

# Differences in biochemical, physiological and molecular response mechanisms of rice, weedy rice and barnyardgrass subjected to drought<sup>1</sup>

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## ABSTRACT

The drought stress tolerance may differ among species and cultivars. The drought effect on plants depends on the impact on the plant's physiological, biochemical and molecular processes, as well as on its ability to adapt under these conditions. This study aimed to evaluate the physiological and biochemical responses, and determine the expression of the genes *OsAPX2*, *OsHSP24.15*, *OsHSP71.10* and *OsHSP85.88* under drought conditions in rice, weedy rice and barnyardgrass. A greenhouse experiment was performed in a complete randomized design, with four replications and two factors: water conditions (well-watered and water deficit) and plant species [rice (*Oryza sativa* cv. Puitá), weedy rice (*Oryza* spp.) and barnyardgrass (*Echinochloa* spp.)]. Under drought conditions, the rice and weedy rice plants showed more cellular damage than the barnyardgrass, and the three species showed a reduced photosynthetic rate. C<sub>3</sub> plants (rice and weedy rice) increased the damage to lipids and proteins at 5 days of drought. However, for C<sub>4</sub> plants (barnyardgrass), the drought conditions did not affect the biochemical parameters. The expression of the *OsHSP85.88* gene increased in the three plants exposed to water deficit.

**KEYWORDS:** *Oryza* spp., *Echinochloa* spp., photosynthesis, oxidative damage.

## INTRODUCTION

Climate change is characterized by increased carbon dioxide (CO<sub>2</sub>) concentration in the atmosphere, increasing average temperatures and frequent extreme events, including drought periods (Dahal et al. 2019).

Rice is an important staple food for more than 3 billion people, comprising 50-80 % of their daily calorie intake, and it is threatened by climate change because it is a paddy field crop susceptible to water stress, especially in the initial growth stages, before flooding (Serraj et al. 2011, Balbinot et al. 2021).

## RESUMO

Diferenças nos mecanismos de resposta bioquímica, fisiológica e molecular de arroz, arroz daninho e capim-arroz submetidos a seca

A tolerância ao estresse hídrico pode diferir entre espécies e cultivares. O efeito da seca nas plantas depende do impacto nos processos fisiológicos, bioquímicos e moleculares da planta e da capacidade de a mesma se adaptar a essas condições. Objetivou-se avaliar as respostas fisiológicas e bioquímicas, bem como determinar a expressão dos genes *OsAPX2*, *OsHSP24.15*, *OsHSP71.10* e *OsHSP85.88* em condições de seca em arroz, arroz daninho e capim-arroz. Foi realizado experimento em casa-de-vegetação, em delineamento inteiramente casualizado, com quatro repetições e dois fatores: condições hídricas (bem irrigado e déficit hídrico) e espécies de plantas [arroz (*Oryza sativa* cv. Puitá), arroz daninho (*Oryza* spp.) e capim-arroz (*Echinochloa* spp.)]. Em condições de seca, as plantas de arroz e arroz daninho apresentaram mais danos celulares do que o capim-arroz, e as três espécies apresentaram taxa fotossintética reduzida. As plantas C<sub>3</sub> (arroz e arroz daninho) aumentaram os danos aos lipídios e proteínas aos 5 dias de seca. No entanto, para plantas C<sub>4</sub> (capim-arroz), as condições de seca não afetaram os parâmetros bioquímicos. A expressão do gene *OsHSP85.88* aumentou nas três plantas expostas ao déficit hídrico.

**PALAVRAS-CHAVE:** *Oryza* spp., *Echinochloa* spp., fotossíntese, dano oxidativo.

The drought stress tolerance is evident in almost all plants, but it may differ among species and cultivars. The degree of drought on plants depends on the impact on their physiological, biochemical and molecular processes and their ability to adapt under these conditions. The photosynthetic pathway of plants is directly related to water stress tolerance, and C<sub>3</sub> plants are more sensitive to drought (Alfonso & Bruggemann 2012).

The rice crop follows the C<sub>3</sub> photosynthetic pathway and grows in fields with weeds that follow a similar path, such as weedy rice, and C<sub>4</sub> weeds,

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like barnyardgrass (*Echinochloa* spp.), two of the main weeds competing with rice in South America (Radosevich et al. 2007, Silva et al. 2020).

Water deficit may physiologically affect plants in several ways, including their photosynthesis, transpiration rate, stomatal conductance, water-use efficiency, intercellular CO<sub>2</sub>, photosystem II activity and membrane stability index (López-Serrano et al. 2019, Piveta et al. 2021). Another common effect of drought stress is the disturbance between the antioxidant system's generation and the quenching of reactive oxygen species (ROS) (Faize et al. 2011). Furthermore, changes at the molecular level may occur in plants, including stress-responsive genes expression, which, in turn, are responsible for the synthesis of stress tolerance-related proteins, such as ascorbate peroxidase (APX) and heat shock proteins (HSPs) (Oliveira et al. 2019). More than 5,000 genes were identified to be differentially expressed in rice submitted to drought stress (Ray et al. 2011). However, little is known about the effect of drought on the gene expression in weeds.

