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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (15): 2852-2857, 2006
ISSN 1028-8880

Effect of Water-Stress on Some Water Related Traits and Their Relationships with Height and Dry Matter in Maize Early Maturing Inbred Lines

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Abstract: In this study, seven early maturing maize inbred lines were evaluated for some water related traits under 3 different water-stress treatments performed by using 0, 3 and 6% Polyethylen Glycol (PEG) solutions. The experiment was carried out in a split plot design with 3 replications. Main plots and sub plots were allocated to water-stress treatments and inbred lines, respectively. When the inbred lines reached to 6-leaf stage, Water Use Efficiency (WUE), Leaf Relative Water Content (LRWC), Excised Leaf Water Lost (ELWL) and stomata characteristics were measured for all inbred lines under the three treatments. Height and dry matter were also recorded for the all 7 inbred lines. Although inbred lines behaved differently when they were subjected to water-stress, nonetheless, the results indicated that water-stress significantly influence all characters except ELWL. This indicates that ELWL is not a sui criterion for screening water-stress tolerant genotypes. Stomatal area per unit area of leaf and number of stomata were increased due to water stress in most inbred lines. WUE fluctuated based on the inbred lines so that some of the inbred lines had higher WUE under water-stress conditions and some of them had lower WUE at the same conditions. Inbred lines showed significant differences for all characters except the number of stomata on abaxial surface of leaves and height. Among the water-related characters studied, LRWC seemed to be the best character for screening drought tolerant genotypes due to its quick response to water-stress at the two stress treatments. LRWC showed a significant positive correlation with dry matter and height indicating that genotypes having high LRWC under water-stress conditions will produce more dry matter and height.

Key words: Maize inbred lines, water stress, water related traits, height, dry matter

INTRODUCTION

Plants respond and adapt to a variety of environmental stresses to survive. Among these environmental stresses, water-stress is one of the most adverse factors of plant growth and crop production. Maize inbred lines and hybrids are different in their responses to water-stress (Saab *et al*., 1990). Therefore screening the tolerant genotypes is the first step in the breeding programmes for tolerance to water-stress. Comparing genotypes for their responses to water-stress can be done by measuring yield and yield components under normal and water deficient conditions and considering the differences between performance of genotypes under normal and water-stress conditions as an indicator of water-stress susceptibility (Shinozaki *et al*., 1997). In addition to yield and yield components, physiological response of plant genotypes are usually compared under water-stress conditions (Farshadfar *et al*., 2002). Water-stress induces various biochemical and physiological changes in plants (Shinozaki *et al*., 1997). Therefore, understanding physiological responses of plants under stress conditions may results to improvement and production of drought tolerant varieties of crops (Kerepesi *et al*., 2000). Physiolological characters that indicate water status of plants such as Water Use Efficiency (WUE), Leaf Relative Water Content (LRWC) and Excised Leaf Water Lost (ELWL) have been widely used to assess the response of plants to water-stress (Vyn and Hooker, 2002; Golestani and Assad, 1998; Wassome *et al*., 2000). Stomatal characteristics are also related to waeter status of plants and affect the ability of plants to survive under water-stress conditions (Wang and Clarke, 1993).

In regions in which growth season is short, quick establishment of plants leads to reduction in evaporation at the beginning of growth season and therefore causes storage of water for later growth stages. Morphological characters such as root and shoot lengths in a short period after sowing have been used as two indicators of

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plant establishment under environmental stresses (Saab *et al*., 1990). Enhancement in the early growth of roots an shoots can be measured as a total dry matter production (Mohammady-D and Rezaie, 2002).

Performing artificial water-stress treatments is done by various methods including water holding, irrigation with reduced water and using different osmotic solutions. Among osmotic solutions, Polyethylen Glycol (PEG) has been widely used to perform water-stress treatments (Janes, 1974; Hohl and Schopfer, 1991; Ruf *et al*., 1967). Pervious studies indicated that some early maturing maize inbred lines are able to complete their growth cycles in shahrekord regions of Iran where the growth season is short due to low temperatures in the first and second months of spring and the last month of summer (Mohammady and Rezaie, 2002). The response of these early maturing inbred lines to water deficit was unknown. The present study was conducted to evaluate these inbred lines for different aspects of their response to water-stress. Several water related traits were also studied in order to identify the response of the inbred lines to water-stress and to investigate the relationship between the water related traits from one side and height and dry matter production from other side.

