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Maintenance of the water use efficiency in the drought-stressed *Sorghum bicolor* L. as compared to *Zea mays* L. in relation to differential expression of aquaporin genes

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Abstract

Zea mays L. is less tolerant to drought than *Sorghum bicolor* L. In the present study, we investigated the response of both plants to drought stress applied under field conditions by withholding water for 10 d. The plant growth in terms of shoot fresh and dry weights was more severely reduced in maize than in sorghum as a result of drought stress, consistently with reduction of leaf relative water content (RWC). Gas exchange was also more greatly inhibited by drought in maize than in sorghum. As a result, the water use efficiency (WUE) of maize was fluctuated according to the time point during the day and in response to drought stress. In contrast, sorghum was able to maintain largely constant WUE during the day in the well-watered plants as well as under drought stress. This may indicate that sorghum was more efficiently controlled its water status in particular water uptake than did maize. Studying the expression of four aquaporin genes (*PIP1;5*, *PIP1;6*, *PIP2;3* and *TIP1;2*) revealed that most of the genes responded weakly to drought stress except *PIP2;3* which was highly responsive to drought in sorghum but not in maize roots, where it may have supported greater water uptake in sorghum, and thereby maintained higher leaf RWC in sorghum than in maize and hence could account at least in part for the drought tolerance of sorghum as compared to maize. The outcome of this study is that *PIP2*;3 may have role in drought tolerance and maintenance of the WUE of sorghum plants compared to those of maize.

Keywords: *Zea mays*; *Sorghum bicolor*; aquaporin; drought tolerance; water use efficiency; relative water content; gas exchange

Introduction

Marked differences in water use efficiency occur among plants employing the three photosynthetic pathways: C3, C4 and crassulacean acid metabolism (CAM). Plants exhibiting C4 and CAM photosynthesis are more water-use efficient than those exhibiting C3 photosynthesis (Briggs and Shantz, 1914; Fischer and Turner, 1978; Winter *et al.*, 2005). The C4 pathway reduces

photorespiration by elevating the CO_2 concentration at the site of Rubisco using a biochemical CO_2 pump. Thus accelerating net CO_2 fixation in relation to net transpiration, thereby increasing WUE.

Water use efficiency and drought tolerance are often taken loosely as a synonymous, although they are frequently unrelated (Hsiao and Acevedo, 1974). Drought resistance in a genetic/physiological context refers to the ability of one genotype to yield 'better' than another during severe drought stress. On the other hand, WUE is defined as the ratio between diffusion of CO_2 into the leaf (photosynthesis) and loss of H_2O through transpiration (Bassett, 2013).

In C4 plants, drought has been reported to increase WUE as a result of reducing transpiration (Ghannoum *et al*., 2002). However, drought stress led to inhibition of dry matter accumulation and also decreased leaf $_{13}C$ contents. This indicates that drought stress improved the leaf level WUE but may have reduced whole plant WUE. It has also been reported that the high WUE does not necessarily correlate with high growth rates under drought in C4 plants (Maroco *et al*., 2000). Thus, it seems that the relationship between WUE and drought tolerance is still a matter of controversy that need more detailed information to be resolved. Furthermore, the leaf water status in C4 plants appears to be an overriding character that regulates plants growth rate and WUE under normal and drought conditions.

It appears that drought tolerance is a trait linked to many physiological and molecular mechanisms in addition to photosynthetic mechanism. In nature, drought tolerance and drought sensitivity occurs in both C3 and C4 plants. Furthermore, it cannot be ruled out that there is causal relationship between drought tolerance and C4 photosynthesis and hence, no specific correlation can be established between the type of photosynthesis and drought tolerance (Taylor *et al*., 2011).

However, the C4 species differ in their ability to tolerate drought (Kakani *et al*., 2011). Among C4 species, sorghum is known to be more drought tolerant than maize. The drought tolerance of sorghum may be due to its ability to root deeply and thus to draw water from great soil depths (Singh and Singh 1995, Farre and Faci 2006). In contrast, Merrill *et al*. (2007) reported that the depletion in soil water was higher in maize than in sorghum. Singh *et al*. (2010) showed the difference between maize and sorghum in terms of the root systems morphology and architectural development.

At low water potential, the amount of CO_2 entering the leaf reduced because of the loss of turgor of the leaf which led to stomatal closure and so low photosynthesis rate. So that, the maintenance of stomatal opening by osmotic adjustment is necessary for CO_2 fixation by leaves. Sorghum was showed to be one of the plants which can adjust osmotically at a particular low leaf water potential so as to maintain higher rates of photosynthesis than those plants in which hardly any adjustment took place (Jones and Rawson, 1979). In sorghum, Sanchez-Diaz and Kramer (1973) showed a smaller reduction in water content per change in water potential than maize, which they supposed to be due to a lower cell wall elasticity.

The large number of plant aquaporins has been explained by their importance in regulating water flow through the plant body and in maintaining cellular water homeostasis at all developmental stages and in all environmental conditions (Hachez *et al*., 2006).

Under drought stress, the root water uptake through aquaporins has been found to increase (Lu and Neumann, 1999). Cell-to-cell water movement through AQPs is believed to play a pivotal role in coping with environmental stress when transpiration decreases and osmotic flow through membranes is dominant (Vandeleur *et al*., 2005; Kaldenhoff *et al*., 2007).

In the present work, consequences of drought stress on plant growth, gas exchange and WUE in relation to expression of some selected aquaporin genes were studied in *maize* (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.). Maize is widely known as drought sensitive with isohydric response to drought (Tardieu and Simonneau, 1998) whereas sorghum is more tolerant to drought with anisohydric response (Tardieu, 1996).

Materials and methods

Plant material and growth conditions

Two crop plants were used in this study: sorghum (*Sorghum bicolor* L., Hybrid 10) and maize (*Zea mays* L., Hybrid 153). The seeds of both hybrids were supplied by the Agricultural Research Institute (Giza, Egypt). Plants were grown and treated with drought under field conditions in a wire-mesh greenhouse. This experiment was

carried out in the greenhouse of the experimental field of Botany Department (Faculty of Science, Damietta University). The soil was clay with less than 30 mM NaCl soil salinity. The experiment location had coordinates of: 31.4391°N and 31.6821°E and altitude of about 5 m. The climatic conditions over the experiment period were: 27-31/22-25ºC day/night temperature, 65-75% relative humidity during the day (RH), 12 h photoperiod and 2,850 µM m^{-2} s^{-1} maximum light intensity (full sunlight).

