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RESEARCH

Physiological and biochemical analyses of novel drought-tolerant maize lines reveal osmoprotectant accumulation at silking stage

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ABSTRACT

Drought is one of the most limiting factors in agricultural production worldwide. The aim of this work was to evaluate the *per se* response to drought stress of three maize (*Zea mays* L.) semi-inbred lines (CHIH, COAH, and ARZM) during the anthesis and grain filling stages. These semi-inbred lines (BC1S1) are S_1 offspring of crosses between drought-tolerant maize and a high-yield line bred by the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). Materials were evaluated during a 15-d controlled drought and post-drought irrigation reestablishment period. The photosynthetic rate decreased by 50% in CHIH during drought and it recovered after stress was removed. Proline levels increased 2.3 times in COAH during stress and nonsignificant increases were observed in ARZM or CHIH. However, glycine betaine accumulated at 1061-1133 ng mg^{-1} fresh weight (FW) before stress, but decreased to 879-1000 ng mg^{-1} FW during drought stress. The trehalose concentration increased 1.8 times in ARZM (5.60 ng mg^{-1} FW) under drought stress and remained at similar levels in CHIH (6.48 ng mg^{-1} FW) and COAH (8.63 ng mg^{-1} FW) before and during drought stress. In contrast, trehalose-6-phosphate decreased 33% to 38% under drought stress in all three BC1S1 entries and recovered its initial levels after irrigation. Grain biomass loss under drought stress was 54% for ARZM, 48.2% for COAH, and only 26.5% for CHIH. These results showed that CHIH, COAH, and ARZM are drought-tolerant and suggest that osmoprotectant accumulation might play a key role in their physiological performance and grain biomass trait.

Key words: Drought, glycine betaine, maize, proline, trehalose, trehalose-6-phosphate.

INTRODUCTION

Drought is one of the most widespread abiotic limitations in agriculture. The frequency and severity of drought events are increasing, and the overall availability of water is decreasing in many areas due to climate change (Mishra and Singh, 2010). The responses to water stress events are further exacerbated by a greater demand for agricultural products and increased competition for water resources needed for non-agricultural purposes. Maize (*Zea mays* L.) is one of the most important crops worldwide; it is used as feedstock, as food in some countries, and as a bioenergy source. The crop is extremely sensitive to drought during the flowering stage, which causes an impact on both kernel set and grain filling. Drought stress affects growth rates during the vegetative stage of maize by lowering the active photosynthetic leaf area of the crop canopy; this causes a loss in yield at maturity because it extends the anthesis-silking interval (ASI) and limits grain weight (Almeida et al., 2014).

The development of improved maize varieties that are able to withstand drought stress with a minor loss in grain yield is very important; however, the high cost of molecular and genomic techniques are beyond the reach of developing countries. Therefore, interdisciplinary research is essential to develop low-cost techniques and transfer them to small farmers. A successful example is the selection of a shorter ASI together with higher yield under drought stress in Africa (Maiti and Satya, 2014).

The first response to stress is turgor loss that decreases the growth rate, stem elongation, foliar expansion, and stomatal opening; water deficit therefore alters the sink-source relationship and affects the translocation of photosynthates to fruits (Bhargava and Sawant, 2013). The fastest response to water deficit is stomatal closure to protect the plant from water loss. Water deficit produces abscisic acid (ABA) biosynthesis, which triggers stomatal closure and causes a decrease in intracellular CO_2 concentration and photosynthesis inhibition (Chaves et al., 2009). The lack of intracellular CO_2 due to prolonged stomatal closure causes the accumulation of reactive oxygen species and reactive nitrogen species and damages the photosynthetic apparatus (Laxa et al., 2019). There are antioxidant enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, in cellular organelles and the cytoplasm that play an important role in the detoxification of these reactive species. It has been observed under drought conditions that a myriad of genes are expressed, which are involved in osmolytes synthesis, late embryogenesis abundant (LEA) proteins, aquaporins, signaling molecules, and transcription factors (Shanker et al., 2014).