MATERIALS AND METHODS

Genetic materials: Genetic materials were seven early maturing maize inbred lines selected from a collection of inbred lines produced in Karag Institute of Research for Breeding and Preparation of Seed and Seedling Iran. These inbred lines are able to complete their growth cycle during 90 days and are sui for cultivating at Shahrekord region (50° 51' E, 32° 19' N) in Iran.

Experiment: This experiment was carried out in a split plot design with 3 replications in a green house at Shahrekord University in 20 cm diameter plastic pots. Main and sub plots were allocated to water-stress treatments and inbred lines, respectively. The pots were filled with a soil containing 1 part clay and 3 parts compost. Seeds were sowed in the pots each pots containing 4 seedlings. The normal treatment was performed by irrigating the pots with distilled water and stress treatments were performed by using 3 and 6% of PEG solutions. The amount of water and solutions added to the pots were recorded for each pot separately. When the fifth leaf of plants fully emerged (the sixth leaf appeared), the characters under study were measured for the plants in each pot.

Stomatal frequencies and size were measured on the adaxial and abaxial surfaces of the fifth fully expanded leaves for all plants using the impression method (Wang and Clarke, 1993). Stomata counts were made on the impressions from 3 randomly selected microscopic fields of view of each impression for both adaxial and abaxial surfaces and length (SL) and width (SW) of stomatal guard cells were measured on both surfaces from the impressions using a scaled eyepiece of a light microscope. Number of stomata (N) was calculated in one mm^{-2} of leaf and stomatal area in one square millimeter of leaf was also calculated as a product of SL×SW×N.

Leaf Relative Water Content (LRWC) and Excised Leaf Water Lost (ELWL) and Water Use Efficiency were measured as 3 other indicators of water related characters. Leaf Relative Water Content (LRWC) was estimated using the following formula in which Fw, Dw and Tw are Fresh weight, Dry weight and Turgid weight, respectively.

$$\text{LRWC} = [(\text{Fw-Dw})/(\text{Tw-Dw})]\times 100$$

ELWL was also estimated using the following formula:

$$\text{ELWL} = [(\text{Fw-Weigh 6 hr after leaf excision})/(\text{Fw-Dw})]\times 100$$

Fresh weights of leaf samples (5 cm length) were measured immediately after leaf Excision. Then, the leaf samples were put in a tube containing 100 mL distilled water for 24 h and turgid weights were recorded. To obtain dry weights, the leaf samples were oven dried at 70°C for 48 h and then dry weights of all samples were recorded using a digital balance. Finally, WUE was calculated as a ratio of plants dry weight to total water used.

Analysis of variance was performed using SPSS software and comparisons between the means was done using Duncan's test.

RESULTS

Analysis of variance indicated that both water-stress and inbred lines were significant for all characters except ELWL and number of stomata on the abaxial surface of leaves. The interaction of water-stress treatments and inbred lines was also significant for WUE and number of stomata on both surfaces of leaves. No difference was observed between water-stress treatments for stomata area and number of stomata on lower surface of leaves. Nonetheless, water stress treatments influenced the other characters significantly.

As can be seen from the Fig. 1, stomatal area on adaxial and abaxial surfaces of leaves was higher under water-stress conditions imposed by using PEG 6%