The seeds were sown in four main blocks, two blocks for each species. Each block included 60 holes divided into 4 sub-blocks. The distance between holes was 30 cm. Each hole contained two or three seeds. The seeds were sown in holes of the dry soil. The soil was wetted with water. Thereafter, one block from each species was watered regularly and used as control and one block from each species was allowed to dry so that the field capacity (FC: measured by saturating a pre-weighed soil sample with water and calculating the FC% as the soil weight/saturated soil weight %) reached 65%.

Measurement of gas exchange parameters

After 10 days of drought treatment, gas exchange parameters were measured in the control and droughted plants of each species. The photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s) and leaf internal CO_2 (C_i) were measured for the second leaves in each species by using LCi-SD gas exchange system (Analytical Development Company, ADC Ltd, Hertfordshire, UK). The leaf area used was 6.25 cm^2 in each species. The leaf-level water use efficiency (WUE) was calculated as WUE= *A**E*. Gas exchange measurements were made under full sunlight at three different time periods during the day (in the morning which is 3 h after sunrise, in the midday which is 7 h after sunrise and in the afternoon which is 3 h before sunset).

Harvesting of the plant material

At the end of the experiment, whole shoots of five plants from each set were harvested and used for fresh and dry weight determination. Leaf (second leaf) and root samples were also collected, frozen immediately in liquid nitrogen and stored at -80°C until used for subsequent biochemical and molecular analyses. For root samples, the plants were removed carefully from soil, washed briefly in cold distilled water to remove soil remains and frozen immediately in liquid nitrogen. Samples were collected at predawn under dim light (shortly before dawn), at morning, midday and afternoon.

Determination of fresh and dry weights, and relative water contents (RWC)

Samples were collected at midday for measuring RWC. After recording the fresh weights of shoots, they were dried in oven at 60ºC for 2 d and the dry weights were then recorded. To determine the RWC, Fresh leaf samples were weighed (FW) and then incubated overnight in distilled water at 4ºC. Excess water was removed from samples by using absorptive tissue and then samples were weighed (Saturated weight, SW). The samples were then dried in oven at 60ºC for 2 d and weighed (DW). The RWC was then calculated as follows:

$$RWC\% = (FD-DW)/(SW-DW) \times 100$$

Five measurements from different plants were made for each treatment.

Quantification of gene expression by semi-quantitative RT–PCR

Total RNA was extracted from about 50 mg frozen leaves using TRI-reagent (Sigma, UK) according to the manufacture's protocol. To prevent DNA contamination, the extracted RNA was treated with DNA-free kit (Ambion, UK) for 30 min at 37°C. Poly A tail mRNA was then isolated by reacting 10 µl of RNA with 2 µl of oligo dT(18) and 3 µl RNase and DNase free H_2O for 5 min at 70°C and the reaction was terminated on ice for 2 min. The reverse transcription was conducted by using MMLV-reverse transcription kit according to the supplier's recommendations (Promega, UK). Primers for each gene were designed to recognise conserved regions resulting from the alignment of the characterized genes in other species that are related to *Zea mays* and *Sorghum bicolor*. The primers used for amplifying PIP1;5, PIP1;6, PIP2;3, TIP1;2 and 18S rRNA are listed in Table 1. The PCR conditions were as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 45 s. For each gene, the number of PCR cycles was optimized to show the maximal differences among samples within the linear phase of amplification. For each gene, three replicates from different RNA extracts were used. PCR

products were resolved by electrophoresis on 1% agarose gels, stained with ethidium bromide in 1X TAE (Tris–acetic acid-EDTA) using BioRad equipment and visualized and documented using Transilluminator UViTec. The band volumes were measured by using Lab Image V 2.7.2 software. The measurements were normalized for equal 18S rRNA bands.

Table 1. Primer pairs used for amplification of different genes.

Gene name	Forward primer5' to 3'	Reverse primer 5' to 3'
PIP1;5	CATGCAGTGCCTGGGCGC	GTGCCGGTGATGGGGATG
PIP1;6	GTGCCTGGGCGCCGTCTG	GATGGGGATGGTGGCGAG
PIP2;3 Primer I (for sorghum only)	GGCATCTCAGGTGGGCAC	GCCAACACCGGGACGTGGG
PIP2;3 Primer II (for maize only)	ATGGCGAAGCAGGACATCGAAG	CCCGCCGCCGGACTTATTAGG
TIP1;2	GCTCATCTTCGTCTTCGC	AGACGGCGGGGTTCATGG

Statistics

Sigmaplot V 12.0 program was used to run one-way ANOVA followed by LSD analyses.

Results

Responses of the growth of maize and sorghum to drought stress in the greenhouse

Response of the shoot biomass to drought stress

Drought stress led to significant reduction in growth in both maize (Fig 1A) and sorghum (Fig 1B) in terms of shoot FW. The biomass reduction was greater in the droughted maize plants (down to about 25.5% of the control) than in those of sorghum (down to 84.5% of the control). Drought stress led to reduction in DW in maize to about 32.8% of the control but no significant change was observed in sorghum.

The effect of drought stress on shoot RWC

Drought stress reduced the shoot RWC only in maize where it reached 65.8% of the control plants. Drought stress had no significant effect on the shoot RWC in sorghum.

Respones of gas exchange in maize and sorghum to drought stress

The effect of drought treatment on rates of photosynthesis (A)

Drought stress led to a significant reduction of the rates of photosynthesis in maize in the morning and midday with the greatest reduction at midday (down to 78.0% of the control in the morning and 47.7% of the control in the midday) but no significant change was observed in the afternoon maize (Fig 3A). Contrarily, no significant difference was observed in the droughted plants of sorghum in the morning (compared to the control) but significant decrease was observed in the midday and afternoon (83.7% of the control in the midday and 57.5%of the control in the afternoon) (Fig 3B).

