DOI: 10.1007/s10535-016-0656-9 BIOLOGIA PLANTARUM 61 (1): 127-137, 2017

Water use efficiency in the drought-stressed sorghum and maize in relation to 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 masses was more severely reduced in maize than in sorghum, consistently with reduction of leaf relative water content. Gas exchange was also more inhibited by drought in maize than in sorghum. The water use efficiency (WUE) of maize fluctuated during the day and in response to the drought stress. In contrast, sorghum was able to maintain a largely constant WUE during the day in the well-watered plants as well as in the stressed ones. Studying the expression of four aquaporin genes (*PIP1;5*, *PIP1;6*, *PIP2;3*, and *TIP1;2*) revealed that *PIP1;5* in leaves and *PIP2;3* in roots were highly responsive to drought in sorghum but not in maize, where they might have supported a greater water transport. The expression pattern of *PIP1;6* suggests its possible role in CO_2 transport in control but not droughty leaves of both the plants. *TIP1;2* seemed to contribute to water transport in leaves of the control but not droughty plants. We conclude that *PIP1;5* and *PIP2;3* may have a prominent role in drought tolerance and maintenance of WUE in sorghum plants.

Additional key words: gas exchange, plasma membrane intrinsic proteins, relative water content, *Sorghum bicolor*, tonoplast intrinsic proteins, *Zea mays.*

Introduction

Marked differences in water use efficiency (WUE) occur among plants employing the three photosynthetic pathways: C_3, C_4, and crassulacean acid metabolism (CAM). Plants exhibiting C_4 and CAM photosyntheses are more water-use efficient than those exhibiting C_3 photosynthesis (Fischer and Turner 1978, Winter *et al*. 2005). The C_4 pathway reduces photorespiration by elevating CO_2 concentration at the site of Rubisco using a biochemical CO_2 pump, thus accelerating net CO_2 fixation in relation to transpiration, thereby increasing WUE (Way *et al*. 2014).

Water use efficiency and drought tolerance are often taken loosely as synonymous, although they are frequently unrelated (Hsiao and Acevedo 1974). Drought tolerance is an ability of one genotype to yield 'better' than another one during severe drought stress. On the other hand, WUE is defined as the ratio between diffusion of CO_2 into the leaf (net photosynthetic rate, P_N) and H_2O loss (transpiration rate, E) (Bassett 2013). Drought has been reported to increase WUE as a result of reducing transpiration. However, drought stress leads to inhibition of dry matter accumulation and also decreasing leaf ^{13}C content (Ghannoum *et al*. 2002). This indicates that drought stress improves leaf WUE but may reduce dry matter accumulation per amount of water consumed. It has also been reported that a high WUE does not necessarily correlate with high growth rates under drought (Maroco *et al*. 2000). Thus, it seems that the relationship between WUE and drought tolerance is still a matter of controversy that needs more detailed information to be resolved. Furthermore, leaf water status appears to be an overriding character that regulates plant 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 photosynthesis. In nature, drought tolerance and drought sensitivity occur in both C_3 plants and

Submitted 1 December 2015, *last revision* 21 April 2016, *accepted* 2 May 2016.
Abbreviations: AQPs - aquaporins; CAM - crassulacean acid metabolism; c_i - sub-stomatal CO_2 concentration; E - transpiration rate; FC - field capacity of soil; g_s - stomatal conductance; P_N - net photosynthetic rate; RT-PCR - reverse transcriptase polymerase chain reaction; RWC - relative water content; WUE - water use efficiency.
Acknowledgements: The authors would like to thank the University of Damietta for funding this work.
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C_4 plants but it cannot be ruled out that there is a relationship between drought tolerance and C_4 photosynthesis (Taylor *et al*. 2011). However, C_4 species differ in their ability to tolerate drought (Kakani *et al*. 2011). Among C_4 species, sorghum is known to be more drought-tolerant than maize. The drought tolerance of sorghum has been reported to be due to its ability to root deeply and thus to draw water from greater soil depths (Singh and Singh 1995, Farre and Faci 2006) than maize. In contrast, Merrill *et al*. (2007) reported that depletion of soil water was higher in maize than in sorghum. Singh *et al*. (2010) reported differences between maize and sorghum in terms of root system morphology and architectural development that promote a more efficient water uptake by sorghum roots.

At a low water potential, the amount of CO_2 entering a leaf is reduced due to stomatal closure. Sorghum shows a high osmotic adjustment at a low water potential so maintains a higher water uptake and higher P_N 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 in maize, which they supposed to be due to a lower cell wall elasticity in maize. However, it cannot be ruled out that cell wall properties alone could account for differences in water relations between maize and sorghum and hence more investigation in respect to water transport is needed.

