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Article

PHYSIOLOGICAL AND MOLECULAR RESPONSES IN RICE, WEEDY RICE AND BARNYARDGRASS EXPOSED TO SUPRA-OPTIMAL TEMPERATURES

Respostas Fisiológicas e Moleculares em Arroz, Arroz-Vermelho e Capim-Arroz Exposto a Temperatura Supraótimas

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ABSTRACT - The global temperature to rise 0.3 to 4.8 °C to century. Supra-optimal temperatures can affect plants at different organizational levels, causing morphological, physiological, biochemical and gene expression alterations. Rice, weedy rice, and barnyardgrass may to response differently when subjected to supra-optimal temperatures. Thus, the aimed at determining the physiological response and expression of the genes *APX2*, *HSP24.15* e *HSP71.10* in rice, weedy rice, and barnyardgrass when in to supra-optimal temperatures. A greenhouse experiment was conducted in randomized complete desing with four repetitions, with a factorial combination of temperature x plantas, where: factor A consisted of two temperatures (25 °C and 40 °C); and factor B of three plants [rice (*Oryza sativa* cv. Puitá INTA-CL), weedy rice (*Oryza* spp.), and barnyardgrass (*Echinochloa* spp.)]. The 40 °C temperature, in general, caused a reduction in the photosynthesis parameters and in the protein content, and increased the oxidative stress in C3 plants; no damage was observed in the C4 plant subjected to this temperature. In response to the supra-optimal temperatures, rice and weedy rice increased of APX and SOD activity and the expression of Os*APX2*, Os*HSP24.15* and Os*HSP71.10* genes. Barnyardgrass exposed to supra-optimal temperature do not modify the activity of its antioxidant system and increased the Os*HSP71.10* gene expression.

Keywords: *Oryza sativa*, *Echinochloa* spp., photosynthesis, oxidative stress, gene expression.

RESUMO - A temperatura média global pode aumentar de 0,3 a 4,8 °C até o final do século. Temperaturas supraótimas podem afetar as plantas em diferentes níveis organizacionais, causando alterações morfológicas, fisiológicas, bioquímicas e na expressão de genes, e plantas de arroz, arroz-vermelho e capim-arroz tendem a responder de forma distinta quando submetidas a temperaturas elevadas. Assim, os objetivos do estudo foram determinar as respostas fisiológicas e a expressão dos genes OsAPX2, OsHSP24.15 e OsHSP71.10 em arroz, arroz-vermelho e capim-arroz quando submetidos a temperaturas supraótimas. Para isso, foi conduzido experimento em casa de vegetação, utilizando delineamento completamente casualizado com quatro repetições, em esquema fatorial. O fator A constou de duas temperaturas (25 °C e 40 °C) e o fator B, de três plantas [arroz (Oryza sativa cv. Puitá INTA-CL), arroz-vermelho (Oryza spp.) e capim-arroz (Echinochloa spp.)]. Em geral, a temperatura de 40 °C causa redução na fotossíntese, nas trocas gasosas e no teor de proteínas, além de aumento do estresse oxidativo nas plantas C3 testadas, sem efeito negativo sobre a planta C4 submetida a essa temperatura. Em resposta à alta temperatura, o arroz e arroz-vermelho aumentam a atividade das

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enzimas APX e SOD e a expressão dos genes OsAPX2, OsHSP24.15 e OsHSP71.10; no capim-arroz, apenas ocorreu aumento na expressão do gene HSP71.10.

Palavras-chave: *Oryza sativa*, *Echinochloa* spp., fotossíntese, estresse oxidativo, expressão gênica.

INTRODUCTION

Rice is the most important crop worldwide in terms of direct consumption, used as staple food for over 50% of the world's population. As it is a C3 plant grown in the summer, in tropical and subtropical regions, it is extremely vulnerable to damage caused by high temperatures. Studies show that global mean temperature may rise by 0.3 to 4.8 ºC to the end of the century (IPCC, 2017). Increase in the both, average temperature and extreme temperature episodes for short periods cause negative effects on crop growth and development (Ashraf and Harris, 2013).

In addition to abiotic stresses, the biotic stresses such as weeds presence influence the rice development. The two main weeds in rice fields in southern Brazil are weedy rice (*Oryza* spp.) and barnyardgrass (*Echinochloa* spp.). Weedy rice, as well as cultivated rice, is a C3 plant, standing out as the main weed infesting paddy rice cultivation, which can cause a 20-35%, reduction in crop productivity, according to the level of infestation in the area (Marchesan et al., 1994). Barnyardgrass is a highly competitive C4 plant that can cause losses up to 90% in rice fields, in view of its adaptation to flooded environments, large seed production and fast growth (Andres et al., 2007).