Understanding the physiological, biochemical and molecular features of rice plants and weeds when subjected to abiotic stresses is essential to obtain information to predict how these plants will behave under the expected climate changes in the future, and, accordingly, develop management strategies for weed control in these situations. Therefore, this study aimed to evaluate the physiological and biochemical responses, as well as determine the expression of the genes *OsAPX2*, *OsHSP24.15*, *OsHSP71.10* and *OsHSP85.88* under drought conditions on rice (C<sub>3</sub> crop), weedy rice (C<sub>3</sub> weed) and barnyardgrass (C<sub>4</sub> weed).

## MATERIAL AND METHODS

The experiment was performed in a greenhouse with temperature control at the Universidade Federal de Pelotas (Capão do Leão, Rio Grande do Sul state, Brazil), between October and November 2015.

The study was conducted in a completely randomized design, with four replications. The two studied factors were water conditions (well-watered and water deficit) and plant species [rice (*Oryza sativa* cv. Puitá), weedy rice (*Oryza* spp.) and barnyardgrass (*Echinochloa* spp.)]. The species were chosen because they have different photosynthetic pathways, and weed rice and barnyardgrass are the main weeds in rice fields.

The plants were sown in trays containing washed sand and, at 7 days after emergence, they were transferred to a hydroponic solution in 4-L pots (12 plants pot<sup>-1</sup>). The hydroponic solution was changed every 5 days (Yoshida 1981), and drought stress (-0.3 MPa) was imposed at 15 days after transplanting (plants with one tiller) by adding polyethylene glycol 6000 (PEG). The stress concentration of -0.3 MPa was determined in a previous study, representing the highest concentration in which the three species remained alive (data not shown). The osmotic potential was calculated according to Michel & Kaufmann (1973):  $\Psi_{os} = - (1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T$ , where  $\Psi_{os}$  is the osmotic potential (bar), C the concentration of PEG in g kg<sup>-1</sup> H<sub>2</sub>O and T the temperature in °C.

The photosynthetic evaluation and sampling of leaf and roots were carried out at 5 days after the stress onset. The leaf materials were stored at -80 °C until the measurement of the variables, and the roots were dried in a forced air circulation oven. The photosynthetic variables were determined using an infra-red gas analyzer (LI-6400), including the photosynthesis rate (A), substomatal concentration CO<sub>2</sub> (C<sub>i</sub>), stomatal conductance (g<sub>s</sub>) and transpiration (E). The intrinsic water-use efficiency (iWUE) and carboxylation efficiency (CE) were calculated using the equations A/E and A/C<sub>i</sub>, respectively. These parameters were measured between 09:00 and 11:00 a.m. (local time). In addition, the CO<sub>2</sub> concentration inside the leaf chamber was set to 400 μmol mol<sup>-1</sup>, the photons fluxes were 1,200 μmol m<sup>-2</sup> s<sup>-1</sup>, and the vapor pressure deficit (VPD) and leaf temperature were the same as those measured in the surrounding atmosphere of the leaf. The measurements were performed on the two youngest, fully expanded leaves of each replicate.

In order to determine the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), the protein samples were quantified by the Bradford method. The APX and CAT activities were determined according to the method described by Azevedo et al. (1998), and the methodology for determining SOD was adapted from Giannopolitis & Ries (1977). The H<sub>2</sub>O<sub>2</sub> content was determined as described by Sergiev et al. (1997), and the cellular damage to tissues by using thiobarbituric acid reactive substances (TBARS) via accumulation of malondialdehyde (MDA), as described by Heath & Packer (1968). The proline concentration was determined according to

the method by Bates et al. (1973). The chlorophyll a (Cha), chlorophyll b (Chb), total chlorophyll (Chtot) and carotenoid (Cr) contents were calculated by the formula of Lichtenthaler (1987).

For molecular evaluation, the RNA was extracted from plant leaves using the reagent PureLinK™ (Plant RNA Reagent-Invitrogen™), following the manufacturer's recommendations. According to the manufacturer's recommendation, the cDNA was obtained using the SuperScript III First-Strand System Kit for RT-qPCR (Invitrogen). Agarose gel electrophoresis (1 %) assessed the quantity and quality. The amount and purity of RNA were determined on the NanoDrop™ 2000 spectrophotometer (Thermo Scientific), with 260/280 nm ratios in the range of 1.9 to 2.2 and 260/230 nm around 2.0, considered acceptable for use in RT-qPCR.