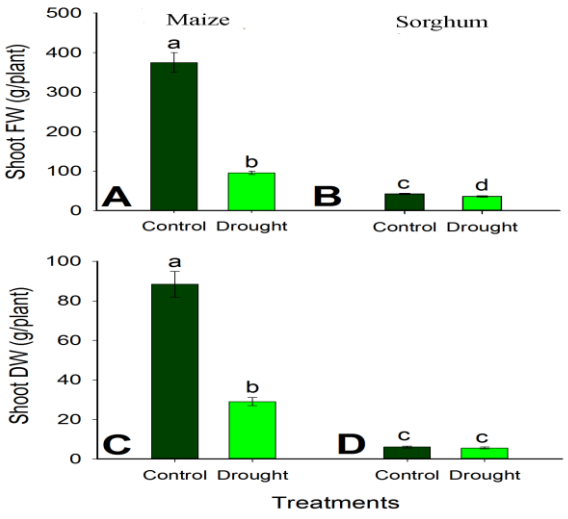

Fig. 1 Effect of drought treatment for 10 d in the green house in terms of shoot fresh weight (A, B) or in terms of shoot dry weight (C, D) on the growth of maize(A, C) and sorghum (B, D). Bars are means of 5 replicates ±SE. Bars ±SE labeled with different small letters are significantly different at $p<0.05$.

The effect of drought treatment on the rates of transpiration (E)

Drought stress led to significant reduction in the rates of transpiration in both plants where *E* decreased (compared to the control) to 83.5% in the morning, 74.9% in the midday and 68.3% in the afternoon in maize (Fig 3C) and to 82.5% in the morning, 89.7% in the midday, 75.1% in the afternoon in sorghum (Fig 3D). The greatest reduction in *E* as a result of drought treatment was observed in maize at midday.

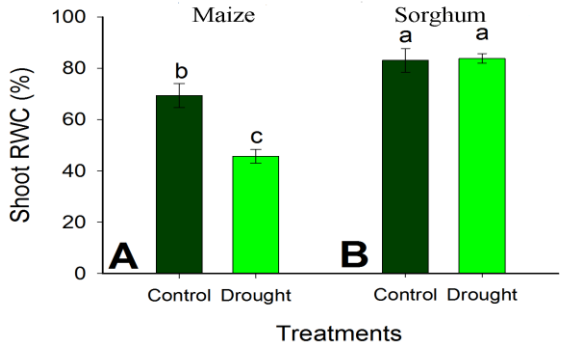

Fig. 2 Effect of drought treatment for 10 d in the green house on the shoot relative water content of maize(A) and sorghum (B). Bars are means of 5 replicates ± SE. Bars ±SE labelled with different small letters are significantly different at $p<0.05$. Samples were collected for measurement of shoot RWC at midday.

The effect of drought treatment on stomatal conductance (g_s)

Drought stress led to significant reduction in the stomatal conductance of maize where it decreased (compared to the corresponding control) to 65.6 % in the morning, 45.7 % in the midday and 61.7 % in the afternoon (Fig 3E). Contrarily, No significant difference was observed in the droughted sorghum plants in the morning or in the afternoon but significant decrease was observed in the midday (82.2% of the control) (Fig 3F).

The effect of drought treatment on the leaf internal carbon dioxide concentration (C_i)

In maize, drought stress led to no significant change in C_i in the morning, increased it significantly in the midday but decreased it significantly in the afternoon (to 39.3% of the control) (p=0.020) (Fig 3G). Contrarily, no significant changes in C_i were observed in sorghum droughted plants at any time point (Fig 3H).

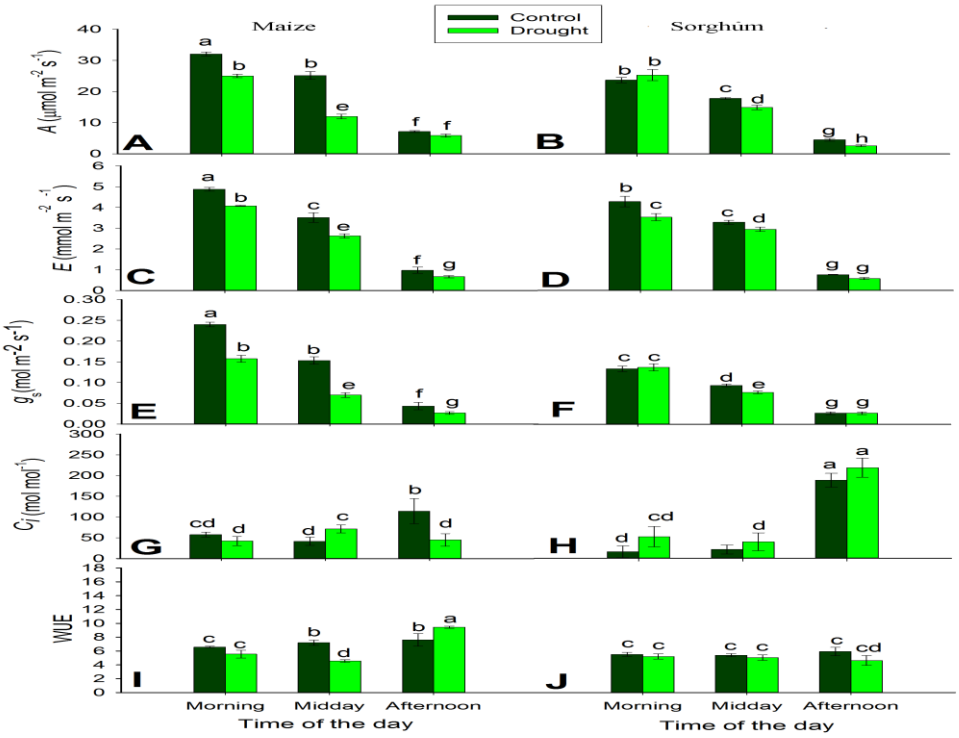

Fig. 3 Changes in photosynthetic rates(A), Transpiration (E), stomatal conductance (g_s), leaf internal carbon dioxide concentration (c_i) and water use efficiency (WUE) at three time points during the day in maize(A, C, E, G, I) and sorghumL.(B, D, F, H, J), respectively, after drought treatment for 10 d. Bars are means or 5 replicates ±standard error. Bars ± SE labelled with different small letters are significantly different at $p<0.05$.