Osmotic adjustment is one of the most efficient processes to protect plant cells from damage caused by abiotic stress and is correlated with higher grain yield under drought stress (Blum, 2017). Polyols such as mannitol, quaternary ammonium salts such as glycine betaine, amino acids such as proline, and sugars such as trehalose, are solutes that are compatible with a metabolism that can accumulate and play an important role in maintaining cellular turgor and protect membranes and proteins from irreversible damage caused by water loss (Dos Reis et al., 2012). Trehalose disaccharide is an osmoprotectant that has been widely studied for its key role in drought tolerance of anhydrobiotic organisms such as resurrection plants, yeasts, many bacterial species, and some invertebrates, which are able to survive months or years in a dehydrated state and revive in a few hours when they are in contact with water again (Iturriaga et al., 2009). It has also been found in many plants, although at relatively low levels, and the genes for trehalose biosynthesis are present in most species. There are five routes of trehalose biosynthesis and the trehalose-6-phosphate synthase-trehalose-6-P phosphatase (TPS-TPP) pathway is the most common in plants, some bacteria, and certain fungi (Figueroa and Lunn, 2016). Trehalose is synthesized in two steps: first, trehalose-6-phosphate (T6P) is synthesized from glucose 6-phosphate and uridine diphosphate-glucose (UDP-glucose) by the TPS enzyme; second, T6P is dephosphorylated by TPP that leads to active trehalose. Trehalose is degraded by trehalase, producing two glucose molecules. It has been reported that genes that encode for the TPS-TPP pathway confer drought tolerance when they are expressed by transgenesis in various crops, including maize (Nuccio et al., 2015).

Previous results have shown that osmoprotectant accumulation in seedlings of a drought-tolerant maize line mainly consists of proline and sugars such as sucrose and trehalose (Velázquez-Márquez et al., 2015). However, drought has an impact on crop yield, mainly at the silking and post-silking stages of flowering grain fill, and especially in the semi-arid regions of the world that depend on the rainy season. In Mexico, drought stress causes significant losses in grain yield and the selection of drought-tolerant maize materials is therefore critical. The Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) has conducted a long-term program in several countries to select and breed drought tolerance in maize. Researchers at the CIMMYT have recently developed breeding lines that display drought tolerance in field tests. These materials were produced by first crossing an open-pollinated landrace to an inbred line, the F_1 was backcrossed to the same inbred line that formed BC1, which formed S1 by selfing. As an S1, these are semi-inbred lines (BC1S1). The aim of the present study was to explore the physiological and biochemical responses of some of these maize BC1S1 entries reported in field tests as drought-tolerant, and treating adult plants at the silking and post-silking stages under irrigation and controlled drought conditions.

MATERIALS AND METHODS

Plant material and treatments

The BC1S1 entries were bred at the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). Using the methodology described by Ruiz Corral et al. (2013) to identify drought-tolerant landraces, 326 subtropical accessions were selected from the CIMMYT Maize Germplasm Bank and the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP) Germplasm Bank for phenotypic evaluation under drought conditions. Briefly, accessions were selected that had the geographical coordinates of field-based collection sites. These coordinates were used to extract data on moisture availability (aridity index; precipitation/potential evapotranspiration) at these sites during the 6 mo of maize cultivation at the relevant sites. Accessions were selected in which the determined aridity index was less than 0.5. The working hypothesis of this selection method is that over generations of cultivation in locations subjected to long-term growing seasons under semi-arid conditions, accessions from these locations will accumulate favorable alleles that confer drought tolerance and/or avoidance. The frequency of such allelic variants should be higher in these accessions due to positive environmental selection compared with materials from more favorable environments (Ruiz Corral et al., 2013).

The landrace accessions were evaluated in the winter season at two locations in western Mexico in 2014 (INIFAP experimental stations at Los Mochis in Sinaloa and Santiago Ixcuintla in Nayarit) and at three locations in 2015 (INIFAP experimental stations at Los Mochis and Santiago Ixcuintla and the CIMMYT experimental station at Ciudad Obregon, Sonora) under managed drought conditions. There was also a normal irrigation treatment in 2015 to compare drought and full irrigation performance. The winter season was selected because there is no rain from October to May in most years. Drip irrigation was used for precise water management and the drought treatment consisted of interrupting irrigation 2 wk before flowering and reestablishing irrigation 2 wk after flowering. The 20 best performing accessions over 2 yr were selected for the drought tolerance breeding project of the CIMMYT Genetic Resource Program. The three selected accessions for the study were CHIH338 (Chihuahua, Mexico), COAH117 (Coahuila, Mexico), and ARZM12236 (Catamarca, Argentina); they were three of the best performing landraces from the evaluations. To develop breeding lines, accessions were crossed and backcrossed to the elite CIMMYT line CML376, followed by one selfing event, and then crossed to the tester CML373. The testcross hybrids were evaluated at three locations in western Mexico in the winter of 2016 (INIFAP experimental stations at Los Mochis and Santiago Ixcuintla and the CIMMYT experimental station at Tlaltizapán, Morelos) under both drought and normal irrigation treatments. At this point in the breeding process, the three semi-inbred lines were selected for phenological and biochemical analyses in the present study. Each evaluated BC1S1 semi-inbred line was assigned an abbreviated name: CML376<2(CHIH338)-1-1 (CHIH), CML376<2(COAH117)-1-1 (COAH), and CML376<2(ARZM12236)-1-1 (ARZM). Plants were grown in a greenhouse in 30 L pots with a homogeneous mixture of sterile sand and peat moss (4:1) with an N (12%), P (11%), and K (18%) mixture. Experimental units (10 plants plot^{-1}) were distributed in split plots (irrigated or drought-stressed treatments) in a randomized complete block design with three replicates of two independent experiments. Irrigation was applied with 1 L distilled water every other day under greenhouse conditions at 30 ± 2 °C, 40% RH, and 16:8 h photoperiod. Adult maize plants at the silking stage were subjected to a drought treatment by interrupting watering for 15 d before a final irrigation event was applied 2 d after providing 2 L distilled water to each plant. Flag leaves were used to measure the photosynthesis rate, relative water content (RWC), and osmoprotectant quantification.