The plant photosynthetic pathway directly interferes with responses to abiotic stresses. The C4 photosynthesis mechanism seems to have evolved as one of the main mechanisms of carbon concentration in terrestrial plants in order to minimize RuBisCO's oxygenase activity and, consequently, carbon loss associated with photorespiration (Ashraf and Harris, 2013). Nonetheless, high temperatures can affect both C3 and C4 plants at different levels, causing morphological, physiological, biochemical and gene expression changes (Wahid et al., 2007).

Physiologically, exposure to supra-optimal temperatures may have direct and indirect effects on the photosynthetic rate, and changes in the photosynthesis parameters have been shown to be good indicators of thermo-tolerance (Wahid et al., 2007). Besides reducing photosynthesis, high-temperature stress can cause loss of plasmatic membrane integrity, denaturation and inhibition of the proteins photosynthicals synthesis, enzyme inactivation and degradation of nucleic acids. This damage is partly due to an increase in oxidative stress caused provoked by a greater production of reactive oxygen species (Noctor et al., 2016). In order to fight against them, plants have enzymatic and non-enzymatic antioxidant systems, and the ability to minimize the effects of oxidative stress depends on the efficiency of their antioxidant system, which varies according to species and also between genotypes (Noctor et al., 2016).

Immediately after exposed to high temperature conditions, plants show significant changes at the molecular level, including changes in gene expression and transcripts accumulation, thus leading to the synthesis of stress tolerance-related proteins. APX enzymes are encoded by a multigenic family; the APX2 isoform is very responsive to abiotic stress, as a 15-fold increase in OsAPX2 gene expression in rice exposed to water deficit is observed (Rosa et al., 2010). Seen in these terms, the production of heat shock proteins (HSPs) is known as an important plant adaptive strategy; plants HSPs comprise five classes, according to their approximate molecular weight. Previous studies have shown that *OsHSP24.15* and *OsHSP71.10* genes the expression increased when rice plants are subjected to water deficit and temperature rise (Grigorova et al., 2011; Ye et al., 2012).

Exposure to supra-optimal temperatures affects plants at various organizational levels, but changes in each species, or even within-species plant genotypes, may be different when caused by this type of stress. Thus, rice, weedy rice and barnyardgrass tend to show different physiological and molecular responses when subjected to abiotic stresses. The hyphotesis of the present study is that plants with a C3 photosynthetic pathways, such as rice and weedy rice, when submitted to supra-optimal temperatures, have a major damege in physiological and molecular variables in comparison with C4 plants, such as barnyardgrass. Hence, this study aimed at

determining the physiological responses and expression of the *OsAPX2*, *OsHSP24.15* and *OsHSP71.10* genes in rice, weedy rice and barnyardgrass when subjected to supra-optimal temperatures.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse between November and December 2014. The study was conducted in randomized complete desing with four repetitions, with a factorial combination of temperature x plantas. Factor A consisted of two temperatures (25 °C and 40 °C), and factor B three plants: rice (*Oryza sativa* cv. Puitá INTA-CL), weedy rice (*Oryza* spp.) and barnyardgrass (*Echinochloa* spp.).

The experimental units consisted of 750 mL pots, filled with soil from rice field, classified as Solodic eutrophic Hydromorphic Planosol. The seeds of each tested plant were sown in trays with commercial soil GerminaPlant®, and the plants were transplanted to the experimental units 7 days after emergence (DAE), the final population was one plant per pot.

The plants grew up until the 30DAE in a greenhouse with a plastic cover and plastic side strips. Where the climatic conditions were: relative humidity of 75%, and mean temperature of 25 °C. They were transferred to 4 m^2 greenhouses, made from the same type of plastic used in the the previous ones, so that the treatments were applied. The temperature inside the greenhouse was controlled by an automated system; it should be noted that the temperatures during the treatments remained constant day and night. The evaluations and the collection of vegetal tissues happened 28 hours after the transfer to the greenhouse at different temperatures. 28h was determined in a previous experiment. The evaluation time was at 11 a.m. (local time).