The effect of drought treatment on water use efficiency (WUE)

In maize, drought stress did not change WUE in the morning, reduced it significantly (p=<0.003) in the midday (63.3% of the control) but increased it significantly in the afternoon (Fig 3I). Contrarily, no significant change in WUE was observed in the droughted sorghum plants compared to the controls at all time points (Fig 3J).

Responses of aquaporin expression in maize and sorghumL. to drought stress

The expression of four aquaporin genes (*PIP1;5*, *PIP1;6*, *PIP2;3* and *TIP1;2*) was investigated after drought stress in maize and sorghum. The transcript abundance of the four genes was quantified based on quantitative amplification of 18S rRNA for leaf and root samples (Fig 4). The expression of each gene was normalized based on the band size in each sample

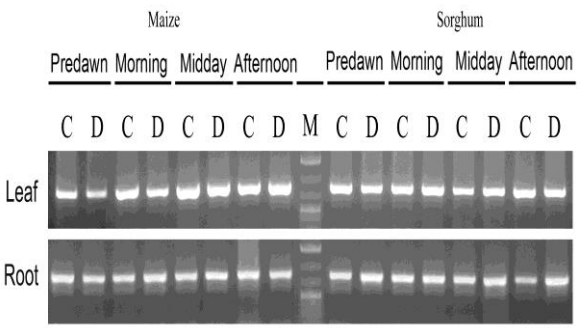

Fig. 4 Amplification of *18 S rRNA* from RNA samples extracted from roots and leaves of maize and sorghum after drought treatment for 10 d in the green house. M is DNA ladder.

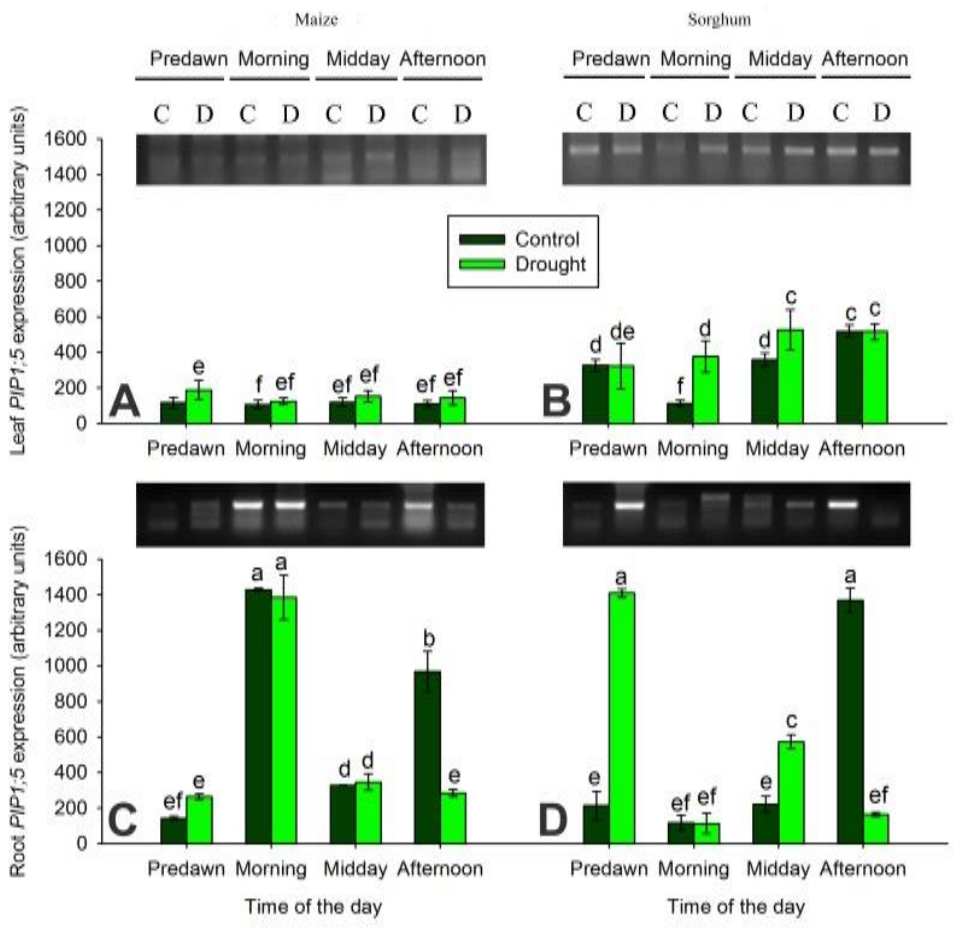

Fig. 5 Changes in the expression of *PIP1;5* in leaves (A, B) and roots (C, D) of maize and sorghum, after drought treatment for 10 d in the green house. Bars are means of 3 replicates ± SE. Bars ± SE labelled with different small letters are significantly different at p<0.05. Statistics was carried out for leaves separately from roots.

The effect of drought stress on PIP1;5 expression

Drought stress did not cause significant change in the expression of *PIP1;5* in the leaves of maize and sorghum compared with their controls (Fig 5A, B) except in sorghum in the morning and midday where there was significant increases (p=<0.001).

In the roots, drought stress didn't lead to significant change in *PIP1;5* expression in maize roots except in the afternoon where the expression significantly decreased (p=<0.001) to 29.4% of the control (Fig 5C). While in sorghum, drought stress caused a significant increase in *PIP1;5* expression at predawn and midday, did not change it the morning, and decreased it significantly (p=<0.001) to 12.1% of the control in the afternoon (Fig 5D). The greatest expression levels of *PIP1;5* were observed in roots of both maize and sorghum..