Aquaporins (AQPs) are a family of membrane intrinsic proteins in plants. They exist in plasma membranes, tonoplasts, membranes of endoplasmic reticulum, and in peribacteroid membranes. They have been proved to facilitate transport of water and small molecules such as urea, glycerol, and CO_2 (Maurel *et al*. 2008). The large number of plant AQPs 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 *via* AQPs has been found to increase as compared to that through apoplast (Lu and Neumann 1999). Cell-to-cell water movement through AQPs is believed to play a pivotal role in coping with environmental stresses when transpiration rate decreases and osmotic water flow through membranes is dominant (Vandeleur *et al*. 2005, Kaldenhoff *et al*. 2007). Aquaporins of maize have been well characterized (Chaumont *et al*. 2001) and assigned a role in cell water permeability and elongation (Hachez *et al*. 2008), root hydraulic conductivity (Hachez *et al*. 2012), and regulation of stomatal movements (Heinen *et al*. 2014). However, little is known about AQPs of sorghum and their role in regulation of its water relations. 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). Maize and sorghum are gaining more interest in view of their importance as potent sources of biofuel (Schittenhelm and Schroetter 2014). Understanding the mechanisms that regulate WUE in these two crops under drought stress is important since the water resources for irrigation are limited and future crops are expected to experience a drought stress (Belder *et al*. 2005, Bouman 2007).

The objectives of the present study were: *1*) to investigate diurnal changes in plant water status and gas exchange in relation to expression of some selected AQPs in the leaves and roots of maize and sorghum plants subjected to drought stress in the field and *2*) to correlate WUE and AQP expression in the well-watered and droughty plants of the two species.

Materials and methods

Plants and growth conditions: Two C_4 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). The plants were grown and treated with drought under field conditions. The soil was clay with less than 30 mM NaCl soil salinity. The experiment location had coordinates of: 31.44°N and 31.68°E and an altitude of about 5 m. The climatic conditions over the experiment period were: 27 - 31/22 - 25 ºC day/night temperatures, 55 - 65 % relative humidity during the day, a 12-h photoperiod, and 2 250 μmol m^{-2} s^{-1} maximum sunlight.

The seeds were sown in 12 randomized blocks, 6 blocks for each species. Each block included 15 holes. The distance between holes was 30 cm. Each hole contained two or three seeds. After 7 d, the plants were thinned to one plant per hole. When the plants were 14-d-old, three blocks from each species were watered every 2 d and used as a control and three blocks from each species were allowed to dry. To measure field capacity (FC), pre-weighed soil sample was saturated with water. Field capacity [%] was calculated as soil mass / saturated soil mass × 100. The drought treatment lasted for 10 d and FC reached 65 %. Then water was added every day to the droughty blocks to maintain soil FC at 65 %.

Measurement of gas exchange parameters and relative water content: After 10 d of the drought treatment, gas exchange parameters were measured in the control and droughty plants of each species. Photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and leaf internal CO_2 (c_i) were measured for the second leaves in each species using the *LCi-SD* gas exchange system (*Analytical Development Company*, Hertfordshire, UK). Leaf water use efficiency was calculated as P_N/E. Gas exchange measurements

were made under full sunlight three times during the day (3 h after sunrise, at midday, and 3 h before sunset). Measurements at each time period lasted for no more than 60 min. A reference sample was measured at 20 min intervals to confirm stability of gas exchange data over the measuring period. Data were collected from five different plants for each treatment.

At the same day, whole shoots of five plants from each set were harvested and used for fresh and dry mass determinations. After recording the fresh mass of shoots, they were dried in oven at 60 °C for 2 d and the dry mass was then recorded. Leaf (the second leaf) and root samples were collected at predawn, in the morning, at midday, and in the afternoon, frozen immediately in liquid nitrogen and stored at -80 °C until used for subsequent analyses. The samples were collected sequentially from different treatments over 1 h to minimize any possible variation due to the time of the harvest.

Samples were collected at midday for measuring relative water content (RWC). The leaf samples were weighed (FM) and then incubated in distilled water at 4 ºC overnight to determine the water saturated mass (SM). The samples were then dried in an oven at 60 ºC for 2 d and weighed (DM). The RWC was then calculated as [(FM - DM)/(SM - DM)] × 100. Five measurements from different plants were made for each treatment.

Quantification of gene expression by semi-quantitative reverse transcriptase chain reaction: The total RNA was extracted from about 50 mg of frozen leaves or roots by using *TRI-reagent* (*Sigma*, St. Louis, USA) according to the manufacture's instruction. To prevent DNA contamination, the extracted RNA was treated with a DNA-free kit (*Ambion*, Paisley, UK) at 37 °C for 30 min. A poly A tail mRNA was then isolated by reacting 10 mm^3 of the RNA with 2 mm^3 of oligo dT(18), and 3 mm^3 of RNase and DNase free H_2O at 70 °C for 5 min and the reaction was terminated on ice for 2 min. The reverse transcription was conducted by using an *MMLV*-reverse transcription kit according to the supplier's recommendations (*Promega*, Southampton, UK). Primers for each gene were designed to recognize 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 18 S rRNA are listed in Table 1 Suppl. The PCR conditions were as follows: an 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. The PCR products were resolved by electrophoresis on 1 % (m/v) agarose gels, stained with ethidium bromide (0.5 μg cm^{-3}) in 1× TAE (40 mM Tris + 20 mM acetic acid + 50 mM EDTA) and visualized by using the *UVI* save documentation system (*U.V. TECH*, Liverpool, UK). The band volumes were measured by using the *Lab Image V 2.7.2* software. The measurements were normalized for equal 18 S rRNA bands (Fig. 1 Suppl.). Three independent measurements were made for each treatment.