The variables analyzed were the following: photosynthesis rate (A), stomatal conductance (Gs), substomatal concentration CO_2 (Ci) and transpiration (E); physiological water effective use (iWUE)) was calculated by the net photosynthesis/transpiration rate ratio. The Infra-Red Gas Analyzer-IRGA, LI-COR, LI-6400 model, was used in the evaluation. Measurements were carried out on the middle third of the last fully expanded leaf. The concentration of CO_2 within the chamber was set up to 400 µmol mol^{-1}, photon flux were 1,200 µmol m^{-2} s^{-1} and and the vapor pressure deficit (VPD). These values were obtained in a previously light and CO_2 curves. Subsequently, tissue samples were collected for biochemical and gene expression analyses, the plant tissues were stored at -80 °C until the analysis.

The activity of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), the protein of the samples was first quantified using the Bradford method (1976). CAT and APX activity was determined according to Azevedo et al. (1998), and SOD activity was determined according to a methodology adapted from Giannopolitis and Ries (1977).

The hydrogen peroxide (H_2O_2) teor was quantified as described by Sergiev et al. (1997), and thiobarbituric acid reactive species (TBARS), via malonic aldehyde accumulation (MDA), as described by Heath and Packer (1968) methodology. The proline content in the tissues was determined according to a Bates et al. (1973). Chlorophyll and total carotenoid contents were calculated by the Lichtenthaler formulas (1987).

For gene expression determination, RNA was extracted from the plant tissue using PureLinK ™ reagent (Plant RNA Reagent - In0vitrogen ™), following the manufacturer's instructions. The quality and quantity of this RNA was determined using the spectrophotometer NanoDrop™ 2000 (Thermo Scientific). The quality of RNA was confirmed by agarose gel electrophoresis (1%). The cDNA was constructed using a commercial SuperScript III First-Strand system kit (Invitrogen™), according to the manufacturer's instructions.

Expression of ascorbate peroxidase 2 target genes (*OsAPX2*); "heat shock protein" 24.15 (*OsHsp24.15*) and "heat shock protein" 71.10 (*OsHsp71.10*) were performed (Table 1). Ubiquitin E2 (OsUBC-E2) and β-tubulin (β-tub) genes (Table 1) were chosen as endogenous after a normalization analysis (data not shown). PCR efficiency for each primer pair was obtained by analyzing four serial dilutions of the cDNA (1: 1, 1: 5, 1:25 and 1: 125) to generate the standard curve. E value was estimated by the equation $E = 10^{(-1/slope)}$, with values between 1.8 and 2.2 considered acceptable for reference and target genes. It should be noted that 35 pairs of primers

Table 1 - Primers used for RT-qPCR in rice, weedy rice and barnyardgrass subjected to temperatures of 25 °C and 40 °C

Gene	Forward (5'-3')	Reverse (5'-3')	Reference
Osβ-Tubulina	GCTGACCACAC CTAGCTTTGG	AGGGAACCTT AGGCAGCATGT	(Zhang e Hu, 2009)
OsUBC-E2	CCGTTTGTAGAG CCATAATTGCA	AGGTTGCCTGAGT CACAGTTAAGTG	(Zhang e Hu, 2009)
OsAPX2	AGAGTCAGT ACGATCAAGAC	TCTTGACAGC AAATAGCTTGG	(Zhang et al., 2014)
OsHsp24.15	GATCAAGGCG GAGATGAAGAAC	ACTCGACGTT GACCTGGAAGA	(Ye et al., 2012)
OsHsp71.10	CCGTGTGCTT CGACATTGAC	CGTTGGTGATG GTGATCTTGTT	(Ye et al., 2012)

were tested, which included several genes from the ascorbate peroxidase families, "heat shock protein", reported as responsive to thermal stress. However, only the genes that were chosen and/or analyzed showed an efficient level within the acceptable value for the three plants evaluated.

Amplification reactions were performed using a total volume of 12 µL containing 6.25 µL LightCycler® 480 SYBR Green I Master (Roche Applied Science), 0.5 µl primer (10 mM); 1 µL cDNA (0.2 µg) and 4.25 µL water. Amplification conditions were the following: one cycle at 95 °C (5 minutes) and 45 cycles of denaturation at 95 °C (20 seconds), 60 °C (60 seconds) and 72 °C (20 seconds). The procedure was completed with the generation of a dissociation curve through denaturation at 95 °C (5 seconds), cooling at 70 °C (1 minute), a gradual heating at 0.11 °C steps up to 95 °C (5 seconds), and cooling at 40 °C (30 seconds). RT-qPCR was performed using a LightCycler 480 system (Roche Applied Science). All reactions were performed in triplicate for each cDNA sample, and the purity of the amplicon was determined when a single melting peak was produced.