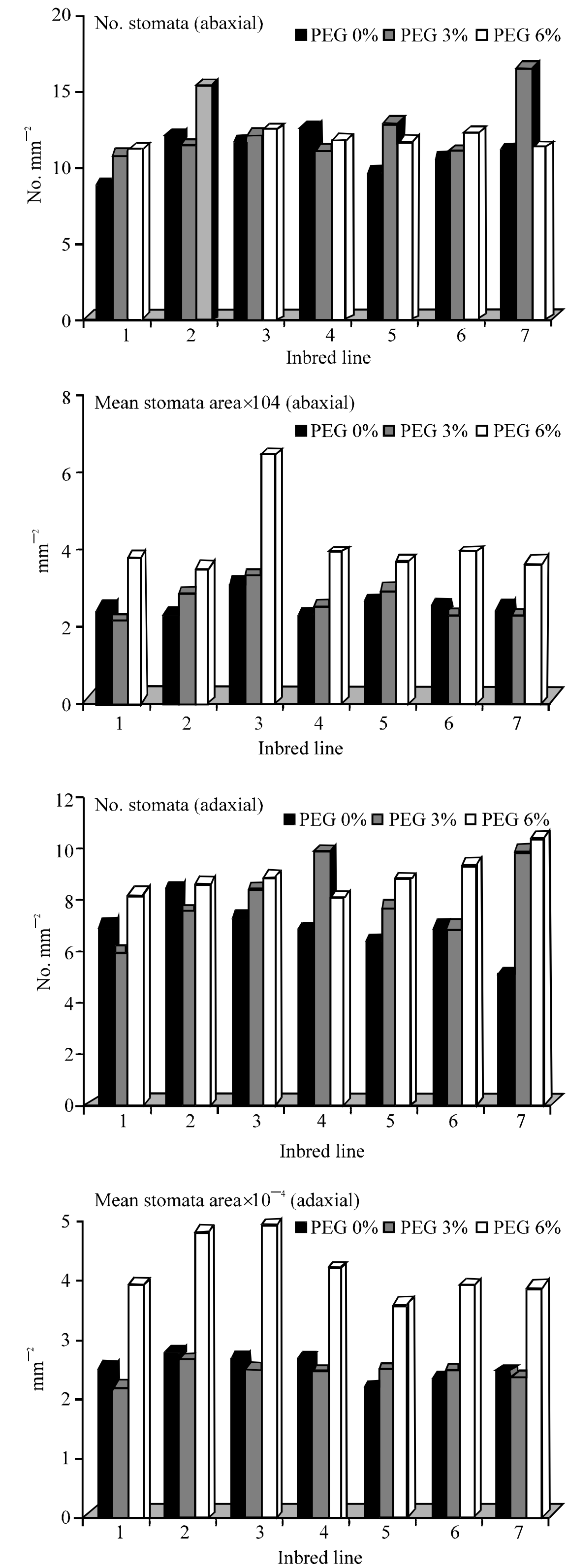

Fig. 1: Effect of water-stress treatments on stomatal characteristics

solution than normal condition for all inbred lines. This is mainly due to increase in stomata number per unite area of leaf. Meanwhile, the stomatal area on both surfaces of leaves under moderate water-stress treatment (PEG 3%) was almost similar to normal condition. In the case of stomatal frequency, inbred lines responded differently to the water-stress treatments. Frequency of stomata on adaxial surface of leaves increased in all inbred lines due to water-stress treatments except inbred line 2. The frequency of stomata on the abaxial surface also increased due to water-stress in all inbred lines except inbred lines 4.

Under moderate water-stress conditions (3% PEG), WUE was highest in all inbred lines except inbred lines 5 and 7 indicating more efficient use of water by the inbred lines under this condition. There was a significant reduction in LRWC under water-stress in all inbred lines. Inbred lines 3 and 4 indicated less reduction in LRWC under stress treatments in comparison with normal condition (Fig. 2). ELWL increased under water-stress in all inbred lines except the inbred line 1. This indicates that water-stress treatments promote water loss after leaf excision. Height and dry matter production were reduced due to water-stress in all inbred lines. Lines 5 and 6 showed the lowest reduction in height and dry matter under water-stress conditions in comparison with normal conditions. This indicates that they tolerate water-stress better than other inbred lines.

The as can be seen from the Table 1, some of the inbred lines had better performance of the traits. At the moderate water-stress treatment, variation was observed between means of inbred lines for all characters except LRWC, Height and stomatal area on the abaxial surface. Conversely, at the severe water-stress conditions (6% PEG), the mean of inbred lines were significantly different for all characters except height. This means that height could not be a sui character using for comparison between maize inbred lines for the effect of water-stress. According the results presented in Table 1 and 2, inbred lines indicated different amount on each character. For instance, some inbred lines were better for some of the characters but not for others. These results imply that all positive characters are not necessarily presented in one particular genotype and that one particular genotype may have only a few positive characters which confer water-stress tolerance to plants.