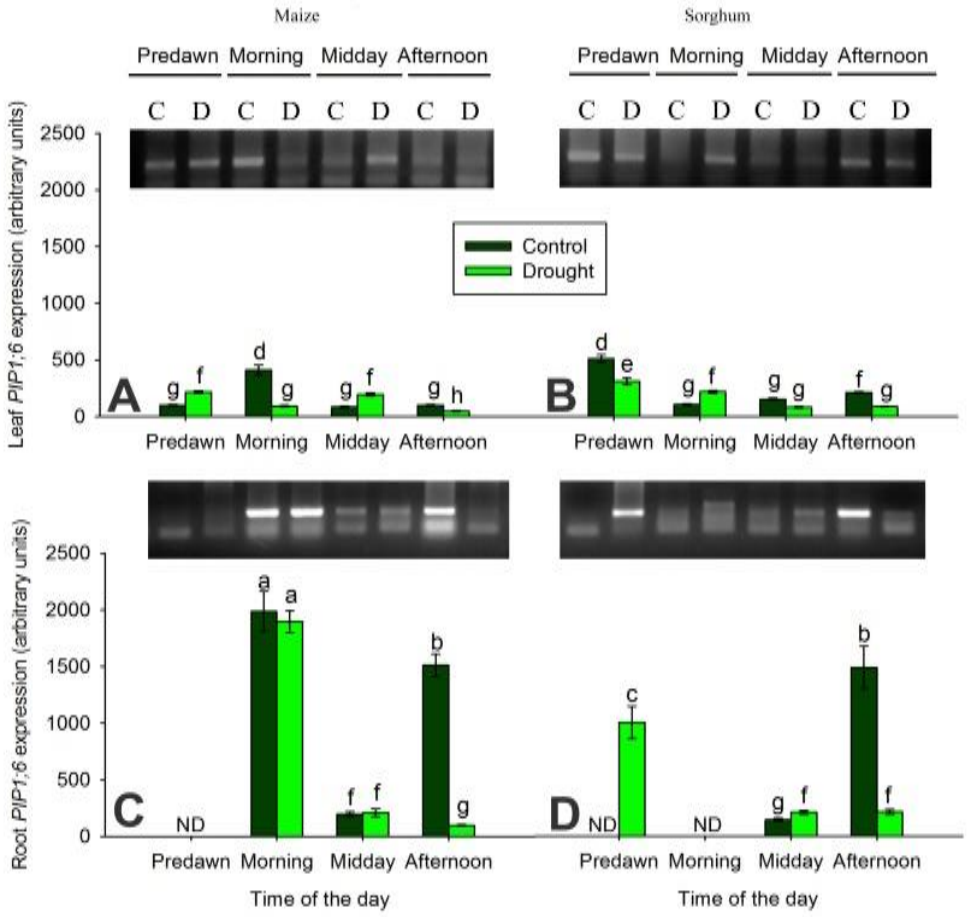

Fig. 6 Changes in the expression of *PIP1;6* in leaves (A, B) and roots (C, D) of maize and sorghum, after drought treatment for 10 d in the green house. Bars are means of 3 replicates ± SE. Bars ± SE labelled with different small letters are significantly different at p<0.05.Statistics was carried out for leaves separately from roots.

The effect of drought stress on PIP1;6 expression

Drought stress did not result in consistent expression pattern for *PIP1;6*, where the expression levels in the droughted leaves were significantly (p=<0.003) higher or lower than in the corresponding controls at different time points in the day in maize and sorghum (Figs 6A& B).

Drought stress resulted in a decrease in PIP1;6 expression in maize roots at afternoon only and reached 6.5% of the control but the transcript abundance remained similar to the control at predawn, morning and midday (Fig 6C). While in sorghum, drought stress increased thePIP1;6 expression in the roots at predawn and midday but decreased it in the afternoon (Fig 6D). Thus, no consistent changes in the expression of PIP1;6

in the roots of maize and sorghum during the day time course or in response to drought

The effect of drought on PIP2;3 expression

Drought stress resulted in increasing the*PIP2;3* expression in maize leaves only at predawn (Fig 7A). In sorghum leaves, drought stress caused a slight decrease in *PIP2;3* expression at predawn to reach 68% of the control. No significant change was observed at morning, midday and afternoon between control and droughted plants (Fig 7B).

No significant change was observed in *PIP2;3* expression in the roots of droughted maize plants at predawn or in the morning but significant (p=<0.002) sharp decreases were observed at midday (so that it was not detected) and in the afternoon (4% of the control) (Fig 7C). In sorghum roots, drought stress caused remarkable increases in *PIP2;3* expression at predawn and morning (with the greatest levels detected in the predawn). Drought stress did not affect *PIP2;3* expression level in the midday but decreased it in the afternoon (14.9% of the control) (Fig 7D). Therefore, *PIP2;3* expression in the roots responded more consistently and strongly to day time and drought stress in sorghum than in maize.

