Investigation note

Thermotolerance in sorghum lines [*Sorghum bicolor* (L.) Moench] for grain

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Abstract

Extreme weather, such as increased drought and high temperatures, has had a significant impact on crop yields. Therefore, it is important to identify characters in sorghum genotypes that help them lessen the impact of these factors and allow them to be productive. The objective of this work was to evaluate the heat tolerance of a group of granite sorghum B and R lines under two humidity conditions to select the best heat tolerant lines. 28 experimental lines B and R were planted on August 30, 2017 in Marin, NL, Mexico. The experiment was sown in a completely randomized design with an arrangement in divided plots and with two repetitions the moisture treatment under water stress was applied 43 days after sowing (DDS) for a period of four weeks without irrigation. Data were taken from days to flowering, damage to the cell membrane at 40 °C and relative leaf water content (CRA). The results showed that the CRA did not allow to establish differences between genotypes, while the heat treatment applied at 40 °C, allowed to establish differences and classify them according to the percentage of damage caused to the cell membrane, since this is one of the features widely used to select tolerant genotypes. Genotypes 20 and 22 were identified as heat tolerant and genotypes 5 and 8 were susceptible.

Keywords: *Sorghum bicolor* (L.) Moench., cell membrane damage, heat tolerance, water stress.

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Climate change has become an obstacle to the development of agriculture worldwide, changes in the intensity of extreme weather such as floods and drought, have had a significant impact on crop yields (Menezes *et al*., 2015). One of the options to strengthen food security is the cultivation of graniferous sorghum [*Sorghum bicolor* (L.) Moench]. It is the fifth most cultivated and consumed cereal in the world and presents adaptation to low rainfall and high temperature environments (FAO, 2016).

The northeast of Mexico, which includes the states of Tamaulipas, Nuevo Leon, Coahuila and San Luis Potosi, is the region with the highest sorghum production with about 800 000 ha sown annually (SIAP, 2019), where sowings with hybrid seeds predominate, which are developed for environments favorable to the crop and are of a high cost in the market because they are produced mainly in the USA, resulting our country dependent on this input (Flores-Naveda *et al*., 2013). The polygenic characteristics of tolerance to abiotic stress in particular water and heat have had limitations for genetic improvement (Bahuguna *et al*., 2015), it is important to use simple, fast and economical techniques that allow obtaining new varieties better adapted to drought conditions and high temperatures (Dhanda *et al*., 2007).

The coexistence of heat and drought stress affects the biochemical and physiological processes of plants, including the function of the cell membrane, so that the increased permeability and leakage of ions outside the cell, have been used as a measurement of cell membrane stability and as a related stress tolerance test (ElBasyoni *et al*., 2017). The thermal stability of the membrane can be an important selection criterion for heat stress tolerance (Hemantaranjan *et al*., 2014) and the relative water content is considered a drought resistance mechanism (Ritchie *et al*., 1990).

The objective of this experiment was to evaluate lines B and R of sorghum for grain under two conditions of humidity, good irrigation and low water stress, through the use of simple and economical techniques that allow greater knowledge about the mechanisms of tolerance to heat and drought, in order to identify sorghum lines for the formation of hybrids with a better response to stress conditions.

The experiment was established on August 30, 2017 in the spring-summer cycle, within the experimental field of the Faculty of Agronomy belonging to the Autonomous University of Nuevo de Leon (UANL). Located in Marin Nuevo Leon, Mexico, with geographical location 25° 52’north latitude 100° 02’ west longitude, at 355 meters above sea level. The average temperatures during the evaluation were 29.4 °C max. and 17.3 °C min. (CONAGUA, 2017). 26 experimental elite lines were used; 11 B lines and 15 R lines and 2 commercial hybrids. The genotypes were established in pots with a diameter of 25 cm and a height of 55 cm, using a soil mixture consisting of 2/3 of river sand and 1/3 of chicken manure. In the sowing three seeds were placed in the center of the pot at a depth of 3 cm and thinned to have one plant per pot.

The experimental design used was completely randomized according to divided plots and two repetitions. The large plot was moisture treatments (good irrigation and low water stress) and the small plot was assigned to genotypes; one pot per genotype was considered an experimental unit. The moisture treatment under water stress consisted of the suspension of potting irrigation 43 days after planting (DDS) for a period of four weeks, contrary to the good irrigation treatment in which water was applied every third day.