Statistical analysis: Measurements were replicated as indicated in each section. To compare the control and drought samples at separate time periods, the *Sigmaplot V 12.0* program was used to run the *t*-test at $\alpha = 0.05$. To test for diurnal variation of a parameter, one-way *ANOVA* was performed also at $\alpha = 0.05$.

Results

The drought stress led to a significant reduction in growth in terms of shoot fresh mass of both maize and sorghum (Table 1). The biomass reduction was significantly greater in the droughty maize plants (down to about 25.5 % of the control) than in those of sorghum (down to 84.5 % of the control). The drought stress led to reduction in dry mass of maize to about 32.8 % of the control, but no significant change was observed in sorghum. The drought stress significantly reduced shoot RWC in maize but had no significant effect on shoot RWC in sorghum (Table 1).

The drought stress led to a significant reduction of P_N in maize in the morning and at midday with the greatest reduction at midday (down to 78.0 % of the control in the morning and to 47.7 % of the control at noon) but no significant change was observed in the afternoon

Table 1. Changes in shoot fresh mass, dry mass, and RWC of maize and sorghum as result of drought treatment for 10 d. Means ± SEs, $n = 5$. Within each column, means labelled with *asterisk*s are significantly different from the corresponding controls at $P < 0.05$.

Species	Treatment	Fresh mass [g $plant^{-1}$]	Dry mass [g $plant^{-1}$]	RWC [%]
Maize	control	375.1 ± 25.3	88.5 ± 6.5	69.3 ± 4.6
	drought	95.7 ± 4.2*	29.0 ± 2.4*	45.6 ± 2.6*
Sorghum	control	42.6 ± 1.2	6.1 ± 0.5	83.9 ± 4.6
	drought	36.0 ± 1.3*	5.5 ± 0.4	84.6 ± 1.8

in maize (Fig. 1*A*). Contrarily, no significant difference was observed in the droughty plants of sorghum in the morning, but a significant decrease was observed at midday and in the afternoon (Fig. 1*B*).

The drought stress led to a significant reduction in E of both the plants: E decreased in maize to 83.5 % in the morning, 74.9 % at midday, and 68.3 % in the afternoon compared to the control (Fig. 1*C*), and in sorghum to 82.5 % in the morning, 89.7 % at midday, and 75.1 % in the afternoon compared to the control (Fig. 1*D*).

The drought stress led to a significant reduction in g_s of maize where it decreased to 65.6 % in the morning, 45.7 % at midday, and 61.7 % in the afternoon compared to the corresponding controls (Fig. 1*E*). Contrarily, no significant change in sorghum g_s was in the afternoon but a significant decrease was observed at midday (82.2 % of the control) (Fig. 1*F*).

In maize, the drought stress led to no significant change in c_i in the morning, increased it significantly at midday but decreased it significantly in the afternoon (to

