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Stress priming enhances drought response in *Sorghum bicolor* potentially involving PIP2;5

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Abstract Stress priming, the exposure to an initial stressor, can positively affect a plant's response to subsequent stresses. Drought priming can induce genetic, biochemical, and physiological responses that enable plants to store information, initiating a memory process that enhances their responsiveness to future drought events. Aquaporin regulation could be among these responses because they have been related to water deficit tolerance. We characterized the physiological drought priming in adult leaves of *Sorghum bicolor* (L.) Moench, and analyzed its relationship with *PIP2;5* aquaporin. Plants were subjected to two events of severe progressive water deficit (SPWD) followed by rehydration. Water status, photosynthesis, antioxidant system, and *PIP2;5* expression were analyzed. The data were collected on the first day of the experiment, during the water deficit events, and at 24 and 72 h after each rehydration. SPWD plants showed improved values of relative water content (RWC), leaf water potential, transpiration (*E*), stomatal conductance (g_s), lipid peroxidation, and H_2O_2 concentration during the second event of water deficiency and rehydration compared to the first stress cycle. This suggests that sorghum promoted physiological responses to increase water deficit tolerance, e.g. strategies in the water-use economy, evidencing the priming of drought stress. Additionally, *PIP2;5* was downregulated during the water deficit period and immediately upregulated when rehydration was applied. Aquaporin regulation during the second stress event was positively correlated to RWC, water use efficiency, intrinsic water use efficiency, and leaf area, which might indicate that *PIP2;5* can impact water status, gas exchange, and growth responses of *S. bicolor* to recurrent SPWD periods.

Keywords Physiological stress response · Physiological stress recovery · Stress sign · Water stress · Plasma membrane intrinsic protein

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1 Introduction

Drought is an important abiotic factor that limits plant development and, consequently, crop yield

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(Rosa et al. 2020). The intensity and frequency of dry periods (Schar et al. 2004; Trenberth et al. 2003) promote the ability of plants to acclimatize to abiotic stresses caused by environmental variations (Lambers et al. 2008; Bruce et al. 2007). Water deficit impacts water status, transpiration, stomatal conductance, and photosynthesis, thereby reducing biomass production (Daryanto et al. 2017). Despite being one of the most drought-tolerant cereals, *Sorghum bicolor* (L.) Moench can experience declines in productivity under moderate to severe water deficits (Reddy et al. 2011; Batista et al. 2017; Sarshad et al. 2021). Consequently, drought priming, which may enhances photosynthesis, antioxidant capacity, and osmotic adjustment, has emerged as a strategy to boost agricultural production during water scarcity (Wang et al. 2014).

Stress priming involves exposing plants to a preliminary stress, enabling them to better withstand subsequent challenges (Liu et al. 2022). Responses to the first drought stress event involve biochemical, molecular, and structural changes, so that tolerance and repair of the damage can occur (Fleta-Soriano and Munné-Bosch 2016; Leufen et al. 2016; Mosa et al. 2017). During the plant's recovery stage, the first stress event leaves a mark, which, through the maintenance of structural changes (Fleta-Soriano and Munné-Bosch 2016; Trewavas 2003) or through signaling proteins and epigenetic modifications, activates the tolerance genes (Bruce et al. 2007; Crisp et al. 2016; Martinez-Medina et al. 2016; Witzany 2018). This activation allows for more efficient responses to future stress events, acting as stress memory (Baluška, Gagliano, Witzany 2018; Bruce et al. 2007; Crisp et al. 2016; Fleta-Soriano and Munné-Bosch 2016; Ogle et al. 2015; Walter et al. 2013). This priming effect has been well-documented in species such as *Arrhenatherum elatius* (Walter et al. 2011), *Dipteryx alata* (Alves et al. 2020), and sugarcane (Ribeiro et al. 2021). In sorghum, stress memory has been observed when drought conditions are applied during the juvenile phase and repeated in the adult phase (Mantoan et al. 2020).

Aquaporins are integral membrane proteins that form channels and allow the passage of water, depending on the concentration gradient. They may participate in different physiological processes linked to plant development, including germination, cell elongation, stomatal movement, photosynthesis, reproduction, and responses to different abiotic stress conditions (Ariani and Gepts 2015). According to subcellular localization, aquaporins are classified into PIPs (plasma membrane intrinsic proteins), TIPs (tonoplast intrinsic proteins), NIPs (nodulin-26-type intrinsic proteins), SIPs (small basic intrinsic proteins) and XIPs (intrinsic proteins X) (Tyerman et al. 2021). In sorghum, there are two subgroups of PIPs (PIP1 and PIP2), five subgroups of TIPs (TIP1-TIP5), four subgroups of NIPs (NIP1-NIP4), and two subgroups of SIPs (SIP1 and SIP2) (Reddy et al. 2015).

Under water deficiency conditions, the regulated activity and abundance of aquaporins modulate the water flow within and between cells (Luu and Maurel 2005; Mahdieh et al. 2008). The expression of aquaporins varies according to isoforms, tissues, species, and stress intensity (Afzal et al. 2016; Park and Campbell 2015). For instance, in *Arabidopsis thaliana* leaves under drought conditions, both *PIPs* and *TIPs* are generally downregulated; however exceptions include the upregulation of *PIP1;4* and *PIP2;5* (Alexandersson et al. 2005; Boursiac et al. 2005; Alexandersson et al. 2010). In contrast, barley (*Pennicetum glaucum*) leaves show upregulation of *PIP2;3* and *PIP1;5* (Iwuala et al. 2020), whereas TIPs show varied response: *TIP3;1* and *TIP4;1* are positively regulated, unlike *TIP1;1, TIP1;2*, *TIP2;1*, *TIP2;2*, and *TIP2;3*, which are negatively regulated (Kurowska et al. 2019). In sorghum leaves, the aquaporins *PIP1;2, PIP2;5*, and *TIP1;1* are downregulated during water deficit (Schley et al. 2022). The diverse responses of aquaporin expression (downregulation, upregulation, or no change) to water deficiency indicate that isoforms contribute distinctively to mechanisms of water transport and regulation (Hachez et al. 2006). Furthermore, during recovery from water deficiency, aquaporins can act as both positive and negative regulators (Jang et al. 2013), where upregulated genes may support plant physiological resilience to stress, whereas those with reduced activity might facilitate adaptation and tolerance to stress conditions (Zargar et al. 2017).

The role of aquaporins in leaf hydraulic conductivity has also been demonstrated (Li et al. 2014). The aquaporin *PIP1;2* in *Arabidopsis thaliana* has been shown to be responsible for a significant portion of leaf water transport (Postaire et al. 2010). In grapevines (*Vitis vinifera*) under reduced irrigation conditions, leaf hydraulic conductivity decreased by approximately 30%, together with the

expression of *TIP2;1* and *PIP2;1* (Pou et al. 2013). The silencing of *PIP1* and *PIP2* genes in tobacco and *Arabidopsis* plants results in a decrease in the recovery capacity after water deficiency (Martre et al. 2002; Siefritz et al. 2002). Schley et al. 2022 revealed that in sorghum *TIP1;1* is related to the recovery of stomatal conductance, transpiration, and leaf hydraulic conductance during rehydration.

Among various aquaporin isoforms, *PIP2;5* stands out due to its remarkable effects when overexpressed in *Zea mays*. This overexpression leads to increased leaf hydraulic conductivity, which positively influences leaf elongation, particularly in conditions of water deficit (Ding et al. 2020). Additionally, it enhances sensitivity to ABA signaling, resulting in stomatal closure (Ding and Chaumont 2020). In sorghum, *PIP2;5* and *PIP2;9* are the only PIP aquaporins significantly upregulated under drought stress, exhibiting the highest expression compared to other members. Additionally, they are the only PIP aquaporins upregulated in all sorghum tissues (Reddy et al. 2015). The same authors suggest that further studies are required to ascertain the functions of the individual aquaporins identified in sorghum. Surprisingly, there is still a notable lack of studies exploring the aquaporin *PIP2;5* and its role in physiological adjustments during stress tolerance.

The objective of this study was to determine if leaf responses to a second water deficit event exhibit improvement over the initial event when both stress episodes occur during the adult phase, thereby characterizing a form of stress priming in *Sorghum bicolor* (L.) Moench. To this end, we subjected adult plants to two cycles of severe progressive water deficiency (SPWD), each followed by a period of rehydration. We assessed physiological variables including photosynthesis, water relations, growth, and antioxidant concentration. Furthermore, we examined the gene expression of the PIP2;5 aquaporin and analyzed its correlation with photosynthetic and water-related parameters.