We evaluated the RT-qPCR of the genes target ascorbate peroxidase (*OsAPX2*), heat shock protein 24.15 (*OsHsp24.15*), heat shock protein 71.10 (*OsHsp71.10*) and heat shock protein 85.88 (*OsHSP85.88*) (Table 1). These genes were chosen because they are reported to be abiotic-stress responsive in plants (Ahuja et al. 2010, Ray et al. 2011) and amplified for rice, weedy rice and barnyardgrass (data not shown). The ubiquitin-conjugating enzyme E2 (*OsUBC-E2*) and ubiquitin 10 (*OsUBQ10*) (Table 1) were used for endogenous genes after normalization analysis (data not shown). The PCR efficiency was obtained from four serial dilutions of cDNA (1:1, 1:5, 1:25 and 1:125) to generate a standard curve for each primer pair tested. The *E* value was estimated using the equation

$E = 10^{(-1/\text{slope})}$ . Values between 1.8 and 2.2 are considered acceptable for the endogenous and target genes (data not shown).

For the RT-qPCR, a total volume of 12 µL containing 6.25 µL of LightCycler® 480 SYBR Green I Master (Roche Applied Science), 0.5 µL primer (10 mM), 1 µL cDNA (0.2 µg) and water (to adjust the final volume) was used. It was amplified for one cycle of 95 °C (5 min), followed by 45 cycles of denaturation at 95 °C (20 s), 60 °C (1 min) and 72 °C (20 s), and a final dissociation curve of denaturation at 95 °C (5 s), followed by cooling to 70 °C (1 min) and gradual heating at 0.11 °C steps to 95 °C and cooling 40 °C (30 s) using the LightCycler 480 system (Roche Applied Science). All reactions were performed in triplicate for each cDNA sample. The produced amplicon purity was assumed when a single melting peak was obtained.

Relative expression data were calculated using the  $\Delta\text{Ct}$ , for the equation  $\Delta\Delta\text{Ct} = (\text{Ct target} - \text{Ct endogenous}) - (\text{Ct calibrator} - \text{Ct endogenous})$ , where  $\Delta\text{Ct}$  was the relative gene expression and the calibrator was the well-watered rice treatment, with the result applied in  $\text{QR} = 2^{-(\Delta\text{Ct})}$ . The data were statistically analyzed by two-way Anova ( $p \leq 0.05$ ). In addition, the plant effects were evaluated by the Duncan test ( $p \leq 0.05$ ), and the water condition was assessed by the t-test ( $p \leq 0.05$ ).

## RESULTS AND DISCUSSION

The data showed an interaction between the water conditions and plant species for the photosynthetic variables *A*, *Ci*, *gs*, *E*, *CE* and

Table 1. Primers used a reference and target primer for RTqPCR in rice, weedy rice and barnyardgrass in response to drought stress.

Gene	Forward (5'-3')	Reverse (5'-3')	Reference
<i>OsUBC-E2</i>	CCGTTTGTAGA GCCATAATTGCA	AGGTTGCCTGAG TCACAGTTAAGTG	Jain et al. 2006
<i>OsUBQ10</i>	ACCACTTCGAC CGCCACTACT	ACGCCTAAGC CTGCTGGTT	Jain et al. 2006
<i>OsAPX2</i>	AGAGTCAGT ACGATCAAGAC	TCTTGACAGC AAATAGCTTGG	Zhang et al. 2014
<i>OsHsp24.15</i>	GATCAAGGCG GAGATGAAGAAC	ACTCGACGTT GACCTGGAAGA	Ye et al. 2012
<i>OsHsp71.10</i>	CCGTGTGCTT CGACATTGAC	CGTTGGTGATG GTGATCTTGTT	Ye et al. 2012
<i>OsHsp85.88</i>	ACGGTGACGG AGGTGATTGA	AACAAAGGATGC CCAAGAGAAC	Ye et al. 2012

iWUE (Figure 1). It was possible to observe that the three plants had reduced  $A$  values under drought (Figure 1A). The photosynthetic rate usually decreases during the exposure of the plant to drought stresses, since, in this condition, stomatal closure imposes limitations on photosynthesis by decreasing the availability of  $\text{CO}_2$  (Chaves et al. 2009, Valença et al. 2020). Furthermore, the  $A$  value was higher in the barnyardgrass than for the rice and weedy rice under both the water conditions (Figure 1A).