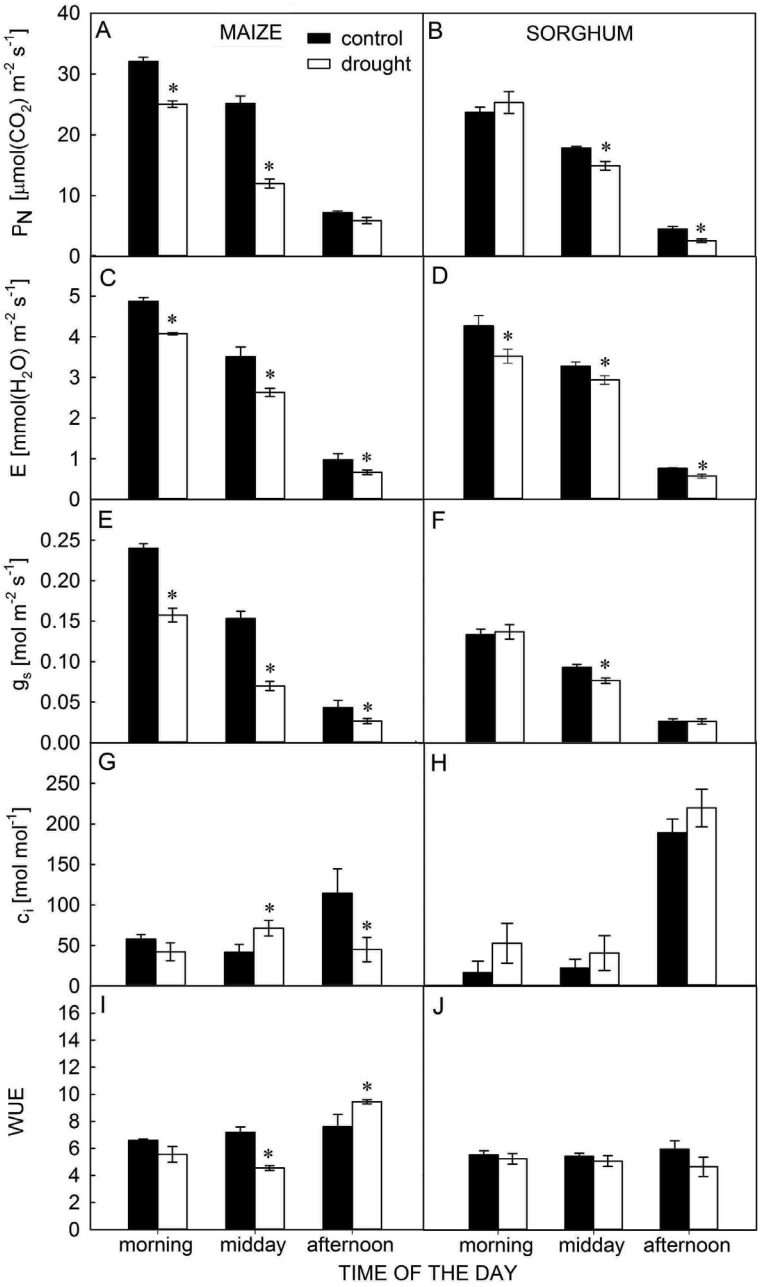

Fig. 1. Changes in photosynthetic rate (P_N), transpiration (E), stomatal conductance (g_s), internal carbon dioxide concentration (c_i), and water use efficiency (WUE = P_N/E) at three time points during the day in maize and sorghum after drought treatment for 10 d. Means ± SEs, $n = 5$. *Asterisks* denote significant differences from the corresponding controls at $P < 0.05$.

39.3% of the control, Fig. 1*G*). Contrarily, no significant changes in c_i were observed in the droughty plants of sorghum at any time point (Fig. 1*H*).

In maize, the drought stress did not change WUE in the morning, reduced it significantly at midday (63.3 % of the control) but increased it significantly in the afternoon (Fig. 1*I*). Contrarily, no significant change in WUE was observed in droughty sorghum compared to the controls at any time point (Fig. 1*J*).

Diurnally, the transcript abundance of *PIP1;5* remained unchanged in maize leaves but decreased significantly from predawn to the morning and then increased gradually at midday and in the afternoon in sorghum (Fig. 2*A*,*B*). The drought stress did not cause a significant change in expression of *PIP1;5* in leaves of maize and sorghum compared with their controls except

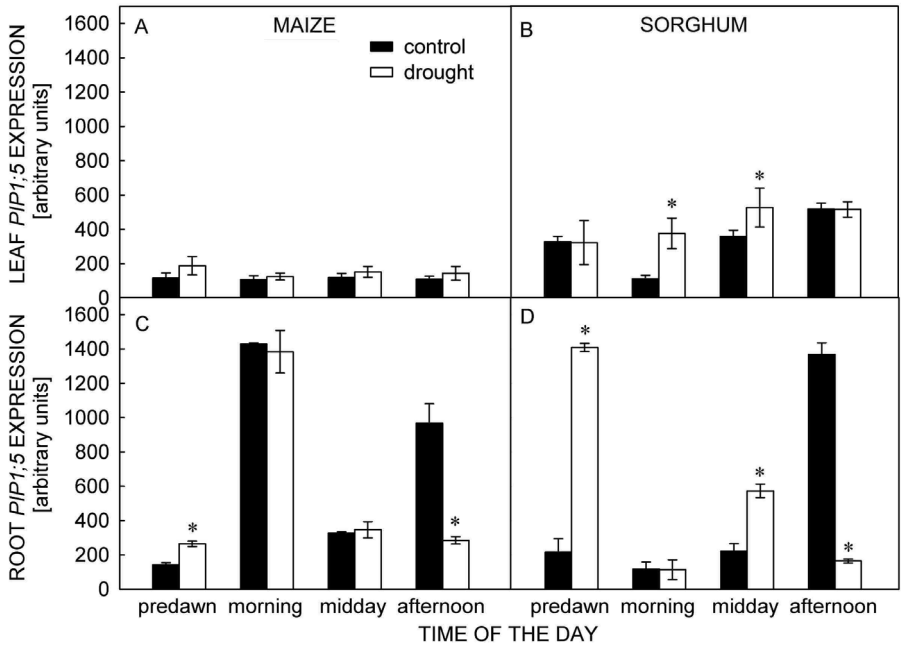

Fig. 2. Changes in expression of *PIP1;5* in leaves (*A*, *B*) and roots (*C*, *D*) of maize and sorghum after drought treatment for 10 d. Means ± SEs, $n = 5$. *Asterisks* denote significant differences from the corresponding controls at $P < 0.05$.