2 Material and methods

2.1 Plant species and growth conditions

We chose *Sorghum bicolor* (L.) Moench (sorghum) due to its tolerant characteristics to water deficiency, being the target of studies on drought tolerance and productivity (Fracasso et al. 2016). The sorghum seeds were hybrids (BRS332) and were acquired in partnership with Embrapa, whose hybrid features high productivity and good tolerance to water deficit.

We utilized a medium soil type, characterized by a combination of clay and sandyloam. To ensure uniform pH, we chemically adjusted the soil with limestone. Each pot was initially weighed, containing an equal amount of soil (10 kg). The volume of the pots was 16 L. Subsequently, we added water and measured the pot mass the following morning after complete drainage of gravitational water had occurred, reaching field capacity.

The sorghum seeds were initially grown on Styrofoam growth plates containing a vegetable substrate within a greenhouse. These seedlings were transplanted into pots 20 days later. Ten days after germination and 1 day after transplantation (21 days after germination), the seedlings were fertilized with 25% and 50% nutrient solutions, respectively, according to Hoagland and Arnon (1950).

2.2 Experimental design

All plants were maintained at field capacity until the beginning of the experiment. They were divided into two treatment groups: control group (C), which remained at field capacity throughout the experiment; and the severe progressive water deficiency group (SPWD), where water supply was abruptly halted until the end of the water deficiency period. The end of the stress period was determined when plants exhibited leaf relative water content (RWC) in predawn of $51.5 \pm 3.5\%$, pre-dawn leaf water potential (ΨPD) of -2.5 ± 3.6 MPa, stomatal conductance (g_s) of 1.2 ± 0.9 mol m-2 s-1, and the maximum quantum efficiency of PSII (Fv/Fm) of 0.57 ± 0.1.

The SPWD plants experienced two periods of water restriction, referred to as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. E1SC began 35 days after seedling transplantation, while E2SC started at

73 days. During the 16-day interim period between cycles, the plants received continuous watering to facilitate full recovery. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. Therefore, the experiment lasted a total of 95 days.

There were 35 pots for each treatment group, with each pot containing two sorghum seedlings. Out of these, five pots were allocated for gas exchange and fluorescence measurements throughout the experiment, while the remaining 30 pots were allocated for destructive analyses, including water relations, gene expression, antioxidant and growth analyses. On each collection day, five plants per treatment were used for destructive analyses.

2.3 Data collection

During water deficit periods, we measured photosynthetic parameters twice a week. The leaf water potential (Ψ MPa), the soil water potential (Ψsoil MPa), and the leaf relative water content (RWC) were obtained on the first day of the experiment, on the last day of stress of the first cycle (E1SC), 24 h and 72 h after rehydration, on the last day of stress of the second cycle (E2SC), and again 24 h and 72 h after rehydration of the second stress cycle. Gas exchange, fluorescence, and leaf hydraulic conductivity (Kleaf) were measured on the first day of the experiment, at E1SC, E2SC, and 24 h after rehydration for both the first and second stress cycles. Leaves were collected for antioxidant analysis at E1SC and E2SC, as well as 24 h and 72 h after rehydration following each stress cycle. For growth analysis, the dry mass of the plants was collected and the leaf area was measured on the last day of stress of the second cycle and on the last day of the experiment (72 h after rehydration). Molecular analysis samples were obtained at E2SC, as well as 24 h and 72 h after rehydration following the second stress cycle.

2.4 Water relations

To determine the leaf relative water content (RWC), we collected the leaves and cut them into rectangle-shaped samples. Immediately after, we weighed the samples to obtain their fresh mass (FM). Subsequently, we placed the samples in Petri dishes on filter paper, immersed them in deionized water, and stored them for 24 h at 5 °C for rehydration, according to Elsheery and Cao (2008). After rehydration, the samples were reweighed to obtain the turgid mass (TM). Then, we dried the samples in an oven at approximately 60 °C until they reached a constant mass, allowing us to determine their dry mass (DM). We used a precision balance with a sensitivity of 0.0001 g to determine FM, TM and DM. We calculated the RWC according to Smart and Bingham (1974), using the formula: RWC (%) = (FM-DM) / (TM-DM) X 100.

Additionally, using the Water Potential Analyzer with Temperature Controller (WP4-T, Decagon Devices, USA), we measured leaf water potential (Ψ in MPa) for the collected leaves at two time points: pre-dawn (PD) at 5:00 am and midday (MD) at 12:00 pm. Soil water potential (Ψ_{soil} in MPa) was also measured at midday using the same equipment. The plant's hydraulic conductivity (K_{leaf}) was calculated according to Tsuda and Tyree (2000), using Ψ_{soil}, ΨMD, and transpiration rate (E) at midday, as per the formula: $E = K_{leaf}$ (Ψ_{soil}—Ψ MD).

2.5 Photosynthetic parameters

We conducted gas exchange measurements using an equipment containing an open photosynthesis system that featured a CO_2 and water vapor analyzer using infrared radiation (Infra Red Gas Analyzer – IRGA model GSF 3000, Walz, Germany). We took measurements between 10:00 and 11:00 am on sunny days, selecting five pots from each treatment and standardized the 2nd or 3rd leaves with a fully expanded limb. We measured the transpiration rate (E, mmol vapor d'água m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}), CO_2 assimilation rate (A_{Net}, µmol m^{-2} s^{-1}), and vapor pressure deficit (VPD Pa/kPa) at a photosynthetic photon flux density (DFFF) of 1500 µmol/m^2s. We calculated the water use efficiency (A/E, µmolCO_2 mmol-1H_2O) and intrinsic water use efficiency (A/g_s).

An IRGA was also employed to measure and calculate the maximum quantum efficiency of PSII (Fv/Fm). To prepare for these measurements, the leaves were covered with aluminum foil and kept in darkness for approximately 30 min (Maxwell and Johnson 2000).

2.6 Growth analysis

We collected all photosynthetically active leaves (green leaves) to measure the leaf area (LA – cm^2) using an Area Meter. To obtain the dry mass (DM—g) of the aerial part of the samples, we collected both leaves and stem, which were then placed in an oven (temperature ≈ 60ºC) until they reached a constant mass. We used a precision scale with a sensitivity of 0.0001 g to determine the dry mass. With the LA and DM data, we obtained the specific leaf area (SLA = LA/ DM leaf).

2.7 Antioxidant analysis

We assessed hydrogen peroxide (H_2O_2) concentration, peroxidase enzyme activity (POD, EC 1.11.1.7), and lipid peroxidation in five samples per treatment. We collected fully expanded leaves, immediately frozen themin liquid nitrogen, stored them in a − 80 ºC freezer, and later macerated them.

The H_2O_2 concentration was obtained using the method determined by Alexieva et al. (2001). Briefly, we used 200 mg of macerated leaf tissue mixed with 2 ml of 0.1% trichloroacetic acid (TCA) for H_2O_2 extraction. After centrifugation at 12,000 rpm for 15 min at room temperature, 500 µl of the supernatant was combined with 500 µl of 0.1 M potassium phosphate buffer (pH 7.0) and 2 ml of 1 M potassium iodide. This mixture was kept in the dark at room temperature for one hour. We measured the absorbance at 390 nm using a spectrophotometer and calculated H_2O_2 concentration based on a standard curve prepared with known H_2O_2 concentrations, expressing the results as µM of H_2O_2 per gram of fresh matter (FM).

The method used to determine the lipid peroxidation was described by Heath and Packer (1968), cited by Rami Devi and Prasad (1998). We ground the plant material in 5 ml of 0.25 thiobarbituric acid (TBA) in 10% TCA and incubated it at 95º C in a water bath for one hour. After centrifuging at 2000 rpm for 5 min, we measured the absorbance of the supernatant at 560 and 600 nm. Lipid peroxidation were calculated using the molar extinction coefficient of malondialdehyde (155 $mmol.L^{-1}$ cm^{-1}) and expressed as TBARS per gram of fresh matter.

POD activity was determined according to Teisseire and Guy (2000). The reaction mixture included 30 µL of diluted enzyme extract (1:10 in extraction buffer), 50 mmol L^{-1} of potassium phosphate buffer at pH 6.5, 20 mmol L^{-1} of pyrogallol (1,2,3-benzenetriol), and 5 mmol L^{-1} of hydrogen peroxide H_2O_2, making a total volume of 1.0 mL. The reaction was carried out at room temperature for 5 min. The formation of purpurogaline was measured at 430 nm using a UV–Visible spectrophotometer and we calculated POD enzyme activity in µmol of purpurogaline produced per minute per mg of protein, using a molar extinction coefficient of 2.5 mmol L^{-1} cm^{-1}. Total soluble protein was determined using the Bradford method (1976).