The  $C_i$  and  $g_s$  of rice and weedy rice decreased under water stress, while they did not change for barnyardgrass (Figures 1B and 1C). The  $C_i$  and  $g_s$  values for barnyardgrass were the lowest among the

three plants under both the water regimes (Figures 1B and 1C). The  $C_i$  and  $g_s$  reduction indicate stomatal closure, which occurs due to decreased leaf turgor and atmospheric vapor pressure along with root-generated chemical signals (Chaves et al. 2009). Therefore, stomatal conductance is the main factor limiting  $\text{CO}_2$  diffusion from the atmosphere to the Rubisco in most cases, and it is responsible for reducing carbon fluxes and lowering the photosynthesis rates in plants, and this resistance to  $\text{CO}_2$  flux depends on the plant species (Akram et al. 2013). Furthermore, a reduction in  $E$  values for the species in this study was observed (Figure 1D), similarly to the results observed in Basmati rice cultivars, where stomatal

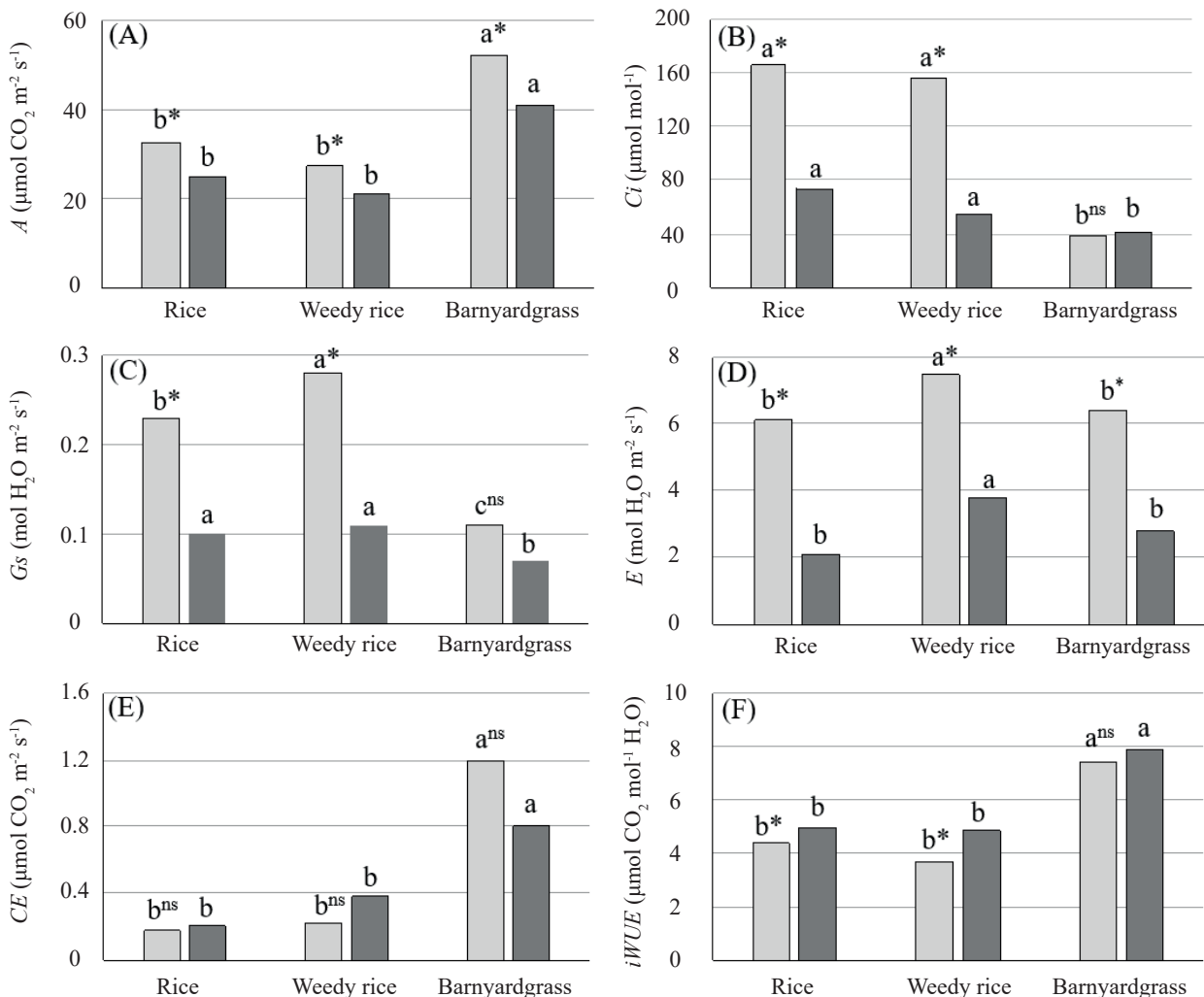


Figure 1. Photosynthesis rate ( $A$ ; A), substomatal  $\text{CO}_2$  concentration ( $C_i$ ; B), stomatal conductance ( $g_s$ ; C), transpiration ( $E$ ; D), carboxylation efficiency (CE; E) and intrinsic water-use efficiency (iWUE; F) in rice, weedy rice and barnyardgrass under water deficit or well-watered conditions. \* or <sup>ns</sup>: means differ or not between the water conditions in the same plant, respectively, according to the t-test ( $p \leq 0.05$ ). Letters compared different plants in the same hydric treatment, according to the Duncan test ( $p \leq 0.05$ ).

closure had inhibitory effects on transpiration (Akram et al. 2013).