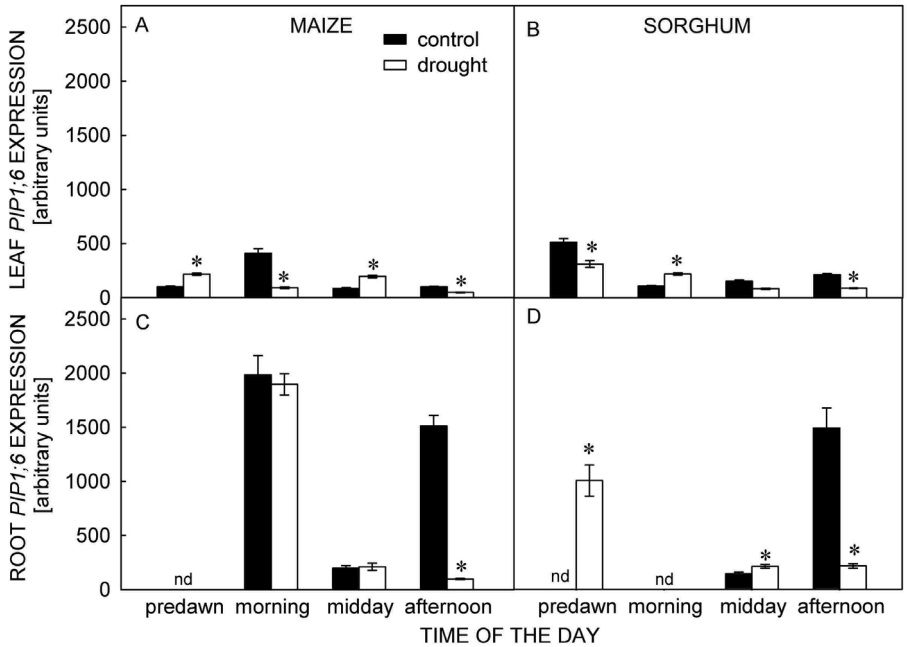

Fig. 3. Changes in expression of *PIP1;6* in leaves (*A*, *B*) and roots (*C*, *D*) of maize and sorghum after drought treatment for 10 d. Means ± SEs, $n = 5$. *Asterisks* denote significant differences from the corresponding controls at $P < 0.05$; nd - not detectable.

in sorghum in the morning and at midday where it was significantly increased. Expression of *PIP1;5* in sorghum leaves was significantly higher or at least equal to that in the corresponding maize leaves. In roots, no consistent diurnal pattern for *PIP1;5* expression was observed in maize, whereas in sorghum it remained unchanged at all the time periods except in the afternoon where it increased significantly (Fig. 2*D*). The drought stress led to a significant increase in *PIP1;5* expression in maize roots at predawn caused no changes in the morning and at midday but significantly decreased it to 29.4 % of the control in the afternoon. Contrarily in sorghum, the drought stress caused a significant increase in *PIP1;5* expression at predawn and midday, did not change it in the morning, and decreased it significantly to 12.1 % of the control in the afternoon (Fig. 2*D*).

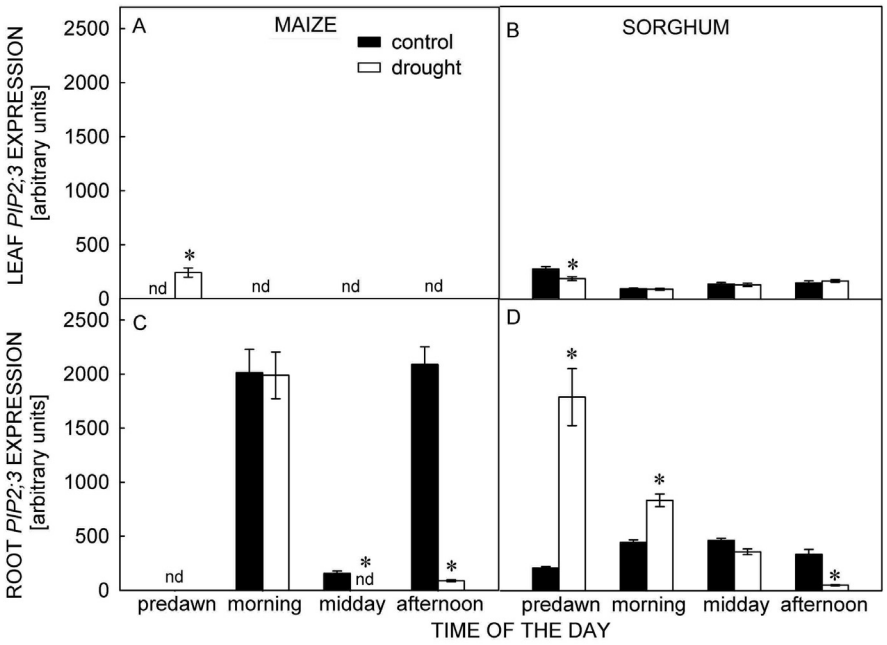

Fig. 4. Changes in expression of *PIP2,3* in leaves (*A*, *B*) and roots (*C*, *D*) of maize and sorghum after drought treatment for 10 d. Means ± SEs, $n = 5$. *Asterisks* denote significant differences from the corresponding controls at $P < 0.05$; nd - not detectable.

Diurnally, expression of *PIP1;6* was maximum in maize leaves in the morning but minimum at other time periods. In sorghum leaves, expression of *PIP1;6* was higher at predawn and in the afternoon than in the morning and at midday (Fig. 3*A*,*B*). The drought stress did not result in consistent changes in expression pattern for *PIP1;6* in leaves of both the plants. In roots, no consistent diurnal pattern was observed for *PIP1;6* expression in maize, whereas in sorghum, no transcripts were detected at predawn and in the morning, but expression increased significantly at midday and sharply in the afternoon (Fig. 3*C*,*D*). The drought stress resulted in a decrease in *PIP1;6* expression in maize roots only in the afternoon to 6.5 % of the control. In sorghum, the drought stress increased *PIP1;6* expression in roots at predawn and midday but decreased it in the afternoon (Fig. 3*C*,*D*). Thus, no consistent changes in expression of *PIP1;6* in roots of maize and sorghum were observed during the day time or in response to the drought.