2.8 PIP2;5 transcriptional profile

We collected photosynthetically active and fully expanded leaves from five samples per treatment, immediately freezing them in liquid nitrogen, and storing them in a − 80 °C freezer until RNA extraction. Total RNA isolation was performed using the Total RNA Purification kit (Norgen Biotek, Thorold, Canada (USA)) following the manufacturer's recommendations. The RNA was quantified using NanoDrop, and 1000 ng of RNA was utilized for DNAse treatment (RQ1 DNAse, Promega, Madison, Wisconsin (USA)) and subsequent cDNA synthesis. For DNAse treatment, we compared amplification profiles with and without treatment to ensure the absence of genomic DNA qPCR was performed using the GoTaq qPCR mastermix kit (Promega, Madison, Wisconsin (USA)), targeting the genes of interest (*PIP2;5*) and endogenous (*PP2A* and *CYP*).

We initiated cDNA synthesis using total RNA treated with the enzyme DNaseI. For each sample, we used 5µL of RNA and cDNA synthesis was performed with the GoScript Reverse Transcriptase kit (Promega) using a random primer mix. We added 1µL (~ 1000 ng) of random primer to the RNA, followed by incubation at 65 °C for 5 min. Subsequently, we added 4µL GoScript Buffer, 9µL of water, and 2µL of GoScript enzyme, exactly in that order. We further incubated the reactions at 25 °C for 5 min, followed by 42 °C for 60 min, and finished by heating at 70 °C for 5 min. The reaction mixtures were then stored at − 20 °C.

For *RT-qPCR*, we used two endogenous genes, *PP2A* (*Serine/threonine-Protein Phosphatase*) and *CYP* (*Peptidylprolyl isomerase*), as referenced in

Reddy et al. (2016), in contrast to the *PIP2;5* gene (Reddy et al. 2015), for which primer details can be found in Supplemental Table S1.

We performed an efficiency curve for all presented genes by using dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32 along with the primers, Master Mix, RNA, and water. The genes showed efficiency greater than 90%. We performed the qPCR amplification reactions with the final volume, using a 7500 Fast Real-Time PCR System thermocycler (Applied Biosystems, Waltham, Massachusetts (USA)). Amplification was programmed as follows: an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each reaction was run in technical triplicates, and a control sample containing only nuclease free water was included. For data analysis, we employed the Fold Change calculation method, based on delta delta Ct (ΔCt—*threshold cycle*) between the treated and control groups. Log2 Fold Change values were subjected to significance test using the *T*-Test ($p < 0.05$). Notably, there was no statistically significant difference observed between the endogenous genes *PP2A* and *CY*.

2.9 Data analysis

We submitted our data of water relations, photosynthesis, and growth analysis to a normality test. We then compared these datasets by *T*-Test or Mann Whitney Rank Sum analysis, with statistical significance set at $p < 0.05$. The data from the antioxidant analysis underwent a preliminary Anderson Darling homogeneity test, performed using Minitab Statistical software version 21.1.0. Subsequently, we compared these results using either a *T*-Test or Mann Whitney Rank Sum analysis, with statistical significance set at $p < 0.05$. A Pearson correlation test was also performed to examine the relationship between *PIP2;5* aquaporin expression and physiological variables.

For the photosynthetic parameters, a normalization calculation was used as described in the results section. This normalization process allows us to express the plant's resistance and resilience potential as a percentage, always comparing the treated group with the control group (Banning and Murphy 2008).

3 Results

3.1 Water relations

During water deficiency periods, the soil water potential (Ψ_{soil}) values of SPWD plants reached -10.1 ± 0.9 MPa in E1SC and -11.3 ± 1.9 MPa in E2SC, whereas the control group maintained values around -0.1 ± 0 and -0.2 ± 0 MPa, respectively (Fig. 1A). Ψ_{soil} during the rehydration periods was quite similar between the control and SPWD groups, with values close to zero (Fig. 1A). The pre-dawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf potentials of SPWD plants exhibited more negative values during E1SC, which subsequently recovered equally to the control upon rehydration (Fig. 1B and C). In E2SC, there was a slight improvement in these values, and there was no statistical difference between SPWD and control groups, which continued during rehydration (Fig.1B and C). The RWC values of SPWD plants demonstrated significant decreases in E1SC, both pre-dawn and midday, reducing approximately 46% and 54%, respectively, compared to the control (Fig. 2A and B). In E2SC, pre-dawn RWC of SPWD plants reduced by approximately 32%, and midday RWC by 35%, indicating an improvement compared to E1SC (Fig. 2A and). Regarding rehydration, SPWD plants rehydrated at the control values in both cycles (Fig. 2A and B).

Leaf hydraulic conductivity (K_{leaf}) was negatively impacted by water restriction in both stress cycles. However, when rehydrating after the second cycle of stress, SPWD plants did not recover K_{leaf} equally to the control, as they did after the first cycle of stress (Fig. 3).

3.2 Photosynthetic parameters

SPWD plants had A_{Net}, g_s and E drastically reduced in E1SC, showing drops of 98%, 97% and 96%, respectively, when compared to control (Fig. 4A, B, and C). Even during the first rehydration, these variables remained lower, with rates still at 83%, 75%, and 73% below the control. In E2SC and second rehydration, A_{Net}, g_s and E showed distinct responses. On the one hand, A_{Net} of SPWD plants followed the same pattern of reduction observed during the first cycle of stress and rehydration, with values 97% and 85% lower than the control, respectively (Fig. 4A). On the other hand,

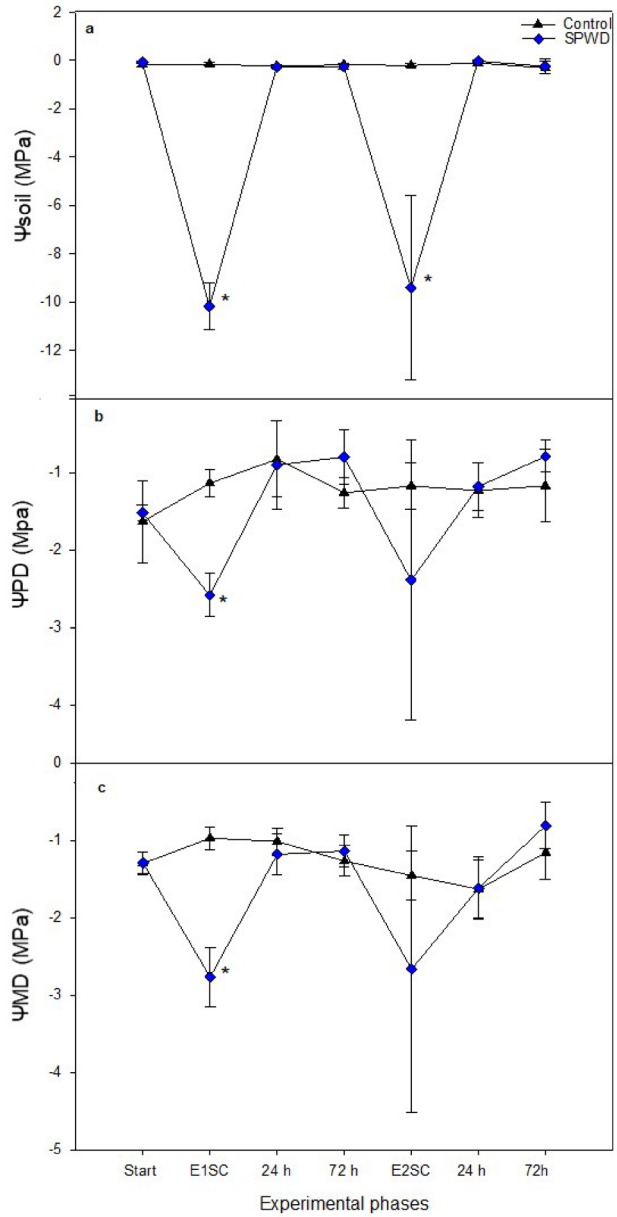

Fig. 1 Soil water potential (**a**), predawn (**b**) and midday (**c**) leaf water potential (**b**) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—blue) conditions. SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference between SPWD and control groups (T-Test or Mann–Whitney analysis, $p < 0.05$)

the drastic reduction in g_s and E observed in E1SC improved in E2SC, presenting values ~ 60% lower than the control, which were maintained during the second rehydration (Fig. 4B and D).

Figure 4C provides information on water use efficiency (A/*E*) for SPWD plants. During E1SC, *A*/*E* dropped by approximately 102% compared to the control. In E2SC, the reduction was again around 100% in relation to the control. However, during both rehydration periods, the *A/E* values for SPWD plants returned to values comparable to those of the control (Fig. 4C). Similarly, intrinsic water use efficiency (*A*/g_s) also exhibited a decrease of around 102% in E1SC and 100% in E2SC for SPWD plants (Fig. 4E). During both rehydration phases, this variable approached values observed in the control group (Fig. 3E).