The humidity levels in the pots were determined with an Aquaterr® EC-350 model tensiometer at average intervals of 5 days, the tensiometer bar was placed at a depth of 15-20 cm; after each measurement, the bar was cleaned to avoid contamination of soil and moisture between experimental units. The variables evaluated were days to flowering (DF); considering the number of days elapsed from sowing until half of the panicle was with the exposed anthers. Percent of cell membrane damage at 40 °C (DMC 40), using the procedure described by Blum and Eberconm (1981) and using the following equation.

$$\%DMC=1-\left\{\frac{\left[1-(\frac{T1}{T2}\right]}{\left[1-(\frac{C1}{C2}\right]}\right\}*100$$

Where: T= treatment; C= control; 1= initial EC measurement; 2= final EC measurement.

Relative content of leaf water (CRA); the technique used by Sade *et al*. (2015) and the following equation was used.

$$\%CRA=\left\{\frac{[(TFW-BW)-DW]}{[TW-DW]}\right\}*100$$

Where: BW= ziploc plastic bag labeled and weighed initially; TFW= fresh leaf weight + BW; TW= Turgent weight and DW= dry leaf weight.

Statistical analyzes were performed using the InfoStat package (Di Rienzo *et al*., 2008). A Student's t-test ($p\leq 0.05$) was performed to compare moisture treatments. An angular transformation was applied $\left[\arcsin (Yi)^{1/2}\right]$ to the percentage data (Yi) of DMC at 40 °C and CRA. An analysis of variance was performed and where necessary a comparison of means by Tukey ($p\leq 0.05$) of the treatments and genotypes was made, presenting the results with the values retransformed to the original scale (percentages).

The moisture treatments applied were different according to the Student t test, which was significant ($p\leq 0.01$ value) with average values of 87.7% humidity for good irrigation and 80.9% humidity under water stress. The analysis of variance showed significant differences for humidity conditions in all variables (DF, DMC 40 and CRA), as well as for genotypes and genotype interaction x humidity condition only in DF and DMC 40 variables.

In Table 1, the test of means per Tukey for the humidity condition for the DF variable, an average value of 69 days in good irrigation and 75 days under water stress. Similar results of flowering delay due to the effect of drought in sorghum were reported by Hammer *et al*. (1989); Muchow and Carberry (1990); Craufurd and Peacock (1993).

Table 1. Tukey averages test of the variables evaluated under two humidity conditions.

Condition	DF (d)	DMC 40 (%)	CRA (%)
Low water stress	75 b	7.8 a	67.6 a
Good watering	69 a	21.3 b	88.6 b

DF= days to flowering; DMC 40= cell membrane damage at 40 °C; CRA= relative water content. Values with the same letter in each column are not statistically different (Tukey, $p \leq 0.05$).

Plants adapt to stress using different mechanisms that involve changes in the morphological and developmental pattern, as well as physiological and biochemical processes (Mutava *et al*., 2011). The delay in the flowering date improves the yield in conditions of drought stress by increasing the number of days available for photosynthesis and the accumulation of dry matter in the sorghum (Alhajturki *et al*., 2012). In the case of damage to the cell membrane at 40 °C under water stress, it showed the lowest average value (7.8) compared to the condition of good irrigation (21.3). This could be due to the existence of an adjustment of the stability of the cell membrane to water stress according to Águeda (2008).

In the case of the variable relative water content, this is an average of 67.6% under water stress because the CRA is the most used expression to measure the level of water of a tissue with respect to the total water that it can store, and is directly proportional to the water availability of the soil, so that the condition of good irrigation obtained a higher average (88.6%). Castro *et al*. (2000) found a similar behavior of the relative leaf water content when evaluating 29 sorghum genotypes for grain in drought and irrigation conditions, with average values of 72.8 and 82.2%, respectively.

The difference in the relative water content of cultivars that suffer from drought stress may be due to their capacity for greater absorption of water from the soil or the capacity of stomata, to reduce water loss (Keyvan, 2010). Because the interaction condition x genotype was significant for the variables DF and DMC 40, the Tukey means test ($p \leq 0.05$) of the genotypes was performed independently for each condition (good irrigation and low water stress) that it is shown in Table 2.

Table 2. Mean values of genotypes and test of means for interaction condition∗genotype.