As expected, the CE was higher for barnyardgrass ( $C_4$  plant) than for the other plants tested under both the water conditions (Figure 1E). None of the tested plants changed the CE significantly when subjected to water deficit during the evaluation period. The CE did not decrease, despite the reduction in the photosynthesis rate, implying that Rubisco continued to perform carbon fixation, otherwise  $CO_2$  would accumulate in the substomatic cavity. The iWUE increased in  $C_3$  plants subjected to water deficit (Figure 1F). However, the iWUE of most  $C_4$  plants was not affected by this stress. Reports showed that stomata usually close during the initial stages of drought stress, resulting in increased iWUE due to decreased transpiration rate (Chaves et al. 2009, Akram et al. 2013).

In the biochemical analysis, an interaction among the factors was only observed for protein,  $H_2O_2$ , TBARS and root dry matter (Figure 2). The rice and weedy rice plants had the highest protein content under both the water conditions, and had about a 10 % decrease in protein content under water deficit, while

the barnyardgrass plants did not show any change in the protein content when subjected to different water conditions (Figure 2B). Total protein reduction is one of the consequences of drought stress, because ROS causes an oxidation of the protein molecule (Gill & Tuteja 2010). This effect has also been reported for abiotic stress in wheat and rice tissues (Sofu et al. 2015).

The  $H_2O_2$  concentration in the well-watered treatment was higher in rice plants, followed by weedy rice and barnyardgrass (Figure 2C). In plants subjected to water deficit, the rice and weedy rice crops did not show a difference in their  $H_2O_2$  content, while the barnyardgrass showed a lower concentration than the other two plants. The  $H_2O_2$  content increased in all the species when exposed to water deficit. On the other hand, the TBARS concentration showed the opposite behavior to the  $H_2O_2$  concentration. Rice and weedy rice had lower TBARS concentrations than the barnyardgrass under both the drought conditions (Figure 2D). The rice and weedy rice plants had different TBARS levels between plants under water stress and control, increasing around 40 % of TBARS under deficit. The barnyardgrass did not change the