In Fig. 5, we observed a reduction in the maximum quantum efficiency of PSII (Fv/Fm) during periods of stress in SPWD plants, with values decreasing by approximately 26% in E1SC and 47% in E2SC. The

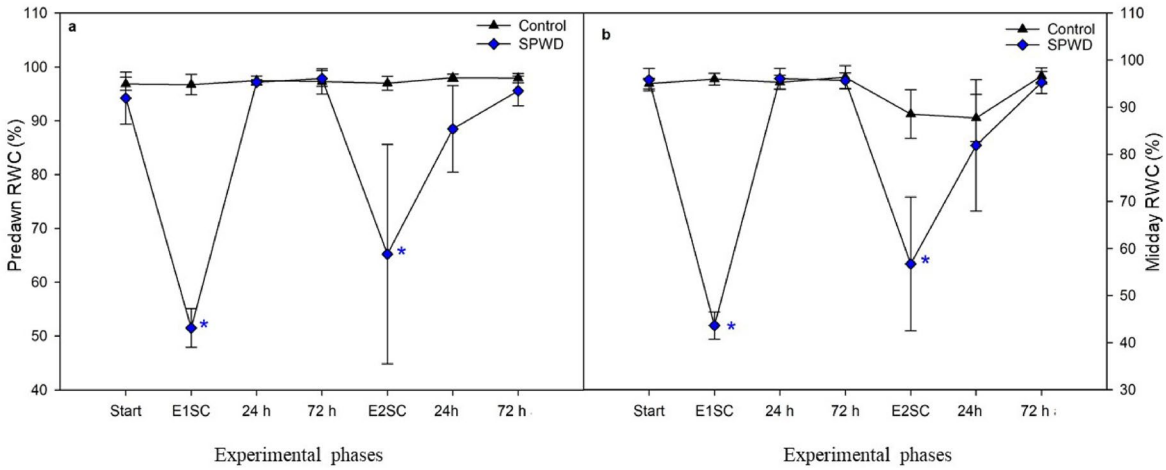

Fig. 2 Predawn (**a**) and midday (**b**) leaf water content (RWC %) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—blue) conditions. The SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference in relation to the control by *T*-Test or Mann Whitney Rank Sum analysis ($p < 0.05$)

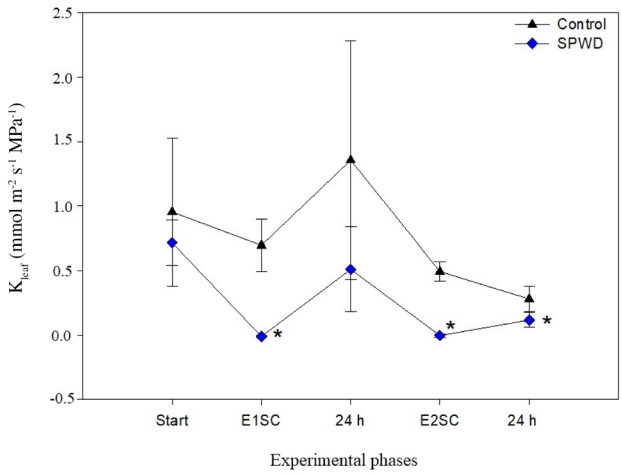

Fig. 3 Leaf hydraulic conductivity (K_{leaf}) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—blue) conditions. The SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference in relation to the control by *T*-Test or Mann Whitney Rank Sum analysis ($p < 0.05$)

values related to rehydration showed some improvement in quantum efficiency, but they were still lower, with reductions around 23% and 20%, respectively (after E1SC and E2SC), compared to the control group (Fig. 5). This highlights the damage in the photochemical stage caused by stress. Moreover, our

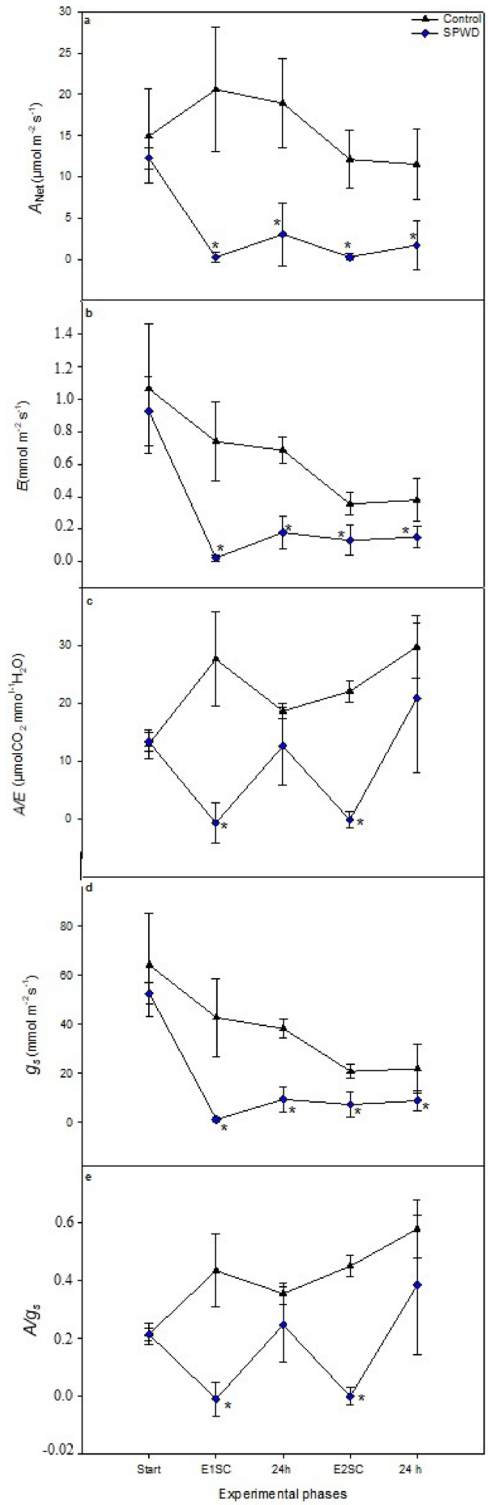

Fig. 4 CO_2 assimilation (**a**), transpiration rate (**b**), water use efficiency (**c**), stomatal conductance (**d**), intrinsic water use efficiency (**e**) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—blue) conditions. The SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference between the treatment in relation to the control by *T*-test or Mann Whitney Rank Sum analysis ($p < 0.05$)

results indicate that Fv/Fm is a photochemical variable sensitive to the type of stress applied in this study, as it showed reductions already from the first stress cycle.

3.3 Growth analysis

On the last day of the second stress cycle, the control group showed a 3 times increase in leaf area compared to the SPWD group, a 2 times increase in leaf dry mass and a 1 time increase in stem + sheath dry mass (Fig. 6A, , and). In the control group, we observed an increase in leaf area, leaf dry mass, and stem + sheath dry mass values after rehydration (Fig. 6A, B, and D). Differently, in the SPWD group, although there was a tendency for improvement after rehydration, they still maintained leaf area, leaf dry mass, and stem + sheath dry mass values approximately 44%, 35% and 31% lower than the control group, even after rehydration (Fig. 6A, B, and C). There were no significant differences in specific leaf area values between the SPWD and control groups (Fig. 6C).

3.4 Antioxidant analysis

The activity of the peroxidase enzyme (POD) in SPWD plants remained relatively stable during periods of water deficiency (E1SC and E2SC) (Fig. 7A). However, it increased compared to the control when rehydration was applied (Fig. 7A). Upon rehydration of E1SC, the increase was gradual, showing a 1.6-fold increase in activity within twenty-four hours and twofold increase within seventy-two hours after rehydration (Fig. 7A). In

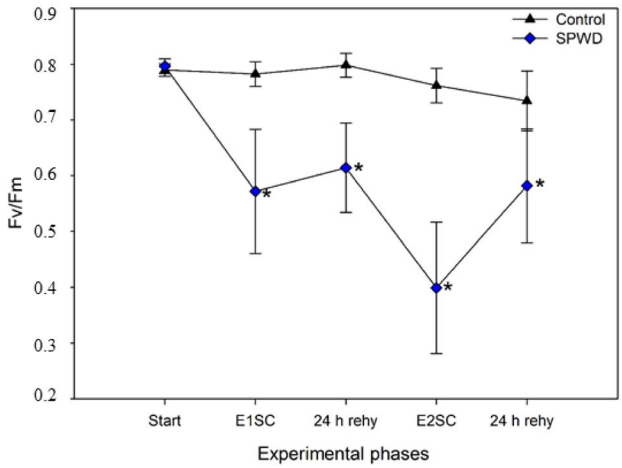

Fig. 5 PSII maximum quantum efficiency (Fv/Fm), of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—blue) conditions. The SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference between the treatment in relation to the control by *T*-test or Mann Whitney Rank Sum analysis ($p < 0.05$)

contrast, during E2SC rehydration, SPWD plants showed a 1.5-fold increase in peroxidase activity within twenty-four hours, followed by a 30% decrease within seventy-two hours after rehydration when compared to control plants. (Fig. 7A).