The correlation between the water related characters from one side and dry matter and height from other side (Table 3) revealed that LRWC had a significant positive correlation with dry matter and height indicating

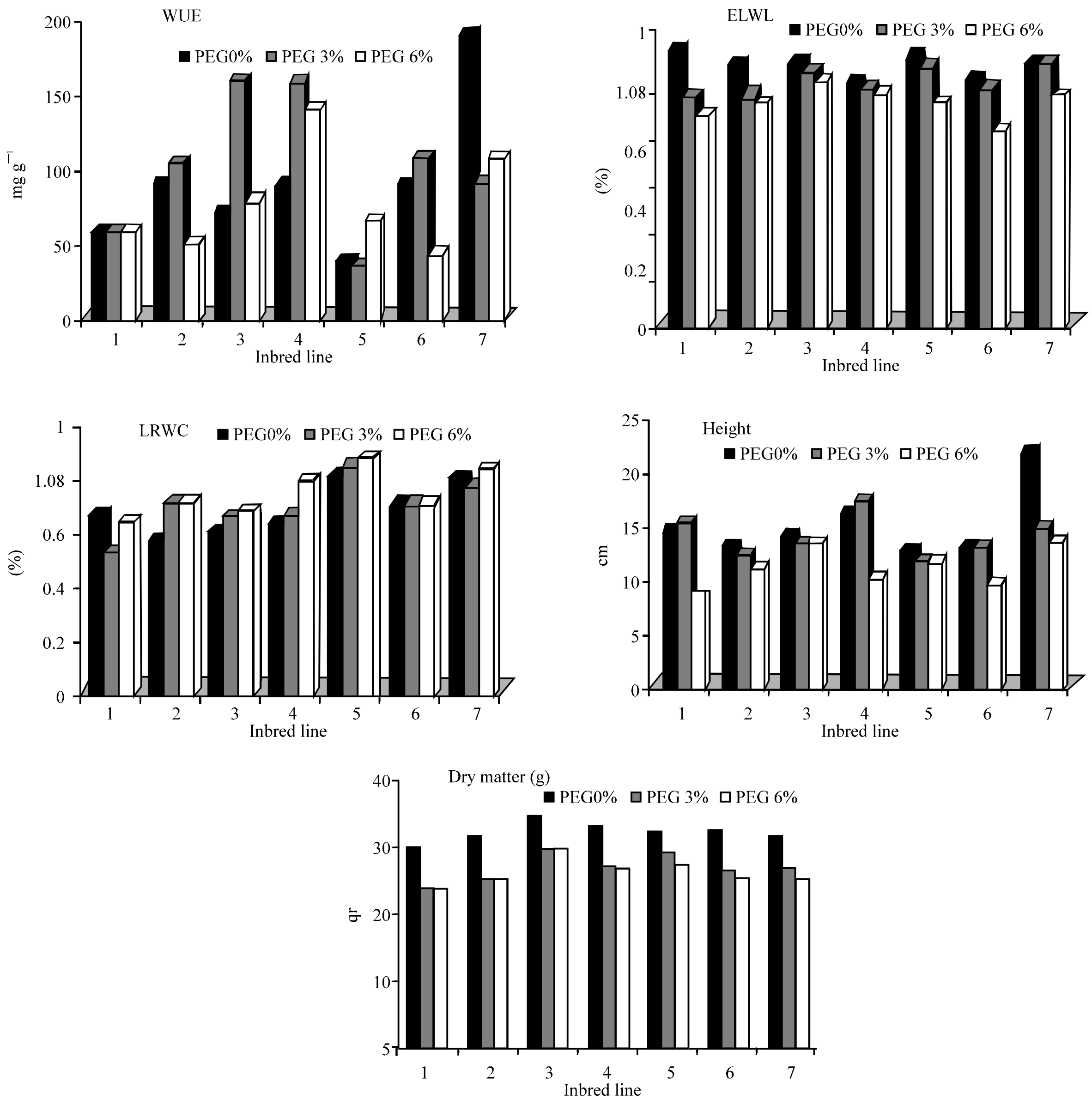

Fig. 2: Effect of Water-stress treatment on WUE, ELWL, LRWC, dry matter and height