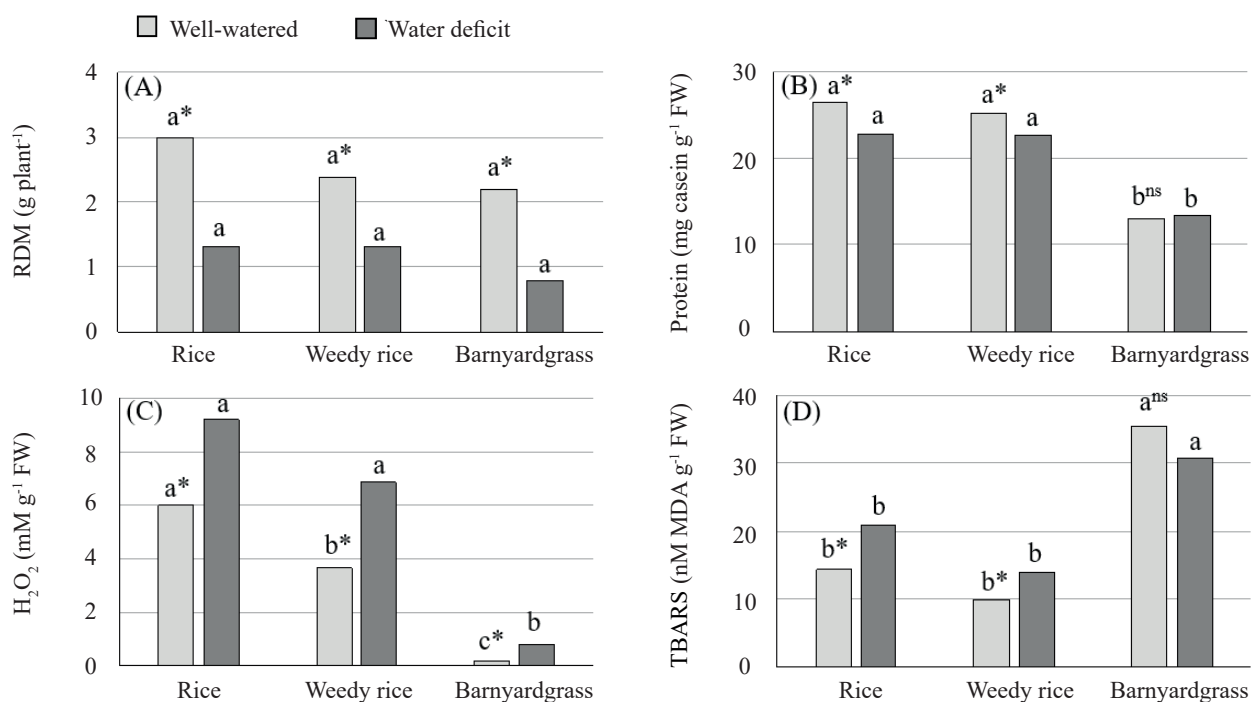


Figure 2. Roots dry matter (RDM; A), protein (B), hydrogen peroxide ( $H_2O_2$ ; C) and thiobarbituric acid reactive substance (TBARS; D) in rice, weedy rice and barnyardgrass under water deficit or well-watered conditions at 5 days of the treatment. \* or <sup>ns</sup>: means differ or not between the water conditions in the same plant, respectively, according to the t-test ( $p \leq 0.05$ ). Letters compared different plants in the same hydric treatment according to the Duncan test ( $p \leq 0.05$ ). FW: fresh weight; MDA: malondialdehyde.



TBARS concentration under water stress conditions, if compared to the control. During environmental stress such as drought, the CO<sub>2</sub> level reduction due to stomatal closure leads to photorespiration and a potential disorder in the electron transport chain, resulting in increased tissue ROS concentration (Faize et al. 2011, Nahar et al. 2018). The ROS levels increased dramatically, triggering an attack on the membrane lipids and increasing lipid peroxidation. In this sense, the drought-induced overproduction of ROS increases the MDA content, which has been considered as an indicator of oxidative damage (Gill & Tuteja 2010).

When evaluating the isolated effects of the factors for other biochemical variables without interaction, the effect of species factor was observed for enzyme activity of CAT, APX and SOD, proline content, chlorophylls a, b and total, and carotenoid content (Table 2). The proline and carotenoid variables showed an isolated effect for the water condition factor (Figure 3). The CAT activity was higher in rice, followed by weedy rice and barnyardgrass (Table 2). The APX enzyme showed a higher activity for rice and weedy rice. However, the barnyardgrass

had SOD activity 2.5-fold higher than for rice and weedy rice. The proline content was about 45 % lower in barnyardgrass than in rice and weedy rice. The chlorophylls a, b, total and carotenoid showed that rice has a greater concentration of chlorophylls a, b and total contents, when compared to weedy rice and barnyardgrass. The carotenoid was higher in rice than in weedy rice and then in barnyardgrass.

The plants subjected to drought accumulated molecules with antioxidant activity, like proline and carotenoid (Figure 3A). The role of proline is to protect from stress by contributing to cellular osmotic adjustment, scavenging free radicals and buffering of the cellular redox potential under stress conditions (Hayat et al. 2012). In wheat, the proline levels in the leaf sheath increases in response to water shortage and play a role as a drought resistance indicator (Karamanos 1995). Likewise, carotene acts as the first line of defense against ROS and is very efficient in quenching <sup>1</sup>O<sub>2</sub>, accumulating in the plants under drought stress (Gill & Tuteja 2010). In this study, although responses of the enzymatic antioxidant system were not verified from the plants under drought conditions, the accumulation of proline

Table 2. Activity of the enzymes catalase, ascorbate peroxide, superoxide dismutase and proline, chlorophyll a, b and total, and carotenoid in rice, weedy rice and barnyardgrass.

Enzymes	Rice	Weedy rice	Barnyardgrass	CV (%)
Catalase	0.34 a <sup>1</sup>	0.24 b	0.02 c	21.31
Ascorbate peroxide	0.24 a <sup>1</sup>	0.18 a	0.08 b	22.16
Superoxide dismutase (AU mg <sup>-1</sup> prot min <sup>-1</sup> )	1.64 b	1.85 b	4.51 a	19.35
Proline (µg proline g <sup>-1</sup> FW)	24.04 a	24.90 a	13.79 b	12.67
Chlorophyll a	2.32 a	1.71 b	1.79 b	15.32
Chlorophyll b	0.82 a	0.67 b	0.68 b	17.29
Total chlorophyll	3.15 a	2.48 b	2.47 b	15.42
Carotenoid (mg g <sup>-1</sup> FW)	0.62 a	0.47 b	0.32 c	16.06

<sup>1</sup>Means followed by the same letter in the row do not differ by the Duncan test (p ≤ 0.05). FW: fresh weight; AU: activity unit.