Regarding hydrogen peroxide (H_2O_2), SPWD plants showed an increase in E1SC approximately 10.7 times higher than the control (Fig. 7B). However, in E2SC, the hydrogen peroxide concentration did not increase compared to the control, indicating acquired tolerance in SPWD plants.

Lipid peroxidation were notably higher in SPWD plants during E1SC, reaching 3.6 times higher than the control (Fig. 7C). After rehydration, these values increased by twofold within twenty-four hours and 1.4-foldwithin seventy-two hours (Fig. 7C). In E2SC, the ratesincreased again1.6 times higher than the control. It is important to note that of peroxidation observed in E2SC was lower than that in E1SC, similar to the corresponding rehydration periods. After the second cycle, lipid peroxidation showed no significant difference compared to the control, indicating the development of stress tolerance (Fig. 7C).

3.5 PIP2;5 transcriptional analysis

We analyzed the expression of the *PIP2;5* aquaporin only during the second water deficit event because it was when we observed attenuated or accentuated physiological and biochemical responses in relation to the first stress event. We observed a 1.5-fold reduction in *PIP2;5* expression in SPWD plants during E2SC compared to the control. However, there was a subsequent increase in expression by 0.27 times compared to the control within twenty-four hours after rehydration. Finally, seventy-two hours after rehydration, the *PIP2;5* expression in SPWD plants returned to similar to those of the control plants (Fig. 8).

Pearson's correlation test between the expression of the aquaporin *SbPIP2;5* and different leaf physiological parameters obtained in E2SC and twenty-four hours after rehydration revealed positive correlations between gene expression and the following variables: RWC PD ($R^2 = 0{,}705$; $p = 0{,}0228$), Ψ_{soil} ($R^2 = 0{,}861$; $p = 0{,}00138$), A/E ($R^2 = 0{,}650$; $p = 0{,}0418$); A/g_s ($R^2 = 0{,}633$; $p = 0493$), e leaf area ($R^2 = 0{,}728$; $p = 0{,}0170$) (Table 1).

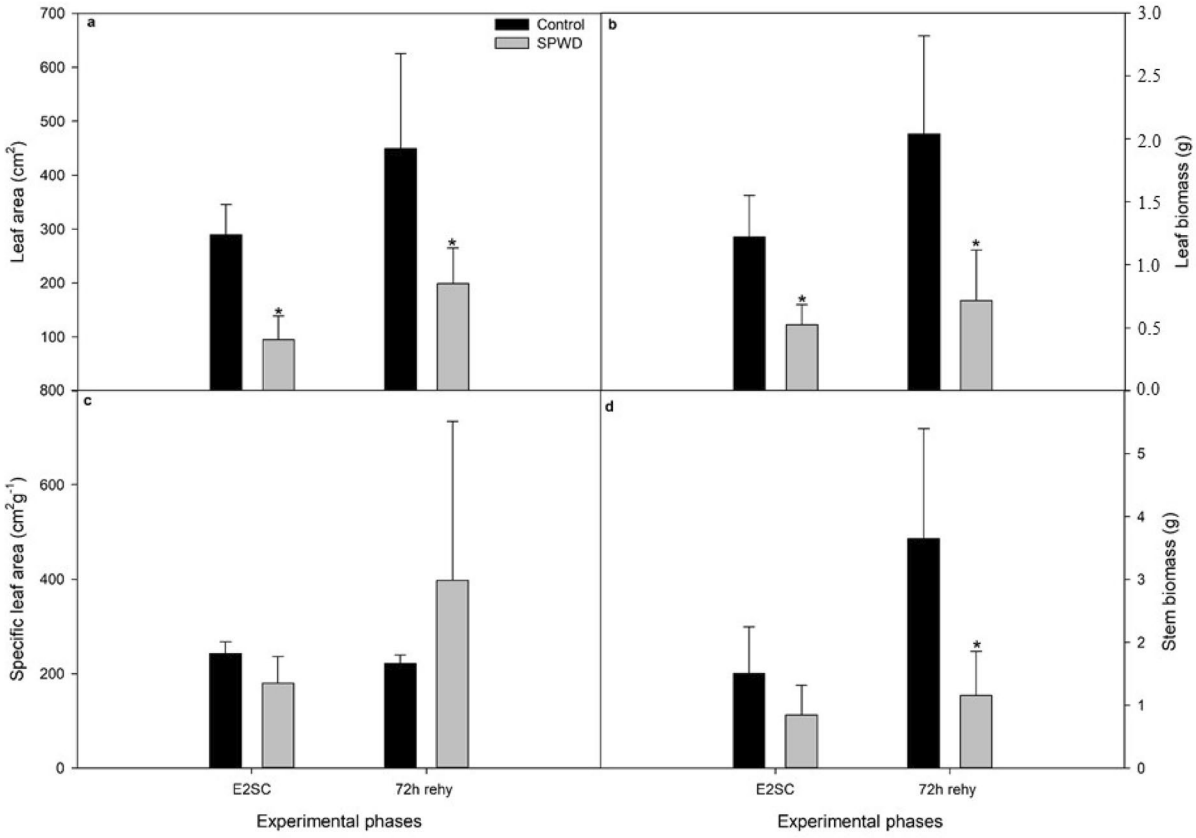

Fig. 6 Leaf area (cm^2 – graphic **a**), leaf biomass (g – graphic **b**), specific leaf area (cm^2 $g.^{-1}$ – graphic **c**), stem biomass (g – graphic **d**) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—gray) conditions. Growth analysis was measured at the end of E2SC and 72 h after rehydration. Asterisks represent statistical difference between the treatment in relation to the control by *T*-test or Mann Whitney Rank Sum analysis ($p < 0.05$)

4 Discussion

Our study showed that *Sorghum bicolor*, subjected to two successive severe progressive water deficit (SPWD) events, displayed enhanced relative water content (RWC), leaf water potential (Ψw) at midday (MD) and predawn (PD), transpiration rate (*E*), stomatal conductance (g_s), lipid peroxidation, and H_2O_2 concentration during the second stress event compared to the first (Figs. 1, 2, 4, and 7). These improvements suggest drought stress priming involving adjustments in water status, gas exchange, and biochemical responses, critical for enhancing sorghum's drought tolerance. Additionally, differential regulation of the *PIP2;5* aquaporin was observed (Fig. 8), which correlated positively with various physiological parameters (Table 1), suggesting its role in physiological adjustments during the subsequent stress event.

A key limitation of our study is the lack of plant groups subjected to variable drought cycles, particularly those experiencing only one cycle of water deficit stress. This absence restricts our capacity to definitively attribute the physiological and biochemical enhancements to the priming effect. Future research should incorporate such treatment groups to clarify the mechanisms of stress priming in sorghum. To contextualize our results, we referenced studies reporting responses to a single drought event.

The regulation of leaf water status during the stress period is essential for developing drought tolerance mechanisms in plants (Galmés et al. 2007; Khanna Chopra and Selote, 2007). One reason is that stomata respond to environmental disturbances that modify the plant's water potential