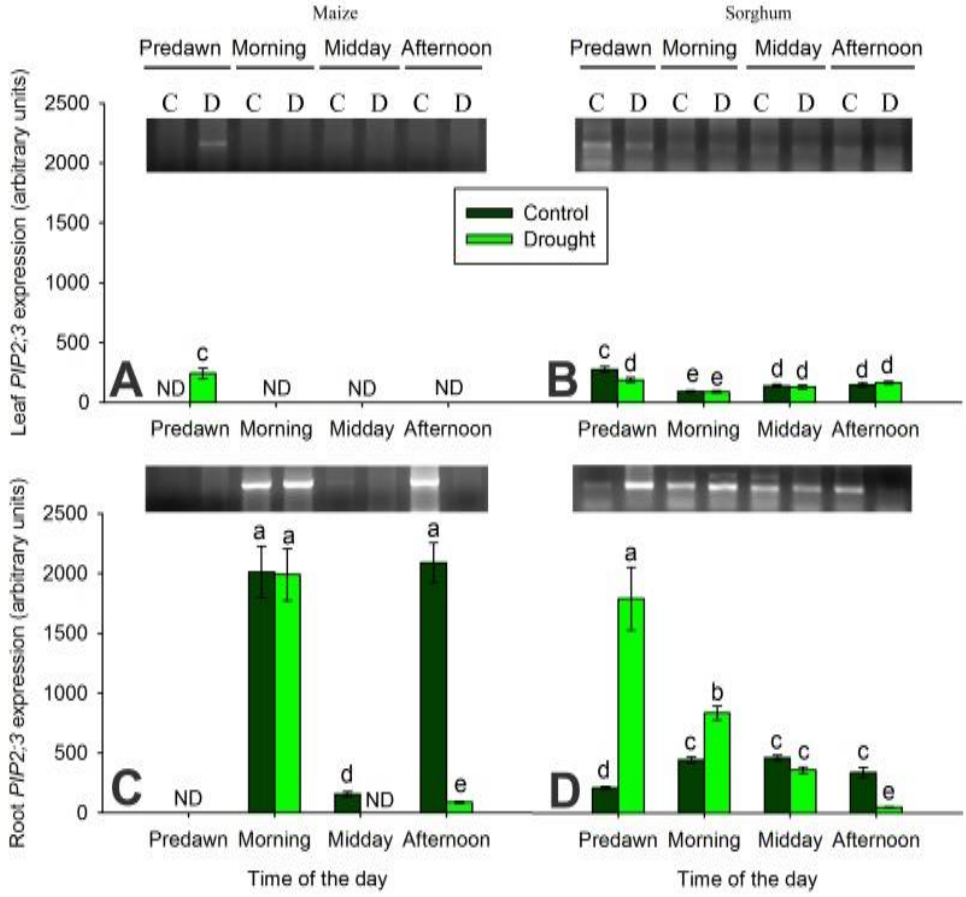

Fig. 7 Changes in the expression of *PIP2,3* in leaves (A, B) and roots (C, D) of maize and sorghum, after drought treatment for 10 d in the green house. Bars are means of 3 replicates ± SE. Bars ± SE labelled with different small letters are significantly different at p<0.05.Statistics was carried out for leaves separately from roots.

The effect of drought on TIP1;2 expression

Drought stress resulted in a significant increase in *TIP1;2* expression in maize leaves at predawn and midday but no significant difference was observed between droughted plants and controls at morning and afternoon (p=<0.027) (Fig 8A). In sorghum leaves, drought stress caused a decrease in *TIP1;2* expression at predawn (18.8% of the control) but no significant difference was observed between droughted and

control plants at morning, midday or afternoon (Fig 8B).

No significant change was observed in *TIP1;2* expression in the roots of droughted maize plants at predawn, morning and midday but a significant decrease was observed in the afternoon so that no transcripts were detected (Fig 8C). In sorghum, drought stress caused a significant increase in *TIP1;2* expression in the predawn and morning but resulted in significant decreases at midday and afternoon where no expression was detected (Fig 8D).

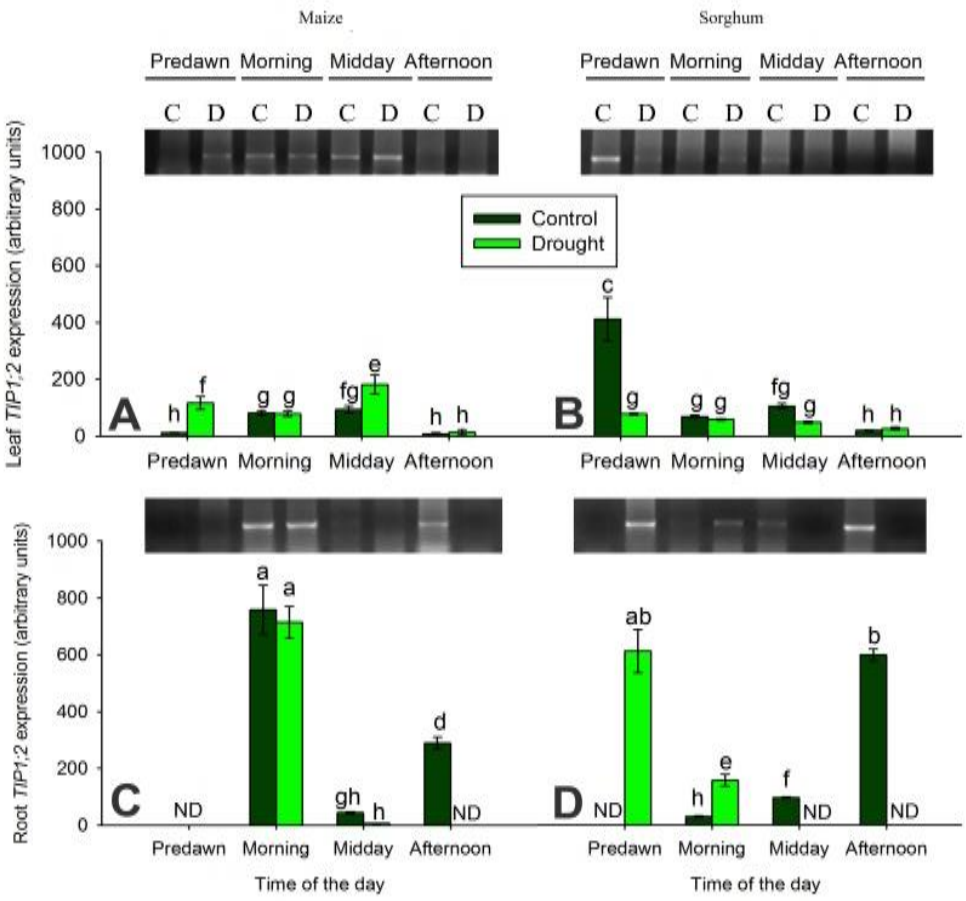

Fig. 8 Changes in the expression of *TIP1;2*in leaves (A, B) and roots (C, D) of maize and sorghum, after drought treatment for 10 d in the green house. Bars are means of 3 replicates ± SE. Bars ± SE labelled with different small letters are significantly different at $p<0.05$.Statistics was carried out for leaves separately from roots.

Discussion

Responses of growth of maize and sorghum to drought stress

Drought stress for 10 days resulted in significantly greater reduction of growth of maize than in sorghum. Similar results were reported by Erdei and Taleisnik (1993); Schittenhelm and Schroetter (2014). The FW of maize plants under drought stress decreased to 25% of the control but decreased by less extent in sorghum (84%) in plants grown in the green house. A similar trend was observed for the DW (Fig 1). This indicates that the dry matter accumulation, which is the result of photosynthesis and nutrient uptake from soil, was more seriously affected by drought in maize than in sorghum. These results showed that sorghum is more tolerant to drought than maize.