Quantification of relative water content (RWC) and leaf temperature

The RWC is a measure of relative turgidity, and has been widely accepted as a reproducible and meaningful index of plant water status. It was determined following a reported protocol (Soltys-Kalina et al., 2016) that measures in triplicate before, during, and after drought stress according to the following formula:

$$\text{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})} \times 100$$

An infrared thermometer was used to measure the temperature at the center and both ends of the leaf blade to obtain a mean temperature of the flag leaf in triplicate every day at noon before, during, and after drought stress.

Photosynthesis measurement

This variable was determined as the QY value, which is the quantum yield of photosystem II (PSII); it is the parameter most used to determine chlorophyll fluorescence. The photosynthetic rate was measured with a photosynthetic fluorescence meter (FlourPen FP100, Photon Systems Instruments, Drasov, Czech Republic); it recorded the QY value, which is equivalent to PSII efficiency. The fluorometer determines the quantum efficiency of PSII ($QY = F_v/F_m$) where F_v is variable fluorescence and F_m is maximum fluorescence when applying continuous actinic light. It was measured on the flag leaf of each plant in triplicate.

Quantification of proline and glycine betaine

Proline was determined according to Ábrahám et al. (2010) using a ninhydrin-acetic acid mixture and ethanol; samples were analyzed with a spectrophotometer at 520 nm using maize flag leaves previously frozen with liquid nitrogen from plants treated under drought, irrigation, and recovery conditions for each plant. Glycine betaine was measured according to a reported protocol using potassium iodine and sulfuric acid with a spectrophotometer at 365 nm (Islam et al., 2009). Quantification was carried out during drought, irrigation, and irrigation reestablishment treatments for each population in triplicate.

Quantification of trehalose and trehalose-6-phosphate

The technique is based on a protocol for trehalose extraction from plant tissues (Velázquez-Márquez et al., 2015). Briefly, the extract was heated to 95 °C and passed through an ion exchange mini-column eluted with deionized water. The eluate was analyzed with a high-performance liquid chromatography (HPLC) system (Agilent/HP 1050 HPLC System, Agilent Technologies, Waldbronn, Germany) and the chromatography column was an Aminex HPX-87H (Bio-Rad, Hercules, California, USA). An infrared (IR) detector was used and analysis conditions were 60 ºC, 0.6 mL min^{-1} flow, and sulfuric acid 0.05 M mobile phase. The trehalose and T6P standards were from Sigma-Aldrich (St. Louis, Missouri, USA). For T6P extraction, the same procedure and HPLC conditions were applied, except for quantification that was determined according to the equation $y = 120x + 5.13$ where y is the area under the curve and x is the T6P concentration.

Grain biomass determination

All maize ears from the experimental units were collected and kernels per row, ear length, ear width, kernels per ear, and ear weight were determined. Once kernels were shelled from the cob, they were weighed every week during 1 mo to obtain the final weight and assuming that weight remains constant when kernels contain 14% moisture. At this point, grain weight per plant and mean per BC1S1 were determined.

Statistical analysis

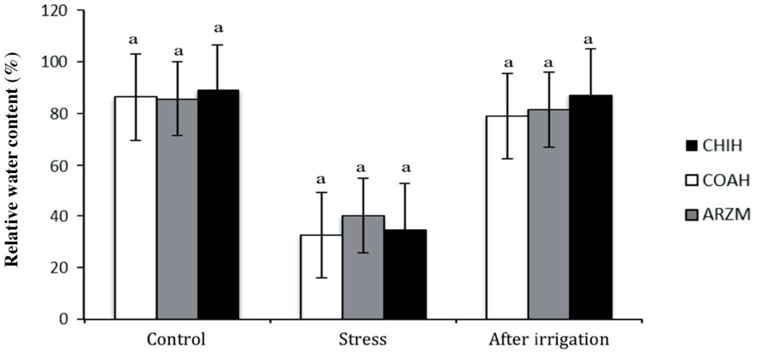
The statistical analysis was performed with the SAS-University-Edition program (SAS Institute, 2012), which consisted of ANOVA for experimental units (10 plants plot^{-1}) distributed in split plots (irrigated or drought-stressed treatments) in a randomized complete block design. Experimental units (10 plants plot^{-1}) were distributed in split plots (irrigated or drought-stressed treatments) in a randomized complete block design with three replicates of two independent experiments, and comparison of means was determined by Tukey's test ($P \leq 0.05$).

