932		0.91	0.92	0.93
Difference between Varieties			0.65								0.018	
difference between Irrigation Levels			0.17								0.04	
Interaction between Variety and Irrigation			0.96								0.70	

common letters in each column indicate no significant difference (according to results of Duncan test)
*values beneath the table indicate the significane levels (P) of the studied traits

Table 7. The mean value of canopy temperature in different stages and under normal and drought stress in maize varieties

	Stem Elongation				Tassel Emergence				Blistering						
Hybrid		Normal	Moderate	Severe		Normal	Moderate	Severe		Normal		Moderate		Severe	
SC704	a	26.00	26.33	26.33	a	27.33	27.33	29.33	a	25.00	f-g	26.67	c-f	28.00	b-d
BC666	a	24.33	26.33	27.67	a	27.00	27.67	28.33	a	25.00	f-g	26.67	c-f	28.33	b-c
ZP599	a	25.00	26.33	26.33	a	26.67	27.33	28.00	a	24.67	f-g	25.33	e-g	27.33	c-e
SC524	a	24.33	26.67	26.67	a	27.00	28.00	28.68	a	24.33	g	25.33	e-g	29.67	a-b
ZP434	a	24.00	27.00	27.67	a	26.67	27.00	28.33	a-b	25.33	e-g	26.00	d-g	26.67	c-f
SC400	a	25.33	26.00	26.33	a	26.67	26.67	28.67	b	25.00	f-g	26.00	d-g	31.00	a
Totall Mean		24.83	26.44	27.06		26.89	27.33	28.56				26.00		28.5	
Difference between Varieties			0.88				0.10					0.02			
difference between Irrigation Levels			0.00				0.00					0.014			
Interaction between Variety and Irrigation			0.13				0.61					0.002			

common letters in each column indicate no significant difference (according to results of Duncan test)
*values beneath the table indicate the significane levels (P) of the studied traits

DISCUSSION

Hybrid SC704 had the highest RWC in all growth stages and under normal irrigation. Hybrid ZP599 has the lowest RWC in all stages and treatments, so it stores little water in its cells. Hybrid SC524 had moderate RWC in all three stages and under severe drought stress. Almost in all treatments, hybrid BC666 had the lowest LWL and ELWL which seems that due to its structure, it losses lower water content. Since the mean value of all hybrids LWL decreases in different growth stages, it can be concluded that as the amount of irrigation water decreases and the plant adjust themselves gradually to the stressful condition, they would suffer less damage. In the blistering stage, plants completely try to overcome the environmental stresses in order to fill their seeds (Campos *et al.,* 2002). Generally, compared to severe stress, moderate stress has a more intensified effect on maize. In other words, the difference between normal and moderate stressful conditions is much more than that of moderate and sever stressful conditions which confirms the hypothesis that maize is vulnerable to drought stress (Stocker, 1986). Results of studying these physiologic traits in three growth stages indicated that selecting the resistant varieties in the blistering stage is more effective, so it is suggested as the best period to distinguish tolerant hybrids.

These results confirm Bolanson (1995). In the first growth stage and under moderate stressful condition, no physiologic trait relevant to leaf water was significant which could be due to maize lower vulnerability to drought stress in stem elongation stage (Emam, 2006). In the blistering stage, four of the five studied indices varied significantly under stressful conditions which could be due to intensification of the effect on the plant in blistering stage. Since in tassel emergence stage, the difference among the hybrids was not significant in any index, it could be concluded that this stage is not suitable for distinguishing hybrids based on physiologic traits, but in the blistering stage, hybrids showed significant differences in terms of three indices. So, it can be considered as the most suitable screening stage. LWL index did not have a significant difference in any stage, therefore it is not a useful index to measure the stress, but RW and canopy temperature had significant difference in all growth stages, so they can be used to measure the level of stress imposed on the plant or lay out the irrigation schedule. According to the results, ELWL is the best index, since its trend of variations is suitable both in tolerant and vulnerable hybrids. Temperature of the canopy did not show a significant difference in any stage and under stressful conditions, so this is not a suitable screening criterion too.

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