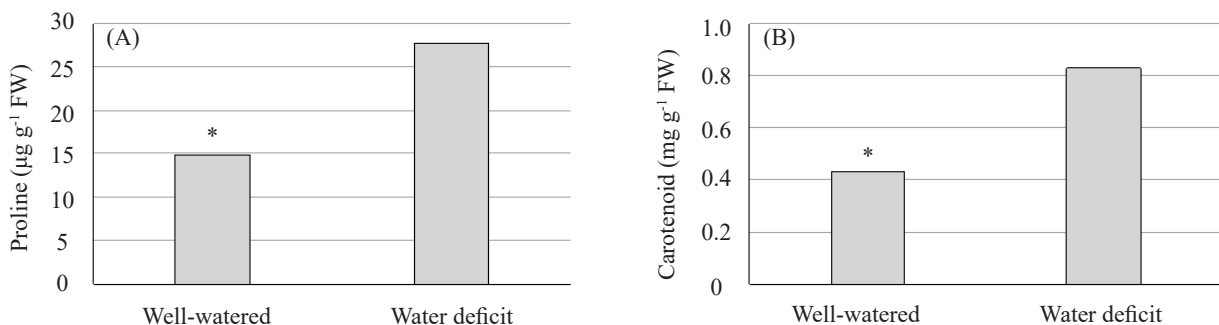


Figure 3. Quantification of the proline (A) and carotenoid (B) levels in well-watered or water deficit plants. \* Means differ between the water conditions according to the t-test (p ≤ 0.05). FW: fresh weight.

and carotenoids was observed, which are part of the non-enzymatic antioxidant system.

An interaction was noted between species and water conditions for expression of the genes *OsAPX2*, *OsHSP24.15*, *OsHSP 71.10* and *OsHSP 85.88* (Figure 4). In both the water conditions, the *OsAPX2* expression was higher for weedy rice (Figure 4A). We observed that the expression in C<sub>3</sub> plants (rice and weedy rice) was higher than in C<sub>4</sub> plants (barnyardgrass). The expression of *APX* is induced by H<sub>2</sub>O<sub>2</sub> (Sofa et al. 2015), what may explain the higher expression in C<sub>3</sub> plants, because a higher concentration of H<sub>2</sub>O<sub>2</sub> was verified in the tissues of these plants than in barnyardgrass (Figure 2C). Rice and barnyardgrass did not have confirmed differences between well-watered or water deficit plants. However, weedy rice plants under drought conditions showed about 4-fold more expression of the *OsAPX2* gene than the well-watered weedy rice plants (Figure 4A). The high expression of *OsAPX2* in rice has been reported as being induced by drought (Rosa et al. 2010). The discrepancy between

our data and other studies could be due to the distinct responses of *APX* in different species and to varying magnitudes of stress.

The *OsHSP24.15* expression in weedy rice plants was higher than for the other plants in both the water conditions (Figure 4B). Only the weedy rice showed a difference for both the water conditions, where the plants subjected to water deficit had 8-fold greater gene expression than well-watered plants. When evaluating the *OsHSP71.10* expression in well-watered plants, rice and barnyardgrass expressed this gene more than weedy rice (Figure 4C). In plants subjected to drought, the weedy rice expressed more *OsHSP71.10* than barnyardgrass, and the rice did not differ from weedy rice and barnyardgrass. The *OsHSP71.10* expression did not change in well-watered or water deficit rice plants. However, the weedy rice showed an increase of about 5-fold when the plants were under drought stress (Figure 4C).

On the other hand, the barnyardgrass showed a 2-fold reduced gene expression when subjected to drought. The *OsHSP85.88* expression in both