RESULTS

Relative water content (RWC) and leaf temperature under stress

The water status was measured by the RWC test. There were nonsignificant differences ($P < 0.05$) among CHI, COAH, and ARZM BC1S1 entries before applying stress, during drought stress, or after reestablishing irrigation (Figure 1). However, there were significant differences ($P < 0.05$) between values obtained before applying drought stress (86% to 88%) and during drought stress (33% to 40%), but not between the RWC values before drought stress and after reestablishing irrigation (78% to 87%); plants recovered their water content at similar levels after applying stress, suggesting that there is no irreversible wilting or cell damage.

Figure 1. Relative water content in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



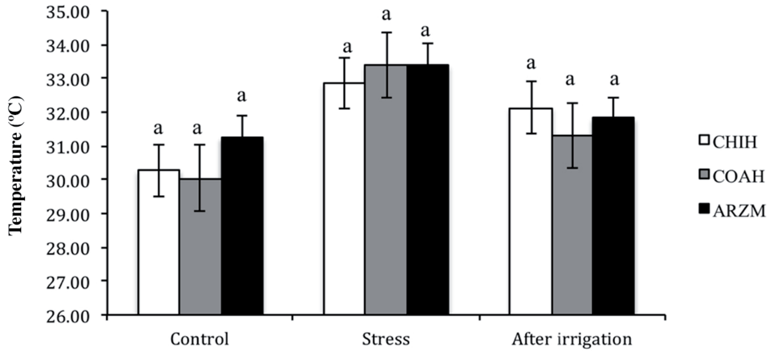
The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

The effect of drought on leaf temperature of the three maize lines before drought stress (30-31.2 ºC), during stress (32.8-33.4 ºC), and after reestablishing irrigation (31.3-32.1 ºC) was then evaluated. In Figure 2, a significant increase in leaf temperature is observed in CHIH, COAH, and ARZM during drought stress. After reestablishing irrigation, leaf temperature of the three lines did not return to its original value.

Effect of drought on photosynthesis

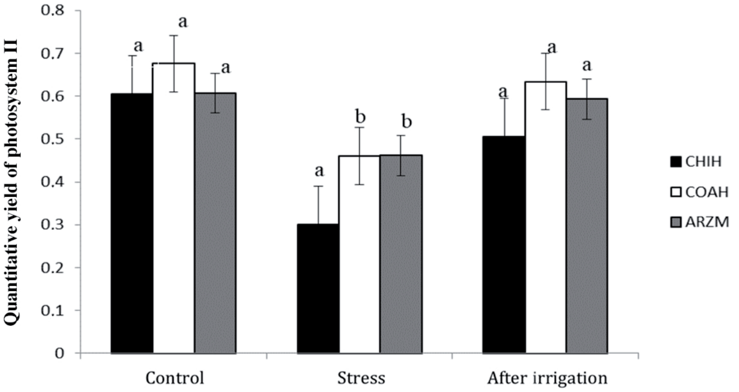
Drought stress affects QY of PSII, which is equivalent to F_v/F_m, and is a parameter used to measure chlorophyll fluorescence (Wang et al., 2012). Light energy used in photosynthesis is lost as fluorescence and mainly from the PSII reaction. Drought decreases fluorescence emission, which makes it a potentially useful tool to detect drought effects on plants (Guo and Tan, 2015). Therefore, we measured the QY of each maize semi-inbred line to determine the change in photosynthesis during drought stress and after reestablishing irrigation. The QY was similar for CHIH, COAH, and ARZM before applying stress (Figure 3) and ranged from 0.60 to 0.68. In contrast, there were significant differences ($P < 0.05$) in QY of the three maize semi-inbred lines during drought stress; CHIH was the most affected (0.30), whereas COAH and ARZM were the least affected and had a value of 0.48 QY (Figure 3). After reestablishing irrigation, nonsignificant difference was observed among the three semi-inbred lines, which varied from 0.51 to 0.64 QY. This suggests that photosynthesis for CHIH was mostly affected by drought stress, but it was restored after reestablishing irrigation as it occurred for CHIH and ARZM. These results indicate that CHIH, COAH, and ARZM maize BC1S1 entries are drought tolerant.