The effect of drought treatment on the relative water content (RWC)

Sorghum RWC was not affected by drought stress this may be because of its roots which could absorb water from deeper soil layers. Drought tolerant species as sorghum has deeper root growth than drought sensitive species as maize. Droughted maize plants showed a reduction in its RWC may be because of the

superficial root growth of maize or less root water uptake capacity or both reasons.

Plants were harvested at midday for measuring their RWC. There is high transpiration demand and the plants especially maize felt moderate drought stress as the water is present in the soil but the plants could not absorb it due to high transpiration rate. This was demonstrated from the results of RWC in the control plants (Fig 2A) as RWC of maize plants decreased although the soil water content is not limited this means that maize plants expresses water deficit although enough water soil content. The reason for the behavior of maize may be because of the excessive transpiration or inadequate water uptake from soil. Inadequate water uptake of root may be due to the root size, or permeability to water or root architecture (deep or superficial) (Schittenhelm and Schroetter, 2014). In both cases, this means that maize is less drought tolerant.

The behavior of sorghum was different as RWC was optimal in the control plant and also drought did not affect sorghum RWC. Drought stress also decreased RWC of maize this means that sorghum is more drought tolerant than maize.

Responses of gas exchange in maize and sorghum to drought stress

The inhibitory effect of drought on *A* is greater in maize than in sorghum (Fig 3A, B) as it led to reduction in *A* at morning to about 78.0% of the control and to 47.7% of the control at midday. While in sorghum no effect of drought was observed on *A* in the morning but decreased slightly at midday to 83.7% of the control and to 57.5% of the control at afternoon. So totally, the reduction in *A* in droughted maize plants is more than that in sorghum. The reason for this result may be the reduction caused by drought in the stomatal conductance in maize more than in sorghum. The reduction in gs means the closure of the stomata and low CO_2 availability for *A* in maize droughted plants than in sorghum and subsequent inhibition of *A* in maize droughted plants than in those of sorghum. Researches confirm this result show that in maize, inhibition of photosynthesis under drought has been attributed mainly to stomatal closure (Foyer *et al*., 1998).

Another possible explanation of the greater inhibition of *A* in the droughted maize compared to sorghum plants could be illustrated by C_i results of the droughted plants in the green house as C_i remained similar to the control at morning and increased at midday while in sorghum no effect of drought was observed on C_i. This increase in C_i in maize droughted plants means that CO_2 was present in the substomatal chamber but did not reach to the sites of carboxylation in bundle sheaths. Drought stress is known to decrease mesophyll conductance to CO_2 so the decrease in mesophyll conductance to CO_2 might be involved in the inhibition of *A* in maize plants (but not sorghum) under drought.

Photosynthesis in the droughted plants is possibly inhibited by the negative water status (see data of RWC) even at high *Ci*. Water stress causes decrease in the RWC of plant tissues so the growth rate of the plant decreases, so the demand for resources decreases and *A* slows down. If this was the case, then the greater reduction of maize growth compared to sorghum could have been a cause and also consequence of more severe inhibition of photosynthesis.

During drought stress, WUE decreased in maize droughted plants at midday because of the reduction in both *A* and *E* but the reduction in *A* was greater than in E. In normal conditions water gets through plants root cells through symplast and apoplast but in drought conditions water moves through the symplastic route more than apoplast so that the total hydraulic conductance of the plant tissues decreased.

Drought stress caused a decrease in WUE at midday in maize but not in sorghum plants (Fig 3I, J) grown in the green house apparently due to high *E* demand combined with limited water availability which may have caused -together with water stress- reduction in g_s and inhibition of *A* and subsequent decrease in WUE. At the morning, drought stress had no effect on maize plants due to lower *E* demand so similar values as control plants while at afternoon WUE in droughted maize plants as the *E* was minimum value so as to high WUE value.

The present study shows that one remarkable feature of drought tolerance (as in sorghum) appears to be the ability to maintain almost constant values of WUE under drought similar to those under control conditions a feature that seems to be missing or less presented by drought sensitive species (such as maize) where WUE shows greater variation during the day in the well-watered plants and under drought stress.

The absolute value of WUE may not an indication to drought tolerance of the plants as it

depends on the conditions in which the plants grow. This is shown by the results of WUE in the green house where it was higher in maize than in sorghum although maize is known as drought sensitive compared to sorghum.

Responses of aquaporin expression in maize and sorghumto drought stress

The effect of drought stress on PIP1;5 expression
The results showed that the expression of *PIP1;5* in the leaves of maize was not affected by neither drought stress nor the circadian rhythm (Figs 5A & B). These results suggest that this gene had no role in water transport or in *A* in the leaves of maize. While in sorghum leaves, its expression was higher than that in maize implying that *PIP1;5* plays a role in water transport in sorghum leaves. Drought stress led to increase in the expression of *PIP1;5* in the leaves of sorghum at morning and midday which confirms that it has a role in water transport under drought conditions. The expression of *PIP1;5* in the roots of both maize and sorghum in control and droughted plants showed no consistent patterns (Fig 5C, D) which indicates that this gene had no role in water uptake by the roots of both plants.

Heinen *et al*. (2014) suggested that *PIP1;5* has a role in CO_2 transport in the leaves. On the other hand, our results indicated that the expression pattern in the control leaves of sorghum was minimum at the morning where the *A* was highest. This means that *PIP1;5* has no role in CO_2 transport but may have an important role in water transport in sorghum leaves. At the morning, water is transported mainly through the apoplastic path due to the high transpiration rate (Steudle and Peterson, 1998) so that it does not depend mainly on aquaporin activity. While under drought stress, water transport depends mainly on aquaporins (Steudle and Peterson, 1998) as shown by the high expression of *PIP1;5* in the droughted sorghum leaves at the morning and midday. In general, the consistent expression pattern of *PIP1;5* in sorghum leaves during the day indicates that it has a role in water transport.