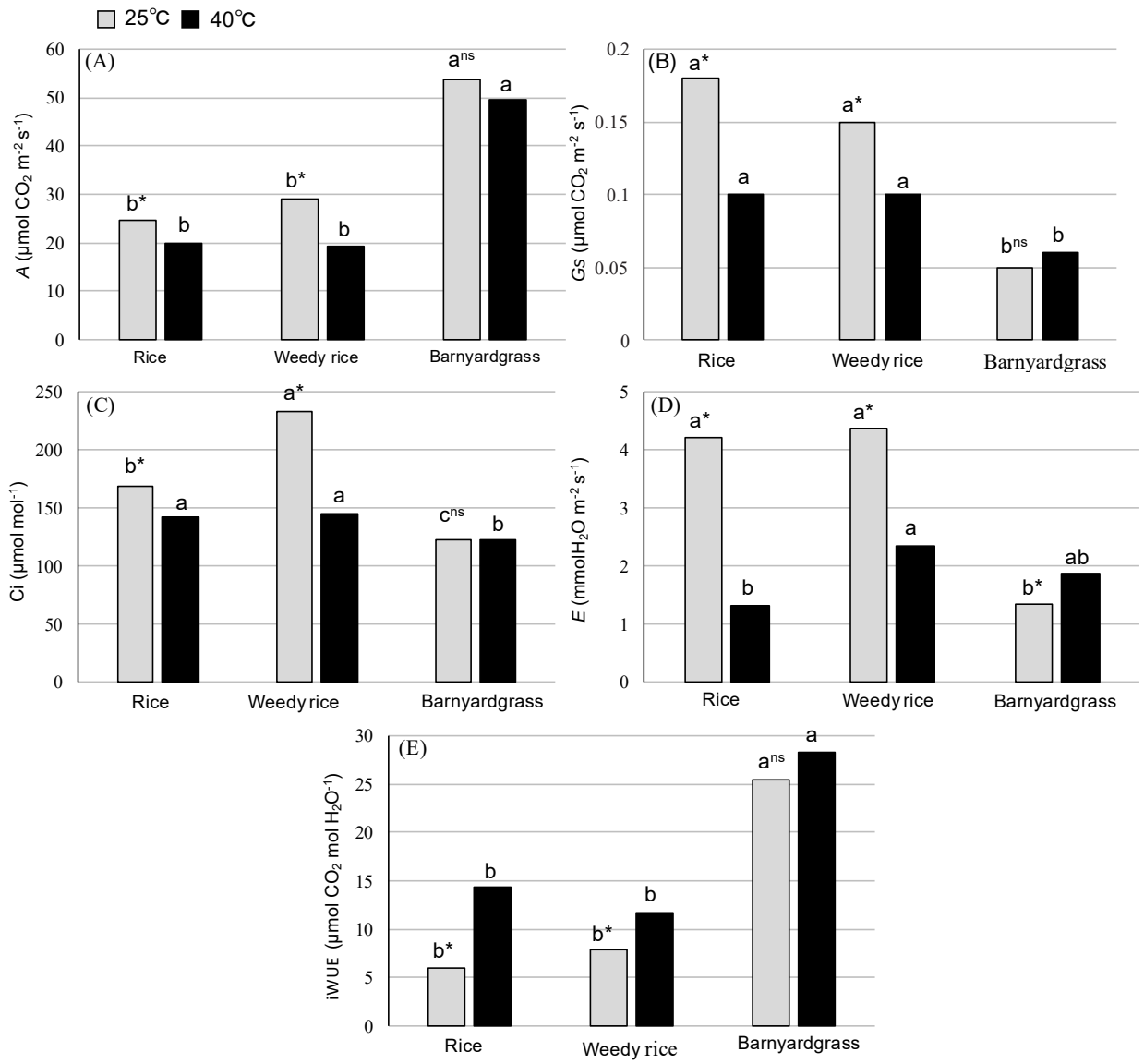
For relative genes quantification of, the calculation of relative expression was carried out using the ΔCt method, by the equation ΔΔCt = (Ct target – Ct endogenous) - (Ct calibrator – Ct endogenous), where ΔΔCt corresponds to the relative expression of the gene and the calibrator corresponds to rice treatment at 25 °C; the application of the result in $2^{-(\Delta\Delta Ct)}$ determined the variation dimension.