Figure 2. Leaf temperature profile in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Figure 3. Quantitative yield of photosystem II (QY) in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



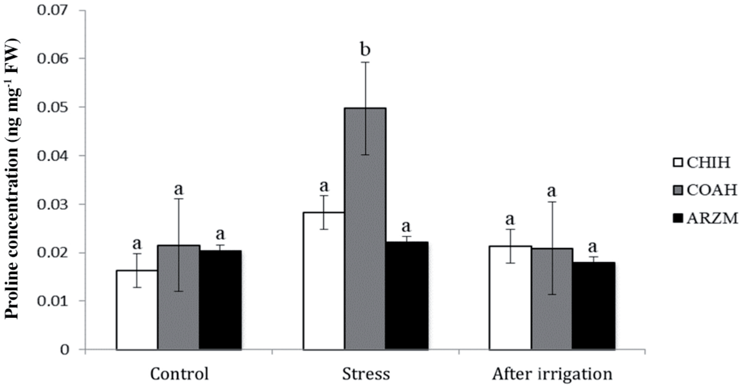
The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Osmoprotectant accumulation under drought conditions

We measured three osmotic active compounds commonly present in drought-tolerant plants: proline, glycine betaine, and trehalose. First, the proline concentration of the three lines was measured before and during drought stress and after reestablishing irrigation. Proline levels before stress, were 160-210 ng mg^{-1} fresh weight (FW), and they were similar in CHIH, COAH, and ARZM (Figure 4). However, the proline concentration during drought stress increased 2.3 times in COAH and 1.75 times in CHIH; however, it did not vary in ARZM. This suggests that drought stress induced proline biosynthesis; proline levels returned to the normal concentration when stress ceased and the plant regained its water content. The ARZM proline levels did not vary significantly before or during drought stress or after reestablishing irrigation (200, 220, and 170 ng mg^{-1} FW, respectively), suggesting that drought did not induce proline biosynthesis in this maize semi-inbred line.

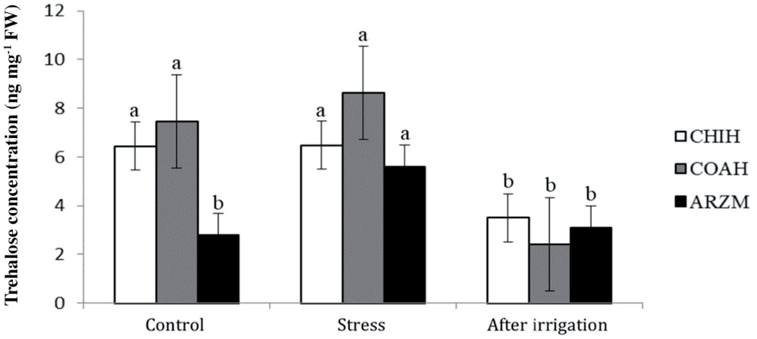
The trehalose concentration was determined in the three BC1S1 at anthesis before and during drought stress and after reestablishing irrigation. Trehalose was present before stress at 6.43, 7.47, and 2.81 ng mg^{-1} FW in CHIH, COAH, and ARZM, respectively, and there was significantly less disaccharide in ARZM. A significant increase ($P < 0.05$) during drought stress in ARZM (5.60 ng mg^{-1} FW) was observed (1.8 times), whereas nonsignificant increase was detected in CHIH (6.48 ng mg^{-1} FW) and COAH (8.63 ng mg^{-1} FW) after drought stress (Figure 5). As expected, trehalose levels decreased after irrigation; however, they decreased to levels lower than the control conditions in CHIH (3.51 ng mg^{-1} FW) and COAH (2.40 ng mg^{-1} FW).

Figure 4. Proline concentration in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Figure 5. Trehalose concentration in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).