Table 1: The mean performances of inbred lines at moderate water-stress treatment (3% PEG) for the characters under study

Inbred line	WUE	LRWC	ELWL	No. stomata (adaxial)	No. stomata (abaxial)	Stomata area (adaxial)	Stomata area (abaxial)	Dry matter	Height
1	58.13^{f*}	76.00^{a}	51.90^{b}	5.89^{b}	10.33^{b}	2.20^{a}	2.15^{b}	24.30^{b}	15.25^{a}
2	103.53^{d}	76.60^{a}	70.10^{ab}	7.44^{ab}	11.22^{b}	2.69^{a}	2.94^{ab}	25.51^{b}	12.33^{a}
3	159.23^{a}	84.20^{a}	65.70^{ab}	8.44^{ab}	12.11^{b}	2.55^{a}	3.38^{a}	29.72^{a}	13.45^{a}
4	157.80^{b}	78.10^{a}	66.00^{ab}	7.97^{a}	11.00^{b}	2.48^{a}	2.58^{ab}	26.31^{b}	17.25^{a}
5	35.33^{g}	86.30^{a}	83.70^{a}	7.67^{a}	12.89^{b}	2.46^{a}	2.96^{ab}	29.13^{a}	11.67^{a}
6	107.60^{c}	79.50^{a}	70.50^{ab}	7.00^{b}	11.10^{b}	2.45^{a}	2.29^{ab}	26.43^{b}	13.00^{a}
7	90.76^{e}	86.00^{a}	76.00^{a}	9.78^{a}	16.33^{a}	2.40^{a}	2.28^{ab}	26.56^{b}	14.83^{a}

*: The means having common letter(s) are not significantly different

that genotypes having high LRWC under water-stress conditions will produce more dry matter and height. A highly positive significant correlation was also observed between stomatal area on the adaxial surface of leaf and the stomatal area on the abaxial surface. This result implies that measuring the stomatal area in one side of leaf is adequate for comparing the genotypes for this character.

Table 2: The mean performances of inbred lines at severe water-stress treatment (6% PEG) for the characters under study

Inbred line	WUE	LRWC	ELWL	No. stomata (adaxial)	No. stomata (abaxial)	Stomata area (adaxial)	Stomata area (abaxial)	Dry matter	Height
1	58.13[e]*	69.6[ab]	63.20[b]	3.93[ab]	3.79[b]	3.93[ab]	3.79[b]	24.20[b]	8.91[a]
2	48.23[f]	74.00[ab]	71.20[ab]	4.79[a]	3.53[b]	4.79[a]	3.53[b]	25.31[b]	10.95[a]
3	78.06[c]	81.20[a]	67.00[ab]	4.95[a]	6.47[a]	4.95[ab]	6.47[a]	29.48[a]	13.58[a]
4	139.56[a]	76.60[ab]	77.80[ab]	4.23[ab]	3.98[b]	4.23[ab]	3.98[b]	26.19[ab]	10.05[a]
5	66.20[d]	74.40[ab]	86.00[a]	3.54[b]	3.75[b]	3.54[b]	3.75[b]	27.01[ab]	11.41[a]
6	42.43[g]	64.80[b]	69.80[ab]	3.89[ab]	3.98[b]	3.89[ab]	3.98[b]	25.16[b]	9.50[a]
7	106.60[b]	76.10[ab]	82.30[ab]	3.88[ab]	3.64[b]	3.88[ab]	3.64[b]	28.01[a]	13.41[a]

*: The means having common letter(s) are not significantly different

Table 3: Correlation between the characters under study using the mean of inbred lines over the two stress treatments