Transcripts of *PIP2;3* were not detected in maize leaves at any time period of the day, whereas in sorghum leaves, the highest expression was observed at predawn and the lowest in the morning (Fig 4*A*,*B*). The drought stress resulted in increasing *PIP2;3* expression in maize leaves only at predawn (Fig. 4*A*). In sorghum leaves, the drought stress caused a decrease in *PIP2;3* expression at predawn to 68 % of the control. No significant differences were observed in the morning, at midday, and in the afternoon between control and droughty sorghum leaves (Fig. 4*B*). In roots of maize, expression of *PIP2;3* changed irregularly during the day. Contrarily in sorghum, expression of *PIP2;3* was lowest at predawn and then increased significantly in the morning but no further changes occurred (Fig. 4*C*,*D*). No significant change was observed in *PIP2;3* expression in roots of the droughty maize plants at predawn or in the morning but a significant decrease was observed at midday and in the afternoon (Fig. 4*C*). In sorghum roots, the drought stress caused remarkable increases in *PIP2;3* expression at predawn and in the morning. The drought stress did not affect *PIP2;3* expression at midday but significantly decreased it in the afternoon (to 14.9 % of the control, Fig. 4*D*). Therefore, *PIP2;3* expression in roots responded more consistently and strongly to the day time and drought stress in sorghum than in maize.

Expression of *TIP1;2* in maize leaves was lowest at predawn and in the afternoon but significantly higher in the morning and at midday. In sorghum leaves, the

transcript abundance of *TIP1;2* was greatest at predawn and then decreased sharply at other time periods (Fig. 5*A*,*B*). The 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 droughty plants and controls in the morning and afternoon (Fig. 5*A*). In sorghum leaves, the drought stress caused a decrease in *TIP1;2* expression at predawn (to 18.8 % of the control) and midday but did not lead to a significant change in the morning or afternoon (Fig. 5*B*). In roots, no consistent diurnal pattern for *TIP1;2* was observed in maize. Contrarily in sorghum roots, no *TIP1;2* transcripts were detected at predawn, but the transcript abundance increased progressively over the next time points where it was maximum in the afternoon (Fig. 5*C*,*D*). No significant change was observed in *TIP1;2* expression in roots of the droughty maize plants at predawn, in the morning, and at midday, but a significant decrease was observed in the afternoon so that no transcripts were detected (Fig. 5*C*). In sorghum, the drought stress caused a significant increase in *TIP1;2* expression at predawn and in the morning but resulted in a significant decrease at midday and in the afternoon where no expression was detected (Fig. 5*D*).

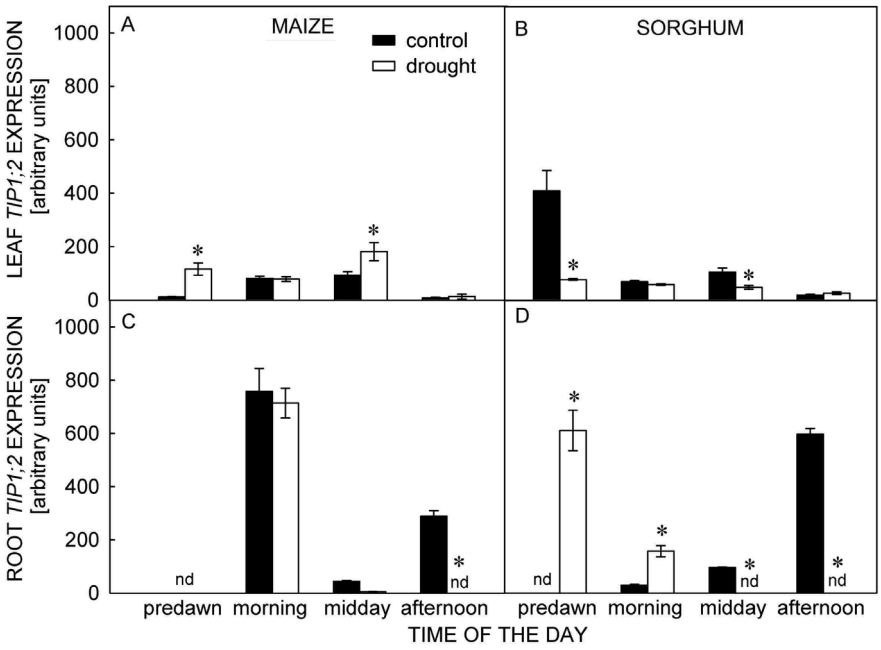

Fig. 5. Changes in expression of *TIP1;2* in leaves (*A*, *B*) and roots (*C*, *D*) of maize and sorghum after drought treatment for 10 d. Means ± SEs, $n = 5$. *Asterisks* denote significant differences from the corresponding controls at $P < 0.05$; nd - not detectable.