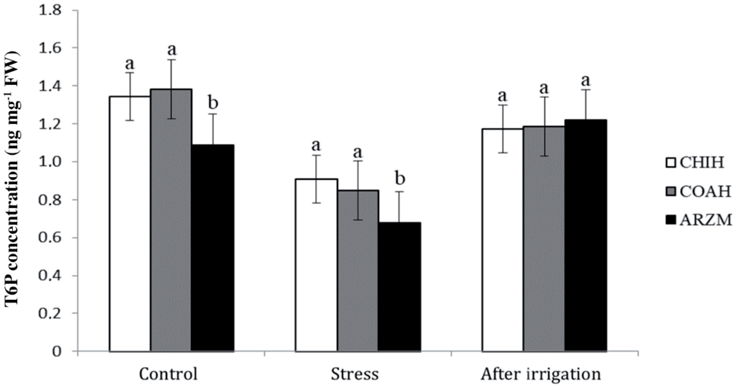


The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

In addition, we measured the T6P concentration of CHIH, COAH, and ARZM maize lines before and during drought stress and after reestablishing irrigation (Figure 6). The T6P levels in CHIH (1.34 ng mg^{-1} FW) and COAH (1.38 ng mg^{-1} FW) were similar before stress, but the level was significantly lower in ARZM (1.10 ng mg^{-1} FW). During drought stress, the three BC1S1 entries showed a significant decrease ($P < 0.05$) in T6P concentration, which was more pronounced in ARZM (0.68 ng mg^{-1} FW) and to a significant lesser extent in CHIH (0.90 ng mg^{-1} FW) and COAH (0.85 ng mg^{-1} FW) (Figure 6). The CHIH (1.17 ng mg^{-1} FW), COAH (1.18 ng mg^{-1} FW), and ARZM lines (1.21 ng mg^{-1} FW) recovered their T6P concentration after reestablishing irrigation to similar levels compared with the control conditions (Figure 6).

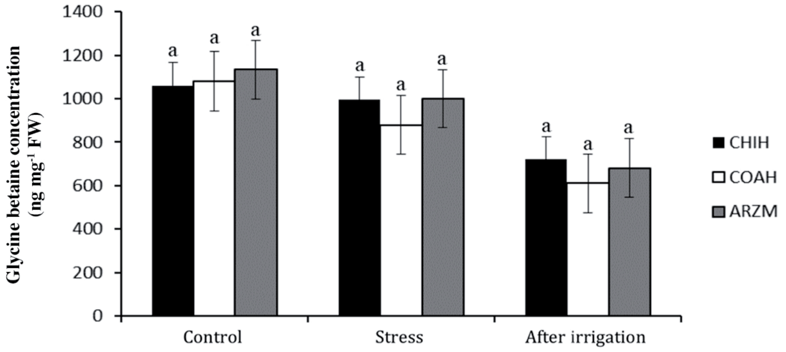
Glycine betaine accumulation has been reported as an effective and efficient osmoprotectant in various crops, including maize (Wani et al., 2013). Therefore, in the present study the glycine betaine concentration of the BC1S1 entries was determined before and during drought stress and after reestablishing irrigation. Before drought stress, glycine betaine varied from 1061 to 1133 ng mg^{-1} FW in the three lines and represented nonsignificant differences ($P < 0.05$) (Figure 7). No increase in glycine betaine was found during drought stress. On the contrary, the glycine betaine concentration decreased to 879-1000 ng mg^{-1} FW and further decreased to 680-720 ng mg^{-1} FW when irrigation was reestablished; this suggests that drought stress did not induce biosynthesis of this osmoprotectant in the maize lines analyzed in this study.

Figure 6. Trehalose-6-phosphate (T6P) concentration in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Figure 7. Glycine betaine concentration in three maize BC1S1 entries under different water treatments (control, stress, and after irrigation).



The values represent the mean ± SE (n = 10). Different letters indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Grain biomass upon stress

We determined the grain biomass of CHIH, COAH, and ARZM maize lines under drought stress and compared them with the irrigated control. Table 1 shows ear traits obtained under drought and irrigation conditions for the three maize BC1S1 entries. After drought, ear rows decreased 16.7% in COAH and ARZM, but remained constant in CHIH; kernels per row decreased 14.3% in CHIH, 23.8% in ARZM, and 26.1% in COAH. Ear length decreased only 6.7% in CHIH, 26.7% in COAH, and 31.2% in ARZM; it remained constant in CHIH but decreased 25% in the two other BC1S1 entries. There was also a significant reduction in kernels per ear in COAH (37.5%) and ARZM (53.8%), while CHIH only decreased by 13.2%. Finally, ear weight decreased in all three lines, but significantly more in COAH (50%) and ARZM (61.9%) than in CHIH (26.3%) (Table 1). All these results clearly show that yield components were significantly less affected in CHIH than in the other two maize materials.

Estimated grain biomass per plant was 44.16, 25.09, and 29.98 g under drought conditions and 60.08, 48.44, and 65.16 g under irrigation for CHIH, COAH, and ARZM, respectively (Table 2). These results show that drought affected the physiological performance of the three evaluated BC1S1 entries. However, under drought conditions, CHIH was the least affected by drought with a decrease in grain biomass of 26.50%, followed by COAH with 48.20% and ARZM, which exhibited the highest decrease (53.99%) (Table 2).