Parameters	LRWC	WUE	ELWL	No. stomata (adaxial)	No. stomata (abaxial)	Stomatal area (adaxial)	Stomatal area (abaxial)	Dry matter	Height
LRWC	1								
WUE	0.341	1							
ELWL	0.191	0.057	1						
No stomata (adaxial)	-0.014	0.224	0.457	1					
No stomata (abaxial)	0.349	-0.239	0.292	0.370	1				
Stomatal area (adaxial)	-0.460	-0.250	0.168	0.314	0.145	1			
Stomatal area (abaxial)	-0.187	-0.132	0.165	0.273	-0.006	0.824**	1		
Dry matter	0.786**	0.210	-0.470	0.419	0.470	-0.093	0.219	1	
Height	0.560*	0.463	-0.274	0.177	-0.013	-0.545*	-0.352	0.324	1

*,**: Significant at 5% and 1% of probability, respectively

DISCUSSION

The effect of water-stress on maize yield and physiological characters has been widely studied (Ahmadi and Baker, 2001; Bergonci *et al.*, 2001; Calvino *et al.*, 2003, Chandrasekar *et al.*, 2000). Maize crop responds to water deficit in the form of changes in various physiological processes. The physiological changes observed in inbred lines under water-stress conditions could be consequences of deleterious effects of water deficit on important metabolic processes as well as responses of various defense mechanisms adapted by the plant under drought stress (Chandrasekar *et al.*, 2000). Among the water related characters studied here, LRWC seemed to be the best character for screening drought tolerant genotypes due to its quick response to water-stress at the two stress treatments. Such a result has been reported in wheat by Golestani and Assad (1998) and Mohammady-D *et al.* (2005). For all inbred lines, the LRWC differences between water-stress and well water conditions were significant indicating the effect of water-stress on leaf water status. Inbred lines 3 and 4 had higher LRWC than other lines under water-stress conditions. Maintaining considerable amount of water into the leaf tissues enables the plants to complete growth metabolism and is therefore a good indicator of tolerance to water-stress (Turner, 1996). High LRWC under water-stress conditions can be achieved by adjusting the stomatal aperture and size. Since line 3 had a high stomatal area, its high LRWC could not be due to smaller size of stomata and it is possibly due to stomatal closure under water-stress conditions. Maize inbred lines have shown variation for LRWC under water-stress treatments. A Possible explanation of variation between inbred lines for LRWC under well water conditions is that this variation might to be due to differences between inbred lines leaf thickness and stomatal conductance. In the present experiment, inbred lines with high LRWC under water-stress conditions generally had high stomata area, which is in agreement with the results of Turner *et al.* (1996) obtained from a study on barley. LRWC is the relationship between fully turgid water content and actual water content of plant tissues when they are subjected to water-stress. Therefore, LRWC indicates the ability of plants to keep their water status at a reasonable level when they experience water-stress. Inbred lines indicated different responses to water-stress in the case of ELWL. This means that this character is less in the genotypes and therefor less reliable than LRWC. Other experiments also support the result of current research (Dhanda and Sethi, 1998; Mohammady-D *et al.*, 2005).

Venora and Calcagno (1991) measured and used stomatal characteristics as an indicator of water-status. This application seems to explain differences between the varieties for water loss only under non-limiting conditions provided varieties with larger size of stomata and higher number of stomata have larger stomatal pores. In unfavorable conditions, particularly under water-stress conditions, stomatal aperture is not the main determination of water lost because it is extremely variable as a result of the influence of atmospheric factors. It is theoretically expected that varieties with higher number of stomata per unit area and greater length and width of stomata lose more water during the growth period. This happens if stomata remain open during the water-stress period. Any response of stomata to water-stress can be discussed in relation to stomatal resistance and LRWC. Reduction in water loss from leaf surfaces during periods of severe water-stress is an important drought tolerance indicator. Low rate of cuticle transpiration, therefore, may reduce leaf dehydration and promote leaf survival (Wang and Clarke, 1993).

To sum up, variation was observed among the inbred lines for some of the characters under study. Therefore, selection between them and using inbred lines that have indicated better performance under water-stress treatments as the parents of hybrids could pave the way for improvement early maturing water-stress tolerant maize genotypes in regions similar to Shahrekord region in Iran. In addition, positive significant correlation was observed between LRWC and height and dry matter production under water-stress conditions. Therefore, LRWC could be a positive water-related characters conferring water-stress tolerance to maize and it can be used for screening water-stress tolerance genotypes.

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