Discussion

The drought stress for 10 d resulted in a significantly greater reduction of growth in maize than in sorghum (Table 2). This indicates that dry matter accumulation, which is the result of photosynthesis and nutrient uptake from soil, was more seriously affected by the drought in maize than in sorghum. These results show that sorghum was more tolerant to the drought than maize. Similar results were reported by Erdei and Taleisnik (1993) and Schittenhelm and Schroetter (2014).

The plants were harvested at midday for measuring RWC. At this time of the day, even well-watered plants may experience some degree of water deficit (Franks *et al*. 2007). This is demonstrated by the results of RWC in the control plants where RWC of the maize plants decreased although soil water content was not limited. Moreover, leaf RWC decreased significantly in maize but not in sorghum under the drought (Table 2) although sorghum maintained a slightly higher *E* at midday compared to maize. This could arise from more efficient water uptake by sorghum roots as a result of a deeper root system of sorghum (Singh and Singh 1995, Farre and Faci 2006, Schittenhelm and Schroetter 2014), and/or a more efficient water transport through sorghum root tissue. In fact, both the features are required for maintaining water uptake by roots under drought because shallow roots are not able to absorb water from the drying superficial layers of soil even if they have a high tissue water permeability. Although we have not had data on root hydraulic conductance, the higher RWC in the droughty sorghum plants compared to those of maize suggest a greater root hydraulic conductance in sorghum than in maize under the drought. However, this point deserves a more detailed investigation.

The inhibitory effect of the drought on P_N was greater

in maize than in sorghum (Fig 1*A*,*B*) presumably due to the greater reduction in g_s (relative to the corresponding control) in maize than in sorghum under the drought. Reduction in g_s means lowering CO_2 availability for P_N. This agrees with previous reports which showed that in maize, inhibition of photosynthesis under drought has been attributed mainly to stomatal closure (Lal and Edwards 1996, Saccardy *et al*. 1996, Foyer *et al*. 1998).

Another possible explanation of the greater inhibition of P_N in the droughty maize plants compared to those of sorghum could be illustrated by c_i results of the droughty plants as c_i remained similar to the control in the morning and increased at midday, but in sorghum no effect of the drought was observed on c_i. This increase in c_i in the maize droughty plants suggests that CO_2 was present in substomatal chambers but was not transported to the sites of carboxylation. Drought stress has been reported to decrease mesophyll conductance to CO_2 (Jones 1973, Flexas *et al*. 2002, Ripley *et al*. 2007), so the decrease in mesophyll conductance to CO_2 might be involved in the inhibition of P_N in maize plants (but not sorghum) under the drought. Photosynthesis in the droughty maize plants was also possibly inhibited by altering photosynthetic enzyme activity and/or expression. It has been reported previously that Rubisco activity decreases when RWC content decreases below 80 %, or if stomatal conductance falls below 0.01 mol m^{-2} s^{-1} (Flexas and Medrano 2002). If this was the case, then the greater reduction of maize growth compared to sorghum under the drought could have been a consequence of a more severe inhibition of photosynthesis.

During the drought stress, WUE decreased in maize at midday because of the reduction of P_N was greater than that of E. The present study shows that one remarkable feature of drought tolerance (as in sorghum) appears to be an ability to maintain values of WUE under drought similar to those under control conditions, a feature that is missing or less present in drought sensitive species (such as maize) where WUE shows a great variation during the day in well-watered and droughty plants. However, the absolute value of WUE *per se* may not well correlate with drought tolerance as shown by the higher WUE in maize than in sorghum in the afternoon (Fig. 1*I*,*J*) although maize is known as more drought sensitive compared to sorghum.

Four aquaporin genes were selected for this study (*PIP1;5*, *PIP1;6*, *PIP2;3*, and *TIP1;2*), with essentially one gene from each main subgroup of the water transporting aquaporins (Fetter *et al.* 2004) except PIP1s, whose functions are still a matter of controversy, where we selected two genes because we expected some variation in expression patterns in the control as well as droughty tissues. The phylogenetic relationships between maize and sorghum aquaporins have been elucidated recently and hence, their functions in both the plants are suggested to be equivalent (Reddy *et al*. 2015). Recently, aquaporins have been reported to be involved in silicon-mediated improved root water uptake by sorghum under osmotic (Liu *et al*. 2014) and salt (Liu *et al.* 2015) stresses, where several *PIP1* and *PIP2* genes are induced by salt and osmotic stresses with or without silicon addition. However, the authors did not study expression of aquaporins in leaves and also the plants were grown in a growth chamber with mild growth conditions. No reports have yet described expression of any tonoplast intrinsic protein aquaporin in sorghum. To our knowledge, this is the first report describing expression of some selected aquaporins in field-grown sorghum plants as compared to maize. Although the selected genes in the present study are not sufficient to draw a full picture on the role of aquaporins in drought tolerance of field-grown maize and sorghum plants, an informative conclusion can be made based on the obtained data.