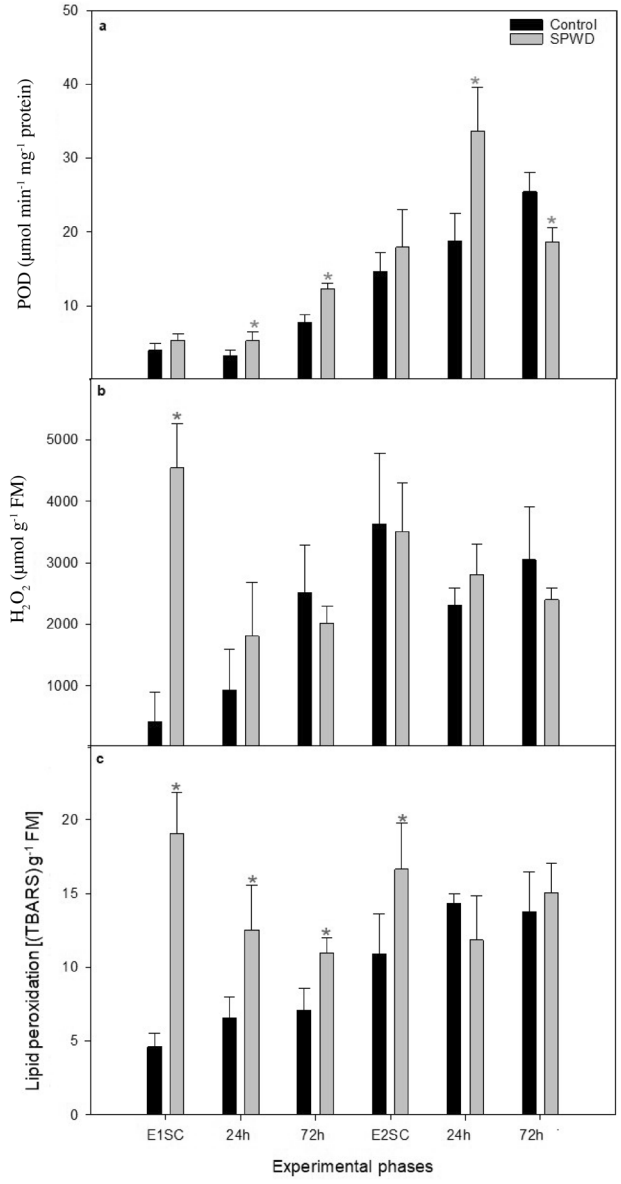

Fig. 7 Activity of the peroxidase enzyme (**a**), H_2O_2 (**b**), and lipid peroxidation (**c**) of *Sorghum bicolor* under control (black) and severe progressive water deficit (SPWD—gray) conditions The SPWD plants experienced two periods of water deficit, referred as E1SC (end of the first stress cycle) and E2SC (end of the second stress cycle), each lasting 22 days. These cycles were followed by rehydration until field capacity, and data were collected 24 h and 72 h after rehydration. 'Start' means when E1SC began (35-day-old plants). E2SC began when SPWD plants were 73-day-old. During the 16-day interim period between cycles, the plants received continuous watering. Asterisks represent statistical difference between the treatment in relation to the control by *T*-test or Mann Whitney Rank Sum analysis ($p < 0.05$)

(Buckley 2019), affecting plant transpiration and potentially influencing carbon assimilation (Yan et al. 2016). Thus, plant water status is commonly linked to stomatal conductance and transpiration rates. Indeed, during the second water deficit event, SPWD plants showed improved RWC, Ψw, E, and *gs* compared to the first event (Figs. 1, 2, and 4). In comparison, when subjected to a single SPWD event, sorghum showed a decrease in leaf RWC, *E*, and g_s by 34%, 99.4%, and 99%, respectively, compared to controls (Schley et al. 2022). Similarly, sugarcane subjected to a single severe drought event experienced decreases in RWC, *gs*, and E by 33%, 83%, and 88%, respectively (Li et al. 2016). Conversely, in our study, two cycles of water deficit resulted in reduced RWC by approximately 32%, g_s by 75%, and *E* by 73% (Figs. 2 and 4), which indicates improved outcomes compared to those reported by Schley et al. (2022) and Li et al. (2016). These observations suggest that sorghum plants can adjust their water-use efficiency and gas exchange responses through stress priming, essential for their drought tolerance process.

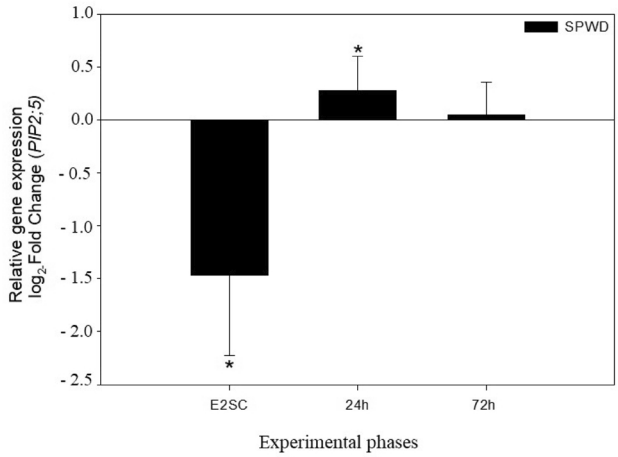

Fig. 8 Relative expression of the aquaporin *PIP2;5* in leaves of *Sorghum bicolor* subjected to severe progressive water deficiency (black—SPWD). The expression of the control treatment is constant and equal to zero. Expression analysis occurred at the end of E2SC, 24 and 72 h after rehydration. Asterisks represent statistical difference between the treatment and the control. Positive values indicate increased expression and negative values indicate low expression

Water scarcity is known to catalyze the accumulation of reactive oxygen species (ROS), such as H_2O_2 (Mirzai et al. 2013; Rai et al. 2012). This accumulation often leads to increased lipid peroxidation, adversely affecting membrane fluidity and integrity (Yadav et al. 2019). Consistently, multiple studies have documented elevated H_2O_2 concentration and lipid peroxidation in various plant species during drought conditions (Sairam and Srivastava 2001; Jubany-Marí et al. 2009; Sun et al. 2016; Khaleghi et al. 2019; Pandey et al. 2010; Irigoyen et al. 1992; Zhang and Kirkham 1994). In our study, we observed a 1070% increase in H_2O_2 concentration and a 267% increase in lipid peroxidation in SPWD plants compared to the control at the end of the first drought event (Fig. 7b and c). Interestingly, subsequent drought events demonstrated a reduction in both H_2O_2 concentration and lipid peroxidation in SPWD plants, with H_2O_2 equivalent to the control and increased lipid peroxidation by only 68% compared to the control by the second event (Fig. 7b and c), indicating acclimation or stress priming. Comparative analysis with single drought event studies further highlights this phenomenon; for instance, in corn, a single drought event increased H_2O_2 and lipid peroxidation by 50.1% and 52.2%, respectively (Sun et al. 2023), and in cucumber, H_2O_2 concentration doubled (Sun et al. 2016). Similarly, *Maclura pomifera* exhibited a 215% increase in lipid peroxidation after one drought event (Khaleghi et al. 2019). Our findings suggest that sorghum may employ regulatory mechanisms for H_2O_2 and lipid peroxidation during repeated water deficit events, indicative of an underlying stress priming mechanism.

Previous studies have demonstrated that the effects of stress priming in crops vary with the type, intensity, duration of stress, and the plant species or genotypes involved (Liu et al. 2022). In sorghum, water deficiency initially applied during the juvenile phase and reapplied in the adult phase has been shown to induce memory effects on RWC, A_{Net}, E, g_s, H_2O_2 concentration, lipid peroxidation, peroxidase activity (POD), and the efficiency of photosystem II (Fv/Fm) (Mantoan et al. 2020). Conversely, our observations indicate that when both water deficit events are applied during the adult phase, sorghum exhibits priming effects in RWC, leaf water potential at midday and predawn, E, g_s, lipid peroxidation, and H_2O_2 concentration (Figs. 1, 2, 4, and 7). These findings suggest that the memory effects on RWC, E, g_s, H_2O_2, and lipid peroxidation are independent of the developmental stage at which stress priming occurs. However, responses in A_{Net}, POD, and Fv/Fm appear to be dependent on the developmental stage. This indicates that certain physiological adjustments related to priming and memory effects are influenced by the stage of development during which the stress priming occurs.

Table 1 Pearson correlations between the expression of the aquaporin *SbPIP2;5* and individual readings of leaf physiological parameters obtained from *S. bicolor* plants collected at E2SC and 24 h after rehydration

SPWD	Fold change *SbPIP2;5*
RWC PD	**$R^2 = 0{,}705$ $p = 0{,}0228$**
RWC MD	$R^2 = 0{,}417$ $p = 0{,}230$
Ψ_{PD}	$R^2 = 0{,}452$ $p = 0{,}190$
Ψ_{MD}	$R^2 = 0{,}583$ $p = 0{,}0771$
Ψ_{soil}	**$R^2 = 0{,}861$ $p = 0{,}00138$**
E	$R^2 = 0{,}139$ $p = 0{,}701$
A_{Net}	$R^2 = 0{,}355$ $p = 0{,}315$
g_s	$R^2 = 0{,}162$ $p = 0{,}654$
VPD	$R^2 = 0{,}0581$ $p = 0{,}873$
A/E	**$R^2 = 0{,}650$ $p = 0{,}0418$**
A/g_s	**$R^2 = 0{,}633$ $p = 0493$**
K_{leaf}	$R^2 = 0{,}573$ $p = 0{,}0835$
Fv/Fm	$R^2 = 0{,}500$ $p = 0{,}142$
Leaf area	**$R^2 = 0{,}728$ $p = 0{,}0170$**
Leaf biomass	$R^2 = 0{,}180$ $p = 0{,}619$
Specific leaf area	$R^2 = 0{,}571$ $p = 0{,}0844$
Stem biomass	$R^2 = -0{,}171$ $p = 0{,}637$

The results demonstrating correlations between the variables are highlighted in bold

Chinnusamy and Zhu (2009) and Skirycz and Inzé (2010) have identified that one potential adverse effect of stress marking is the inhibition of maximum plant growth potential. In line with this, sorghum plants exposed to repeated SPWD events exhibited reduced growth, with leaf area and leaf dry mass decreased by 44% and 35%, respectively (Fig. 6). However, these plants maintained similar specific leaf area (SLA) values to the control (Fig. 6). Conversely, when subjected to a single SPWD event, the same species showed a 73% reduction in leaf area, a 20% decrease in leaf biomass, resulting in a 65% reduction in SLA (Schley et al. 2022). Similarly, studies on soybeans exposed to a single drought event have also reported reductions in SLA (Basal and Szabó 2020; Basal et al. 2024). These findings suggest that recurrent drought events may have a more pronounced negative impact on leaf dry mass than on leaf area, resulting in relatively improved SLA values compared to those observed after a single drought event. This adaptation likely reflects an optimization of leaf structure to enhance water use efficiency and photosynthetic capacity under recurrent stress conditions, thereby supporting the plant's survival and productivity in challenging environments.