Genotypes	Type of line	With interaction				Without interaction CRA
		Good watering		Low water stress		
		DF	DMC 40	DF	DMC 40	
LES-1	R	71 ac	39.9 a	93 ab	5.4 ab	80.5
LES-2	R	75 bc	15.7 a	67 ab	5.6 ab	82.4
LES-3	B	64 ab	13.4 a	61 ab	10.1 ab	78.8
LES-4	B	72 bc	12.6 a	92 ab	13.5 ab	77.7
LES-5	B	69 ac	33.0 a	68 ab	14.7 ab	91.6
LES-6	B	70 ac	5.1 a	78 ab	5.9 ab	67.2
LES-7	B	67 ab	7.6 a	58 ab	12.9 ab	96.5
LES-8	B	59 ab	31.5 a	67 ab	20.4 b	88.5
LES-9	B	63 ab	5.9 a	61 ab	2.9 ab	65.7
LES-10	R	59 ab	20.3 a	69 ab	22 b	62

Genotypes	Type of line	With interaction				Without interaction CRA
		Good watering		Low water stress		
		DF	DMC 40	DF	DMC 40	
LES-11	R	65 ab	19.2 a	78 ab	8.1 ab	68.3
LES-12	R	69 ac	15.7 a	94 ab	12.3 ab	81.4
LES-13	R	68 ab	22.1 a	61 ab	10.8 ab	80.4
LES-14	R	68 ab	26.2 a	101 b	8.9 ab	68
LES-15	R	73 bc	6 a	88 ab	3.8 ab	77.3
LES-16	R	69 ac	37.2 a	66 ab	11.7 ab	80.6
LES-17	R	67 ab	39.3 a	65 ab	11.7 ab	88.1
LES-18	R	64 ab	16.3 a	64 ab	12.4 ab	77.4
LES-19	R	89 c	16.7 a	104 b	13.3 ab	78.5
LES-20	R	60 ab	24 a	63 ab	0.55 a	73.4
LES-21	B	75 bc	34.7 a	86 ab	8 ab	87.6
LES-22	B	70 ac	43.3 a	80 ab	0.77 a	91.7
LES-23	B	77 bc	32.3 a	85 ab	2.9 ab	67.6
LES-24	B	74 bc	23.5 a	86 ab	6.2 ab	74.6
LES-25	R	51 a	35.4 a	51 a	9.6 ab	88
LES-26	R	69 ac	25.6 a	80 ab	3.6 ab	73
TES 1-27	Hybrid	71 ac	5.9 a	85 ab	3.5 ab	73
TES 2-28	Hybrid	79 bc	21.9 a	54 a	14.5 ab	73.2

DF= days to flowering; DMC 40= cell membrane damage at 40 °C and CRA= relative water content. Values with the same letter in each column are not statistically different (Tukey, $p \leq 0.05$).

The test of means for genotypes in the DF variable under irrigation conditions, shows two groups, one of 20 genotypes (early) with a flowering range of 51-71 days and another of eight genotypes (late) with a range of 72- 79 days In the case of water stress, the genotypes were also grouped into two categories, 26 genotypes (early) in a flowering range of 51-94 days and 2 genotypes considered late with 101 and 104 days to flowering.

Highlights genotype 25 with the lowest average value (51 days) for both humidity conditions, this is explained because being the earliest genotype and starting water stress 43 DDS could not affect this variable. The differences in flowering days between genotypes in each of the conditions is since they are adapted experimental lines in different regions of the country such as Central High Valleys, Bajio and Northeast of the country.

In the case of the DMC variable 40 and the interaction being significant, the analysis of means in the condition of good irrigation did not show significant differences between genotypes, while under water stress two groups of genotypes were identified; the first with two genotypes (20 and 22) with values of 0.55 and 0.77% that represented the one with the least damage; the other group with 26 genotypes included a range of 2.9-22% of DMC 40.

This parameter is an indicator of heat tolerance, so low values indicate high membrane thermostability, while high values indicate low thermostability, which may be an indirect selection criterion for heat tolerance (Blum *et al*., 2001; Morales *et al*., 2015). Also, it is one of the secondary traits that is used to study drought and heat stress, since it is a quantitative trait that is moderately heritable with a high genetic correlation with grain yield, so it is widely used for select tolerant genotypes (ElBasyoni *et al*., 2017).

In the CRA variable, the means test of genotypes did not show significant differences between genotypes, presenting a mean of 78.3%. According to Yamasaki and Rebelo (1999), the relative leaf water content is the measure of the current water state of the leaf related to its maximum water retention capacity in complete turgidity, which may be indicative of the degree of stress expressed under drought and heat.

Conclusions

Damage to the cell membrane at 40 °C managed to classify genotypes 20 and 22 as tolerant and genotypes 5 and 8 as susceptible. The relative water content was lower in the condition of low water stress; however, among genotypes I have no significant differences that help identify their tolerance to water stress. The days at flowering of the 28 genotypes were lower in the condition of good irrigation. The means test for genotypes in the DF variable shows genotype 25 with the lowest average (51 days) in both conditions, while genotype 19 had the highest average (89 days for irrigation and 104 days for drought respectively.

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