Expression of *PIP1;5* in leaves of maize did not respond to the drought stress or diurnal rhythm (Fig 2*A*,*B*) suggesting that this gene had no role in water transport or in P_N in leaves of maize. Contrarily in sorghum leaves, its expression was higher than in maize at midday and in the afternoon suggesting that *PIP1;5* played a role in water transport in sorghum leaves under well-watered conditions at least during periods of low E. The results in Fig 2*B* suggest that *PIP1;5* has an even more important role in water transport in sorghum leaves under drought conditions either directly or *via* increasing water transport activity of other PIP2 aquaporins (Fetter *et al.* 2004). This conclusion is supported by the increase in the transcript abundance of *PIP1;5* in the morning in droughty leaves of sorghum but not in the control ones. Previous studies showed that under well-watered conditions, water transport through plant tissues is mainly through apoplast during periods of a high transpiration (Steudle and Peterson 1998). However, under drought stress (or presumably at midday in well-watered plants where transpiration decreases), the contribution of the cell-to-cell path to water transport increases mainly depending on aquaporins (Steudle and Peterson 1998, Lu and Neumann 1999), a feature that typically applies to *PIP1;5* induction by drought in the morning in droughty sorghum leaves. If this is true then the response of *PIP1;5* to the drought in sorghum leaves seems only to facilitate water transport through leaves but did not contribute to improving water uptake by roots since there was no comparable response for root *PIP1;5* in sorghum under the drought. Expression of *PIP1;5* in roots of both maize and sorghum in the control and droughty plants shows no consistent patterns (Fig. 2*C*,*D*), which indicates that this gene has little role if any in water uptake by roots of both the plants. Heinen *et al*. (2014) suggested that *PIP1;5* has a role in CO_2 transport in leaves. On the other hand, our results indicate that the expression pattern in control leaves of sorghum was minimum in the morning where P_N was highest. This suggests that *PIP1;5* had no role in CO_2 transport but may have an important role in water transport in sorghum leaves. In contrast, no similar role could be assigned for *PIP1;5* in maize leaves.

The high expression of *PIP1;6* in control maize leaves in the morning (Fig. 3*A*) suggests that it had a minor role in water transport but it may have a role in

CO_2 transport implying that high expression of *PIP1;6* was a response to the low c_i (Fig. 1*G*). The highest expression of *PIP1;6* at predawn in sorghum control leaves could be explained in view of the previous findings of Sakurai-Ishikawa *et al*. (2011) who reported that a lag time of 4 h exists between the maximum gene expression and the maximum aquaporin protein content. If this applies to *PIP1;6*, then the protein content and activity should peak in the morning (where P_N was maximum) suggesting a role for *PIP1;6* in photosynthesis in sorghum.

The inconsistent expression pattern in roots of both the plants (Fig. 3*C*,*D*) suggests that *PIP1;6* was not involved in water uptake by roots. Hachez *et al*. (2006) also showed that all ZmPIP mRNAs are detected in most cell types in the meristem, elongation, and mature zones of maize roots except for *ZmPIP1;6* and *ZmPIP2;7* transcripts, which were not detected.

The expression patterns of *PIP2;3* in leaves and roots of maize under the control and drought conditions (Fig. 4*A*,*C*) suggests no prominent role in water movement through leaves or water uptake by roots. In contrast, the remarkably higher expression of *PIP2;3* in sorghum roots than in leaves under the drought suggests that this gene had an important role in balancing water uptake in the sorghum plants especially under the drought conditions. This was shown by expression of the gene at predawn in roots and leaves of sorghum under the drought where the expression increased in roots but no comparable increase was seen in leaves. It can be concluded that the sorghum plants depended on aquaporin (*PIP2;3*) for water transport to a limited extent under the control conditions but to a greater extent under the drought conditions.

One of the known reasons for sorghum plants to be more drought-tolerant than maize is root length density as the roots of sorghum have the ability to grow vertically deeper in the soil, but maize roots grow horizontally, a feature which increases the ability of sorghum to absorb more water compared to maize (Schittenhelm and Schroetter 2014). This characteristic of sorghum roots makes sense of the high expression of plasma membrane intrinsic proteins (*PIP2;3*) in roots of sorghum particularly in view of previous data which show that PIP2;3 has a high water transport activity compared to PIP1s (Fetter *et al*. 2004) where deeper roots with a high permeability to water are expected to have an enhanced water uptake. However, activity of PIPs (if exists) in maize roots with their superficial growth would be beneficial only under moderate drought stress.

The expression pattern of *TIP1;2* in control leaves of maize and sorghum (Fig. 5*A*,*B*) suggests a role in water transport in both the plants under the 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 vacuole 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 signaling and responses (Maurel 2007). However, the inconsistent expression pattern in maize roots (Fig. 5*C*) indicates no role of *TIP1;2* in root water uptake. In contrast, expression of *TIP1;2* in the droughty sorghum roots (Fig. 5*D*) showed a similar kinetics as that of *PIP2;3* (see above) suggesting a role (though minor) in water transport under the drought.

We conclude that drought tolerance of sorghum as compared to maize was manifested by the maintenance of stable WUE either under the well-watered or drought conditions in sorghum but not in maize. Such a difference seems to depend on maintaining efficient water extraction from dry soil by higher responsiveness of aquaporins (mainly *PIP1;5* and *PIP2;3*) in roots and leaves of sorghum than of maize and in particular, a stable induction of *PIP2;3* expression in roots of sorghum, a response that was absent in maize.

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