Our study found that the aquaporin *PIP2;5* was downregulated during the second event of drought stress and upregulated upon rehydration (Fig. 8). This regulation of *PIP2;5* could be a direct response to water deficiency and re-watering, as observed in many PIPs in other plant species (Alexandersson et al. 2005; Galmés et al. 2007; Zupin et al. 2017). However, when comparing our findings with those of Schley et al. (2022), in which a single drought event was applied, *PIP2;5* expression was downregulated twice as much as in our study, and this downregulation persisted 72 h after rehydration. In contrast, in our study, *PIP2;5* expression had already recovered to control values within 72 h after rehydration (Fig. 8). This indicates that sorghum differently regulates *PIP2;5* expression when is subjected to a single or recurrent water deficit events, which might be an effect from stress priming.

This differential *PIP2;5* regulation might also indicate a potencial role in varying physiological responses. Indeed, when there is only one event of water deficit and rehydration, *PIP2;5* does not appear to be related to physiological responses in sorghum (Schley et al. 2022). However, in cases of recurrent water deficit stress, this correlation became evident, and *PIP2;5* expression was positively correlated with various parameters, including RWC, Ψ_{soil}, *A/E*, A/g_s, and leaf area (Table 1). This further supports the idea that not only the intensity of stress but also its frequency influences the responses of aquaporins and their impact on the plant's physiological responses.

Additionally, *PIP2;5* could be involved in the improvement of the physiological responses observed

during the second event of stress compared to the first event of stress (Figs. 1, 2, 4, and 7). This involvement could be through directly helping to improve RWC (as indicated by the positive correlation in Table 1), enhancing water status, and consequently indirectly helping to improve Ψw leaf MD and PD (MPa), *E*, and g_s (Figs. 1, 2, and 4), as there is a strong interdependence between the water status of the leaves and water loss through stomatal transpiration, especially during drought scenarios (Yan et al. 2016; Sengupta et al. 2013). However, this hypothesis requires further investigation in future studies, such as investigating these physiological responses in *pip2;5* knockout mutant submitted to one and multiple events of water deficit, as well as include *PIP2;5* as a protein of interest in stress memory studies, possibly involving its interaction with other stress-responsive genes and proteins.

Considering that epigenetic mechanisms, such as DNA methylation and chromatin remodeling, play critical roles in the development of stress memory in sorghum (Mantoan et al. 2020), another valuable future direction would be to investigate the potential involvement of these epigenetic modifications in regulating the expression of aquaporins like *PIP2;5*. Additionally, it would be beneficial to include PIP aquaporins as key proteins of interest in stress memory studies, exploring their interactions with other stress-responsive genes and proteins.

5 Conclusion

Drought priming enhances the physiological adjustments of *Sorghum bicolor* to subsequent water deficits, which may involve the modulation of PIP2;5. The regulation of *PIP2;5* after rehydration seems to play a role in optimizing water status and gas exchange, contributing to the plant's resilience and growth during recurrent drought conditions. These genetic and physiological strategies underscore the potential of exploiting stress memory for developing drought-tolerant crops.

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Author contributions PP and LFRA proposed the research and analyzed data. PP and TRS designed the research and wrote the manuscript. PP conducted the experiments. BCR and JP provided resources for gene expression analyses. All authors read and approved the manuscript.

Data availability Our data will be available upon request. Furthermore, we are studying what would be the appropriate repository to deposit our data.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Afzal Z, Howton TC, Sun Y, Mukhtar MS (2016) The roles of aquaporins in plant stress responses. J Develop Biol 4:9

Alexandersson E, Fraysse L, Sjövall-Larsen S et al (2005) Whole gene family expression and drought stress regulation of aquaporins. Plant Mol Biol 59:469–484

Alexandersson E, Danielson JA, Råde J et al (2010) Transcriptional regulation of aquaporins in accessions of *Arabidopsis* in response to drought stress. Plant J 61:650–660

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell Environ 24:1337–1344

Alves RDFB, Menezes-Silva PE, Sousa LF et al (2020) Evidence of drought memory in *Dipteryx alata* indicates differential acclimation of plants to savanna conditions. Sci Rep 10:1–16

Ariani A, Gepts P (2015) Genome-wide identification and characterization of aquaporin gene family in common bean (*Phaseolus vulgaris* L.). Mol Gen Genom 290:1771–1785

Baluška F, Gagliano M, Witzany G (2018) Memory and learning in plants, 1st edn. Springer International Publishing, Cham

Banning NC and Murphy DV (2008) Effect of heat-induced disturbance on microbial biomass and activity in forest soil and the relationship between disturbance effects and microbial community structure. Appl Soil Ecol 40:109–119

Basal O, Szabó A (2020) Ameliorating drought stress effects on soybean physiology and yield by hydrogen peroxide. Agric Conspec Sci 85(3):202

Basal O, Munkhbat U, Veres S (2024) Enhancing drought tolerance in two soybean genotypes with varied susceptibilities through foliar application of acetic acid. J Plant Growth Regul 43(4):1304–1315

Batista PSC, Menezes CB, carvalho AJ, et al (2017) Performance of grain sorghum hybrids under drought stress using GGE biplot analyses. Gen Mol Res 16:1–12

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bruce TJ, Matthes MC, Napier JA, Pickett JA (2007) Stressful "memories" of plants: evidence and possible mechanisms. Plant Sci 173:603–608

Buckley TN (2019) How do stomata respond to water status? New Phytol 224:21–36

Chinnusamy V, Zhu JK (2009) Epigenetic regulation of stress responses in plants. Curr Opin Plant Biol 12:133–139

Crisp PA, Ganguly D, Eichten SR et al (2016) Reconsidering plant memory: intersections between stress recovery, RNA turnover, and epigenetics. Sci Adv 2:e1501340

Daryanto S, Wang L, Jacinthe P-A (2017) Global synthesis of drought effects on cereal, legume, tuber and root crops production: a review. Agric Water Manag 179:18–33

Devi SR, Prasad MNV (1998) Copper toxicity in *Ceratophyllum demersum* L. (Coontail), a free floating macrophyte: response of antioxidant enzymes and antioxidants. Plant Sci 138:157–165

Ding L, Chaumont F (2020) Aquaporin mediating stomatal closure is associated with water conservation under mild water deficit. bioRxiv, 04

Ding L, Milhiet T, Couvreur V et al (2020) Modification of the expression of the aquaporin *ZmPIP2;5* affects water relations and plant growth. Plant Physiol 182:2154–2165

Elsheery NI, Cao KF (2008) Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. Acta Physiol Plant 30:769–777

Fleta-Soriano E, Munné-Bosch S (2016) Stress memory and the inevitable effects of drought: a physiological perspective. Front Plant Sci 7:143

Fracasso A, Trindade L, Amaducci S (2016) Drought tolerance strategies highlighted by two *Sorghum bicolor* races in a dry-down experiment. J Plant Physiol 190:1–14

Galmés J, Flexas J, Savé R, Medrano H (2007) Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. Plant Soil 290:139–155

Hachez C, Moshelion M, Zelazny E et al (2006) Localization and quantification of plasma membrane aquaporin expression in maize primary root: a clue to understanding their role as cellular plumbers. Plant Mol Biol 62:305

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Hoagland DR (1950) Arnon DI (1950) The water-culture method for growing plants without soil. Circular. Calif Agri Exp Stat Circ 347:1–32

Irigoyen JJ, Emerich DW, Sánchez-Díaz M (1992) Alfalfa leaf senescence induced by drought stress: photosynthesis, hydrogen peroxide metabolism, lipid peroxidation and ethylene evolution. Physiol Plant 84:67–72

Iwuala E, Odjegba V, Sharma V, Alam A (2020) Drought stress modulates expression of aquaporin gene and photosynthetic efficiency in *Pennisetum glaucum* (L.) R. Br. genotypes. Curr Plant Biol 21:100131