Table 1. Maize ear traits under drought stress and control of BC1S1 entries.

	CHIH		COAH		ARZM	
	Control	Stress	Control	Stress	Control	Stress
Rows	12a	12a	12a	10b	12b	10b
Kernels per row	21a	18b	23a	17b	21a	16b
Ear length, cm	15a	14a	15a	11b	16a	11b
Ear width, cm	4a	4a	4a	3b	4a	3b
Kernels per ear	68a	59b	48a	30c	65a	30c
Ear weight, g	19a	14b	16b	8c	21a	8c

The values represent the mean ± SE (n = 10). Different letters in the same row indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

Table 2. Plant grain biomass under drought stress and control of three maize BC1S1 entries.

	CHIH		COAH		ARZM	
	Control	Stress	Control	Stress	Control	Stress
Grain biomass per plant, g	60.08a	44.16b	48.44a	25.09b	65.16a	29.98b
Grain loss, %	0a	26.50b	0a	48.20b	0a	53.99b

The values represent the mean ± SE (n = 10). Different letters in the same row indicate significant differences between treatments and semi-inbred lines (Tukey's test, $P \leq 0.05$).

DISCUSSION

Drought stress severely limits agricultural production because crop productivity is largely dependent on water availability. We used novel maize materials, namely, three BC1S1 semi-inbred lines (CHIH, COAH, and ARZM) previously developed from landraces with the highest performing phenotypic evaluation under drought conditions and crossed with the CML376 elite line (CIMMYT). Previous work allowed the selection of a series of accessions with higher performance under drought in the field using the approach described by Ruiz Corral et al. (2013). These materials were crossed to appropriate CIMMYT maize lines with a heterotic pattern and then backcrossed to the same line. The BC1S1 used in the experiment were subjected to further multi-location field experiments with appropriate controls, and the most drought-tolerant materials were selected. The materials evaluated in the present study were three of the most drought-tolerant materials; their tolerance was defined through field evaluation rather than in a greenhouse study. For greenhouse experiments and since our materials were drought tolerant, we measured the relative stress tolerance between them using the RWC test, which showed a significant recovery of turgor after dramatic water loss (Figure 1).

It is well known that RWC indicates water status in plants, reflecting the balance between water supply to the leaf tissue and the transpiration rate (Lugojan and Ciulca, 2011). We showed that adult flowering maize plants can recover from severe wilting (33% to 40% RWC) without apparent cell damage. To our knowledge, the current work is the first study to report this phenomenon of total RWC recovery after drought stress in flowering maize plants. These results agree with Chen et al. (2016), who evaluated the RWC of 10 maize lines at the seedling stage and found a significant decrease in RWC (44.8% to 64.3%) of all seedlings that were subjected to drought compared with the controls, and all lines had a significant recovery after rewatering plants.

Analysis of leaf temperature in the maize semi-inbred lines showed an increase under drought stress that did not recover the original temperature, but it was within a range that is not harmful to plants. It is known that temperatures greater than 35 °C significantly decrease the activity of ribulose 1,5-bisphosphate carboxylase oxygenase (RuBisCO), thus limiting photosynthesis and respiration (Yamori et al., 2014). In chloroplasts, high temperatures reduce the photochemical efficiency of PSII, which is the photosynthesis component most sensitive to high temperatures.

The photosynthesis rate also decreased from 50% to 70% during drought stress, but it was recovered after reestablishing irrigation, indicating that the CHIH, COAH, and ARZM maize lines are drought tolerant. Photosynthesis is affected under drought stress, but tolerant plants manage to restore this process after stress ends. Hayano-Kanashiro et al. (2009) measured the photosynthesis rate of three Mexican maize landraces; Michoacán21 and Cajete were both drought tolerant and recovered after severe stress, whereas 85-2 did not recover because it was drought sensitive.

Osmoprotectant accumulation is a key biochemical trait in plants that are tolerant to abiotic stress (Dos Reis et al., 2012; Wani et al., 2013), and there is clear evidence that osmotic adjustment sustains crop yield under drought stress (Blum, 2017). Therefore, we measured two common osmoprotectants that accumulate in drought-tolerant crops, that is, proline and glycine betaine, as well as trehalose that is rarely present in crops. Under drought stress, proline accumulated in the COAH and CHIH lines but not in ARZM. Drought stress causes changes in amino acid metabolism. It has been shown that proline accumulation is correlated with osmoprotection and its biosynthesis is an important factor in drought tolerance in Mexican maize landraces such as Michoacán21 and Cajete (Hayano-Kanashiro et al., 2009). In contrast, drought stress did not induce glycine betaine accumulation; however, its concentration before and after drought stress was at levels comparable with osmotic-tolerant maize lines that accumulate glycine betaine (Peel et al., 2010). Therefore, it appears that glycine betaine is accumulated constitutively in the CHIH, COAH, and ARZM lines and can contribute to their drought-tolerant phenotype.