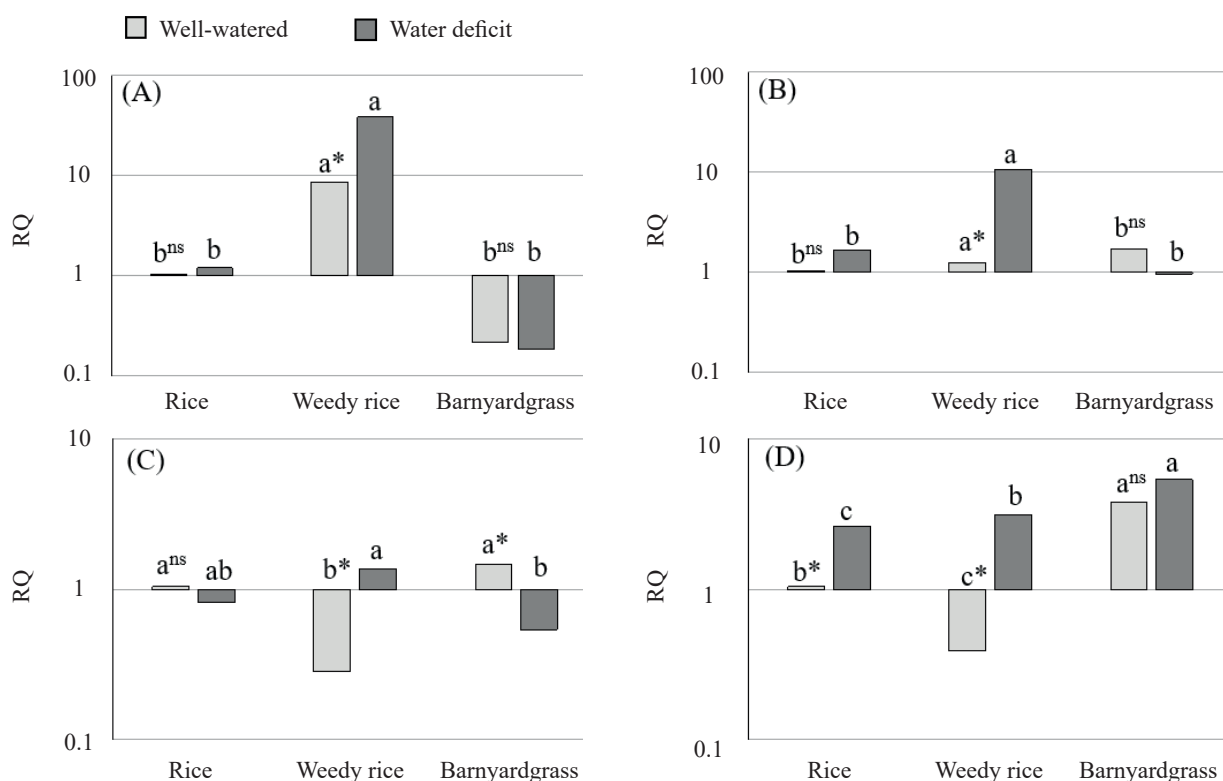


Figure 4. Relative expression (RQ) profiles of *OsAPX2* (A), *OsHSP24.15* (B), *OsHSP71.10* (C) and *OsHSP81.88* (D) in rice, weedy rice and barnyardgrass subjected or not to water deficit. \* or <sup>ns</sup>: means differ or not between water conditions in the same plant, respectively, according to the t-test ( $p \leq 0.05$ ). Letters compared different plants in the same hydric treatment according to the Duncan test ( $p \leq 0.05$ ).

the water conditions was higher for barnyardgrass, followed by weedy rice and rice (Figure 4D). When subjected to drought, the three tested plants showed an increase in the *OsHSP85.88* expression of about 3-, 8- and 1.5-fold for rice, weedy rice and barnyardgrass, respectively.

The response of the *HSPs* gene expression under stress is fast and transient, as the gene expression increases fast to reach the maximum levels after 10-15 min (Sung-Ryul & An 2013). Nevertheless, some genes peak slightly later (after 20 min), and others do not increase until after 2 h (Matsuura et al. 2010). However, the expression kinetics for individual stress-inducible genes is quite diverse when analyzed in detail. In this way, each plant presents an expression pattern of different *HSPs* that varies according to the development stage, tissue, type of stress and exposure time to this stress. Moreover, *HSPs* were associated with thermotolerance in plants (Amano et al. 2012). Analyzing the three tested plants, it was verified that the water deficit stimulated the expression of the *OsHSP81.88* gene in all these plants, being possible to use it as a stress marker in later studies (Figure 4D).

In contrast to the physiological variables, the rice and weedy rice plants differed in gene expression of protein responsive to drought stress. The evaluated genes expressions were more responsive to drought for weedy rice than rice. Weedy rice under drought conditions increased the expression of the genes *OsAPX2*, *OsHSP24.15*, *OS71.10* and *OsHSP81.88*. In rice, only *OsHSP81.88* was expressed more under drought conditions. Despite this difference in gene expression up to five days under drought conditions, it was impossible to observe differences between rice and weedy rice, regarding reducing the damage caused by stress, because the genetic responses are faster than the physiological ones.

Nevertheless, it was observed in both the stressed plants a decrease of approximately 10 % of proteins and an increase in 40 % of TBARS, when compared to the control (Figures 2B and 2C). However, for barnyardgrass, there was no evidence of stomatal closure, since the gs reduction in plants under water deficit did not happen. At the same time, there was a decrease in the photosynthetic rate of stressed barnyardgrass, but this plant continued about 40 % more photosynthetically efficient than the  $C_3$  tested (Figure 1). The water deficit caused an increase in the  $H_2O_2$  content for barnyardgrass,

but this increase seems to be more connected to cell signaling than to oxidative damage (Figure 2C). There was no increase for TBARS or reduction for protein in barnyardgrass (Figure 2C).

## CONCLUSIONS

1. Rice, weedy rice and barnyardgrass show a reduced photosynthetic rate under drought conditions, mainly due to stomatal closure;
2. Under drought conditions, rice and weedy rice show more cellular damage than barnyardgrass.  $C_3$  plants (rice and weedy rice) increase the damages to lipids and proteins at five days of drought. However, for the  $C_4$  plant (barnyardgrass), the drought does not affect the biochemical parameters;
3. The expression of the *OsHSP85.88* gene increases in rice, weedy rice and barnyardgrass exposed to water deficit.

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