Jang HY, Yang SW, Carlson JE et al (2013) Two aquaporins of Jatropha are regulated differentially during drought stress and subsequent recovery. J Plant Physiol 170:1028–1038

Jubany-Marí T, Munne-Bosch S, Lopez-Carbonell M, Alegre L (2009) Hydrogen peroxide is involved in the acclimation of the Mediterranean shrub, *Cistus albidus* L., to summer drought. J Exp Bot 60:107–120

Khaleghi A, Naderi R, Brunetti C et al (2019) Morphological, physiochemical and antioxidant responses of *Maclura pomifera* to drought stress. Sci Rep 9:1–12

Khanna-Chopra R, Selote DS (2007) Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than - susceptible wheat cultivar under field conditions. Environ Exp Bot 60:276–283

Kurowska MM, Wiecha K, Gajek K, Szarejko I (2019) Drought stress and re-watering affect the abundance of TIP aquaporin transcripts in barley. PLoS ONE 14:e0226423

Lambers H, Chapin FS, Pons TL (2008) Plant physiological ecology. Springer, New York

Leufen G, Noga G, Hunsche M (2016) Drought stress memory in sugar beet: mismatch between biochemical and physiological parameters. J Plant Growth Regul 35:680–689

Li C, Nong Q, Solanki MK, Liang Q, Xie J, Liu X, Li Y (2016) Differential expression profiles and pathways of genes in sugarcane leaf at elongation stage in response to drought stress. Sci Rep 6(1):25698

Liu H, Able AJ, Able JA (2022) Priming crops for the future: rewiring stress memory. Trends Plant Sci 27(7):699–716

Luu DT, Maurel C (2005) Aquaporins in a challenging environment: molecular gears for adjusting plant water status. Plant, Cell Environ 28:85–96

Mahdieh M, Mostajeran A, Horie T, Katsuhara M (2008) Drought stress alters water relations and expression of PIP-type aquaporin genes in *Nicotiana tabacum* plants. Plant Cell Physiol 49:801–813

Mantoan LPB, Correa CV, Rainho CA, de Almeida LFR (2020) Rapid dehydration induces long-term water deficit memory in sorghum seedlings: advantages and consequences. Environ Exp Bot 180:104252

Martinez-Medina A, Flors V, Heil M et al (2016) Recognizing plant defense priming. Trends Plant Sci 21:818–822

Martre P, Morillon R, Barrieu F et al (2002) Plasma membrane aquaporins play a significant role during recovery from water deficit. Plant Physiol 130:2101–2110

Maxwell K and Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668

Mirzai M, Moeini A, Ghanati F (2013) Effects of drought stress on the lipid peroxidation and antioxidant enzyme activities in two canola (*Brassica napus* L.) cultivars. J Agri Sci Technol 15:593–602

Mosa KA, Ismail A, Helmy M (2017) Plant stress tolerance: an integrated omics approach. Springer, Cham

Ogle K, Barber JJ, Barron-Gafford GA et al (2015) Quantifying ecological memory in plant and ecosystem processes. Ecol Lett 18:221–235

Pandey HC, Baig MJ, Chandra A, Bhatt RK (2010) Drought stress induced changes in lipid peroxidation and antioxidant system in genus *Avena*. J Environ Biol 31:4

Park WJ, Campbell BT (2015) Aquaporins as targets for stress tolerance in plants: genomic complexity and perspectives. Turk J Bot 39(6):879–886

Postaire O, Tournaire-Roux C, Grondin A et al (2010) A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of *Arabidopsis*. Plant Physiol 152:1418–1430

Pou A, Medrano H, Flexas J, Tyerman SD (2013) A putative role for *TIP* and *PIP* aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine

under water stress and re-watering. Plant Cell Environ 36:828–843
Rai AC, Singh M, Shah K (2012) Effect of water withdrawal on formation of free radical, proline accumulation and activities of antioxidant enzymes in ZAT12-transformed transgenic tomato plants. Plant Physiol Biochem 61:108–114
Reddy BVS, Kumar AA, Ramesh S, Reddy PS (2011) *Sorghum* genetic enhancement for climate change adaptation. Crop Adapt Clim Change. https://doi.org/10.1002/9780470960929.ch23
Reddy PS, Rao TSRB, Sharma KK, Vadez V (2015) Genome-wide identification and characterization of the aquaporin gene family in *Sorghum bicolor* (L.). Plant Gene 1:18–28
Reddy PS, Srinivas Reddy D, Sivasakthi K et al (2016) Evaluation of sorghum [*Sorghum bicolor* (L.)] reference genes in various tissues and under abiotic stress conditions for quantitative real-time PCR data normalization. Front Plant Sci 7:529
Ribeiro RV, Vitti KA, Marcos FC et al (2021) Proposal of an index of stability for evaluating plant drought memory: a case study in sugarcane. J Plant Physiol 260:153397
Rosa L, Chiarelli DD, Rulli MC et al (2020) Global agricultural economic water scarcity. Sci Adv 6:eaaz6031
Sairam RK, Srivastava GC (2001) Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J Agron Crop Sci 186:63–70
Sarshad A, Talei D, Torabi M et al (2021) Morphological and biochemical responses of *Sorghum bicolor* (L.) Moench under drought stress. SN Appl Sci 3:1–12
Schar C, Vidale PL, Lüthi D et al (2004) The role of increasing temperature variability in European summer heatwaves. Nature 427:332–336
Schley TR, Franco DM, Junior JPA et al (2022) *TIP1; 1* expression could modulate the recovery of stomatal opening during rehydration in *Sorghum bicolor*. Environ Exp Bot 200:104908
Sengupta D, Guha A, Reddy AR (2013) Interdependence of plant water status with photosynthetic performance and root defense responses in *Vigna radiata* (L.) Wilczek under progressive drought stress and recovery. J Photochem Photobiol, B 127:170–181
Siefritz F, Tyree MT, Lovisolo C et al (2002) *PIP1* plasma membrane aquaporins in tobacco: from cellular effects to function in plants. Plant Cell 14:869–876
Skirycz A, Inzé D (2010) More from less: plant growth under limited water. Curr Opin Biotechnol 21:197–203
Smart RE, Bingham GE (1974) Rapid estimates of relative water content. Plant Physiol 53:258–260
Sun Y, Wang H, Liu S, Peng X (2016) Exogenous application of hydrogen peroxide alleviates drought stress in cucumber seedlings. S Afr J Bot 106:23–28
Sun Y, Miao F, Wang Y, Liu H, Wang X, Wang H, Yang Q (2023) L-Arginine alleviates the reduction in photosynthesis and antioxidant activity induced by drought stress in maize seedlings. Antioxidants 12(2):482
Teisseire H, Guy V (2000) Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (Lemna minor). Plant Sci 153:65–72
Trenberth KE, Dai A, Rasmussen RM, Parsons DB (2003) The changing character of precipitation. Bull Am Meteor Soc 84:1205–1218
Trewavas A (2003) Aspects of plant intelligence. Ann Bot 92:1–20
Tsuda M, Tyree MT (2000) Plant hydraulic conductance measured by the high pressure flow meter in crop plants. J Exp Bot 51:823–828
Tyerman SD, McGaughey SA, Qiu J et al (2021) Adaptable and multifunctional ion-conducting aquaporins. Annu Rev Plant Biol 72:703–736
Walter J, Nagy L, Hein R et al (2011) Do plants remember drought? Hints towards a drought-memory in grasses. Environ Exp Bot 71:34–40
Walter J, Jentsch A, Beierkuhnlein C, Kreyling J (2013) Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. Environ Exp Bot 94:3–8
Wang X, Vignjevic M, Jiang D, Jacobsen S, Wollenweber B (2014) Improved tolerance to drought stress after anthesis due to priming before anthesis in wheat (*Triticum aestivum* L.) var. Vinjett. J Exp Bot 65(22):6441–6456
Witzany G (2018) Memory and learning as key competences of living organisms. Memory and learning in plants. Springer, Cham, pp 1–16
Yadav DK, Kumar S, Choi EH et al (2019) Molecular dynamic simulations of oxidized skin lipid bilayer and permeability of reactive oxygen species. Sci Rep 9:4496
Yan W, Zhong Y, Shangguan Z (2016) A meta-analysis of leaf gas exchange and water status responses to drought. Sci Rep 6(1):20917
Zargar SM, Nagar P, Deshmukh R et al (2017) Aquaporins as potential drought tolerance inducing proteins: towards instigating stress tolerance. J Proteomics 169:233–238
Zhang J, Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol 35:785–791
Zupin M, Sedlar A, Kidrič M, Meglič V (2017) Drought-induced expression of aquaporin genes in leaves of two common bean cultivars differing in tolerance to drought stress. J Plant Res 130:735–745