In the present study, trehalose was found to accumulate in ARZM at significant levels in adult plants at the reproductive stage; however, it was also found in CHIH and COAH before and after drought stress with similar concentrations. Thus, together with proline and glycine betaine, trehalose accumulation might be responsible for the recovery of turgor and drought tolerance. In a recent study, trehalose accumulation occurred in maize seedlings of a VS-22 drought-tolerant line, but not in the AMCCG-2 drought-sensitive line (Velázquez-Márquez et al., 2015). To our knowledge, the present study is the first case in which trehalose was accumulated in adult maize plants at the flowering stage (silking and post-silking). It is well established that trehalose accumulation in several anhydrobiotic organisms, including ‘resurrection plants’, confers drought tolerance (Iturriaga et al., 2009). Genomic studies have shown the trehalose biosynthesis gene family in several crops, including maize; however, the presence of trehalose was not reported (Henry et al., 2014). Transgenic maize overexpressing trehalose biosynthetic enzymes exhibited improvements in drought tolerance and yield (Nuccio et al., 2015).

Drought, after silking and until the maturity stages, affects grain weight and severely decreases maize grain biomass (Maiti and Satya, 2014). Regarding yield components, it is worth mentioning that ears from the three BC1S1 entries displayed important differences under drought stress. Grain biomass of each maize BC1S1 was estimated under drought and irrigation conditions. Under drought conditions, ears exhibited incomplete development, whereas they were fully developed under constant watering. The CHIH maintained the number of rows and constant ear width and had a relatively small decrease in kernels per row (14.3%), kernels per ear (13.2%), and a 26.3% decrease in ear weight; all yield components decreased significantly in COAH and ARZM (Table 1). Similarly, CHIH grain biomass loss under drought conditions was 26.50% compared with COAH (48.20%) and ARZM (53.99%) grain loss (Table 2). This reduction in the yield components was correlated with the observed lower water content and photosynthetic activity under drought stress (Figures 1 and 2). It has already been shown in other maize landraces and hybrids that drought stress led to a lower number of kernels per row, reduced the number of kernels per ear, and reduced grain biomass (Mazvimbakupa et al., 2015).

We also tried to establish a correlation between osmoprotectant concentration and yield components. It has been reported that during drought stress, T6P levels decrease and recover after stress ceases (Lawlor and Paul, 2014). A similar pattern was found in the T6P concentration of the three maize BC1S1 entries before and during drought stress and after reestablishing irrigation (Figure 6). In addition to being an intermediary in trehalose biosynthesis, T6P has played a key role in plants as a signal molecule by integrating the use of sucrose with growth and development related to environmental conditions, thus providing a major contribution in maintaining the energy balance. Anabolism dominates catabolism during the day due to the inhibition of the SnRK1protein kinase by T6P, while inhibition ceases during photorespiration at night and catabolism predominates (Nunes et al., 2013). The relationship between T6P and grain yield in wheat under drought is also clearly established. Genetic manipulation of T6P levels in meristems and freshly fertilized wheat grains potentially increases grain size by improving crop yield both under irrigation and drought conditions, although it is lower in the latter (Paul et al., 2018). The ARZM, which had the lowest grain biomass under drought stress, showed the lowest accumulation of trehalose and T6P and similar levels of proline and glycine betaine compared with CHIH and COAH; this suggests that trehalose and T6P are important to promote yield components under stress conditions. It has recently been shown that genes involved in the biosynthesis of trehalose, raffinose, and proline are induced by drought stress in the ear leaf (Wang et al., 2019). The CHIH had the highest yield components and lower grain loss under drought stress; however, it accumulated similar concentrations of trehalose and T6P compared with COAH, which had 1.8 times less grain biomass under stress than CHIH. A transcriptomic analysis could probably shed light on which other molecules and genes are involved in the higher performance and yield of CHIH under drought stress.

CONCLUSIONS

The study results indicate that maize CHIH, COAH, and ARZM semi-inbred lines exhibit a drought-tolerant phenotype. In all three maize BC1S1 entries, the photosynthetic apparatus and water content recovers after drought stress and leaf temperature remains within a normal range. This study demonstrated that trehalose, proline, and glycine betaine accumulate in adult maize plants at the silking and post-silking stages. They presumably help to alleviate drought stress in this crop and, together with trehalose-6-phosphate, might be responsible for the reduction of grain biomass loss during stress.

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