The effect of drought stress on PIP1;6 expression in the green house

The high expression of *PIP1;6* in control maize leaves at the morning (Fig 6A) suggests that it had no role in water transport but it may have a role in CO_2 transport meaning that the high expression of *PIP1;6* was a response to low C_i (Fig 3G). The high expression of *PIP1;6* at predawn in sorghum control leaves could be explained as the gene spend time to be translated at the morning. So the translation and post translation processes may be different in maize and sorghum, they may be taking short time in maize and long time in sorghum. The decrease in its expression in the leaves of both plants means that CO_2 concentration was high and no need for that PIP to transport it.

The inconsistent expression pattern in the roots of both plants (Figs 6C & D) suggests that *PIP1;6* was not involved in water uptake by roots. Hachez *et al* (2006) also showed that all ZmPIP mRNAs were detected in most cell types in the meristem, elongation, and mature zone of maize roots except for ZmPIP1;6 and ZmPIP2;7 transcripts, which were not detected.

The effect of drought on PIP2;3 expression in the green house

The expression of *PIP2;3* in the leaves of maize under control and drought conditions (Fig 7A) was low or even undetected indicating that it has minor or no role in the leaves of maize. While in the maize roots, the inconsistent pattern of the gene expression and the unreasonable increased expression in the control and droughted plants at the morning, gives no explanation at least in terms of water uptake or CO_2 transport.

In contrast, the higher expression of *PIP2;3* in sorghum roots than that in their leaves means that this gene has an important role in the balancing of water uptake in sorghum plants especially under drought condition. This was shown by the expression of the gene at predawn in the roots and leaves of sorghum, as at drought condition, the expression increased in the roots of sorghum such that this increase was not seen in the leaves. It can be concluded that sorghum plants depend on aquaporin (*PIP2;3*) for water transport to a limited extent under control conditions but to higher extent under the drought conditions.

One of the known reasons for sorghum plants to be more drought tolerant than maize is the root length density, as the roots of sorghum have the ability to grow vertically deeper in the soil while maize roots grow horizontally, the reason which increase the ability of sorghum to absorb higher amount of water compared with maize (Schittenhelm and Schroetter, 2014). This characteristic of sorghum roots makes sense of the high expression of PIPs (*PIP2;3*) in the roots of sorghum. While the high expression of PIPs in

maize roots with their superficial growth would make benefit when exposed to moderate drought stress.

The effect of drought on TIP1;2 expression in the greenhouse

The expression of *TIP1;2* in maize and sorghum plants was low (Fig 8), but the increase of the gene expression at the morning and midday of control maize leaves and at the predawn, morning and midday in control sorghum leaves means that it has a role in water transport in both plants in control conditions. The role of *TIP1;2* in CO_2 transport has not been reported previously. So this high expression suggests that the plants may employ *TIP1;2* to transport water from the tonoplast to the cytoplasm so that the tonoplast may act as a temporary store for water. Bienert *et al*., (2007) have used a survival assay in yeast to investigate the capacity of aquaporins to transport H_2O_2. A high transport capacity was determined for *AtTIP1;2*. The ability of plasma membrane and intracellular aquaporins to transport H_2O_2 points to important roles in stress signalling and responses (Maurel, 2007).

Also the inconsistent expression pattern in both maize and sorghum roots (Figs 8C & D) indicates no role of TIP1;2 in the water uptake of their roots.

Generally, the response of aquaporins to drought in leaves of both species appears to be inconsistent at most of the time points during the day. It seems to us that maize and sorghum do not rely predominantly on the studied aquaporins for water transport through leaves but sorghum may employ *PIP2*;3 in roots for this function under drought-based on its expression induction.

The outcome of this study is that PIP2;3 may have role in drought tolerance and maintainance of the WUE of sorghum plants compared to those of maize.

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الملخص العربي

الحفاظ على كفاءة استخدام الماء فى نبات الذره الرفيعه المعرض للجفاف مقارنة بالذره الشاميه من حيث اختلاف التعبير الجينى للاكوابورينات

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من المعروف أن الذرة الشامية هى أكثر تحملا للجفاف من الذرة الرفيعة. فى هذه الدراسة بحثنا فى مدى استجابة كلا النباتين للجفاف بعد نموهما فى بيئة طبيعية (صوبة زراعية) وتعريضهما للجفاف لمدة 10 أيام فتأثر نمو النبات متمثلا فى الوزن الغض للمجموع الخضرى وكذلك الوزن الجاف حيث انخفضا بنسبة كبيرة فى الذرة الشامية عنها فى الذرة الرفيعة كنتيجة للتعرض للجفاف وكذلك انخفض المحتوى المائى النسبى. كذلك حدث تثبيط للتبادل الغازى فى الذرة الشامية بشكل أكبر منه فى الذرة الرفيعة. وكنتيجة لذلك فإن كفاءة استخدام الماء تختلف فى الذرة الشامية على مدار اليوم وتحت تأثير الإجهاد المائى. على العكس فإن الذرة الرفيعة لها قدرة على الحفاظ على كفاءة استخدام الماء خلال اليوم إذا روي جيدا أو تعرض للجفاف, وهذا يوضح أن نبات الذرة الرفيعة له قدرة أكبر فى التحكم فى حالته المائية وخاصة امتصاص الماء مقارنة بالذرة الشامية. بدراسة التعبير الجينى لأربع جينات للأكوابورينات (PIP1;5, PIP1;6, PIP2;3, TIP1,2) كانت استجابتها جميعا ضعيفه أثناء تعرضها للإجهاد المائى ماعدا PIP2;3 والذى استجاب بشكل كبير فى نبات الذرة الرفيعة عند تعرضه للإجهاد المائى وليس فى الذرة الشامية وهذا قد يعنى أن له دور فى امتصاص الماء من الجذر فى الذرةالرفيعة وقد يكون هذا سبب فى أن الذرة الرفيعة حافظ على المحتوى المائى النسبى له أثناء الجفاف. وقد يعزى ذلك لدور هذا الجين من الأكوابورينات فى قدرة الذرة الرفيعة على تحمل الجفاف أكثر من الذرة الشامية.