The study data were analyzed for normality and homoscedasticity and, afterwards, subjected to variance analysis ($p \leq 0.05$). Duncan's test was applied to determine the plant effect ($p \leq 0.05$) and the the t test was used to calculate the temperature effect ($p \leq 0.05$). Correlation between the study variables was analyzed using the Pearson's correlation coefficient ($p \leq 0.05$), as it measures the correlation degree between two variables. Pearson® correlation coefficient ranges from -1 to 1, the sign indicates the positive or negative direction of the relationship and the value suggests the strength between the variables.

RESULTS AND DISCUSSION

Interaction between temperature and plant factors for the variables *A*, *Gs*, Ci, *E* and iWUE (Figure 1) was observed. Rice and weedy rice reduced *A*, *Gs*, Ci and *E*, when subjected to hight temperature; on the other hand, iWUE increased in these two species in this situation. *E* was the only variable affected by temperature change in barnyardgrass, with an increase of 28% in plants subjected to 40 °C, when comparing than plant in the 25 °C temperature.

A similar photosynthetic behavior was observed in rice and weedy rice plants; in a 40 °C temperature of *A* showed a reduction of 20 and 33%, respectively (Figure 1A), in contrast to their reaction when subjected to 25 °C temperature of. Previous studies have shown that rice and wheat plants subjected to supra-optimal temperatures in the vegetative phase show a decrease



* Or ns indicate that means may differ or not between temperatures for the same plant according to the t test ($p \leq 0.05$). Letters compare different plants regarding temperatures according to Duncan's test ($p \leq 0.05$). FW = fresh weight; UA = active unit.

Figure 1 - (A) Liquid photosynthesis (a), (B) stomatal conductance (Gs), (C) substomatal CO_2 concentration (Ci), (D) transpiration rate (e) and (E) efficiency in the physiological water effective use (iWUE) in rice, weedy rice and barnyardgrass subjected to temperatures of 25 and 40 °C.

in their photosynthetic rate, leading to a reduction in the production of photoassimilate compounds and, consequently, a lower yield (Wahid et al., 2007; Barnabás et al., 2008). The ideal average temperature for rice growth and development in the vegetative phase is 25 °C, and the maximum temperature supported by the rice crop is 45 °C (Wahid et al., 2007). This fact explains the reduction in the photosynthesis processes of these plants when they are subjected to the highest temperature.

The *A* changes observed among C3 plants in response to hight temperature can be attributed to both stomatal and non-stomatal factors, and these processes have great inter-species variation (Barnabás et al., 2008). It was observed that in the C3 plants stomatal closure occurred when they were exposed to a supra-optimal temperature, since there was a reduction in *Gs* (Figure 1B). Stomatal control is an important physiological property by which plants limit water loss, decreasing stomatal conductance and, generally, affecting gas exchange. A reduced stomatal opening also caused a decrease in the *E* value (Figure 1D), and a single partial reduction of the

stomatal opening limits the transpiration rate more than the entry of CO_2 through stomata (Azcón-Bieto, 1983).

The transpiratory reduction causes increase in leaf temperature, since one of the main mechanisms used by plants for energy dissipation is the loss of latent heat through water evaporation. The stomatal closure is a typical behavior observed among plants subjected to to supposedly moderate temperatures, i.e., between 30 and 40 °C. Wheat and barley plants showed a 34-64% reduction in *Gs* when subjected to 40 °C temperatures (Rollins et al., 2013).

A lower *E* leads to an increase in iWUE, as occurred in rice and weedy rice (Figure 1E), since a smaller amount of water is exhaled to produce a certain quantity of dry matter mass, a fact indicating that a temperature of 40 °C caused moderate heat stress in rice and weedy rice plants. Moderate stresses cause suppression of mesophyll conductance and stomatal closure, whereas in severe stress the plants increase *E* for leaf cooling (Barnabás et al., 2008).

The rice and weedy rice In both temperatures, plants displayed a lower *A* and a higher *Gs* and *Ci* in comparison with the barnyardgrass (Figures 1A, B, C). At 25 °C, C3 plants had higher *E* than C4 plants; however, at 40 °C the weedy rice showed and increased in *E* value; and rice, the lowest; finally, barnyardgrass did not differ from the other species (Figure 1D). iWUE was higher in barnyardgrass than in the other species at both temperatures (Figure 1E).

We observed that the increase in temperatude had no effect on the photosynthetic variables of barnyardgrass plants; as expected, barnyardgrass presented lower *Gs*, *Ci* and *E*, as well as higher *A* and iWUE, than rice and weedy rice. These responses are inherent characteristics of plants with distinct photosynthetic pathways.

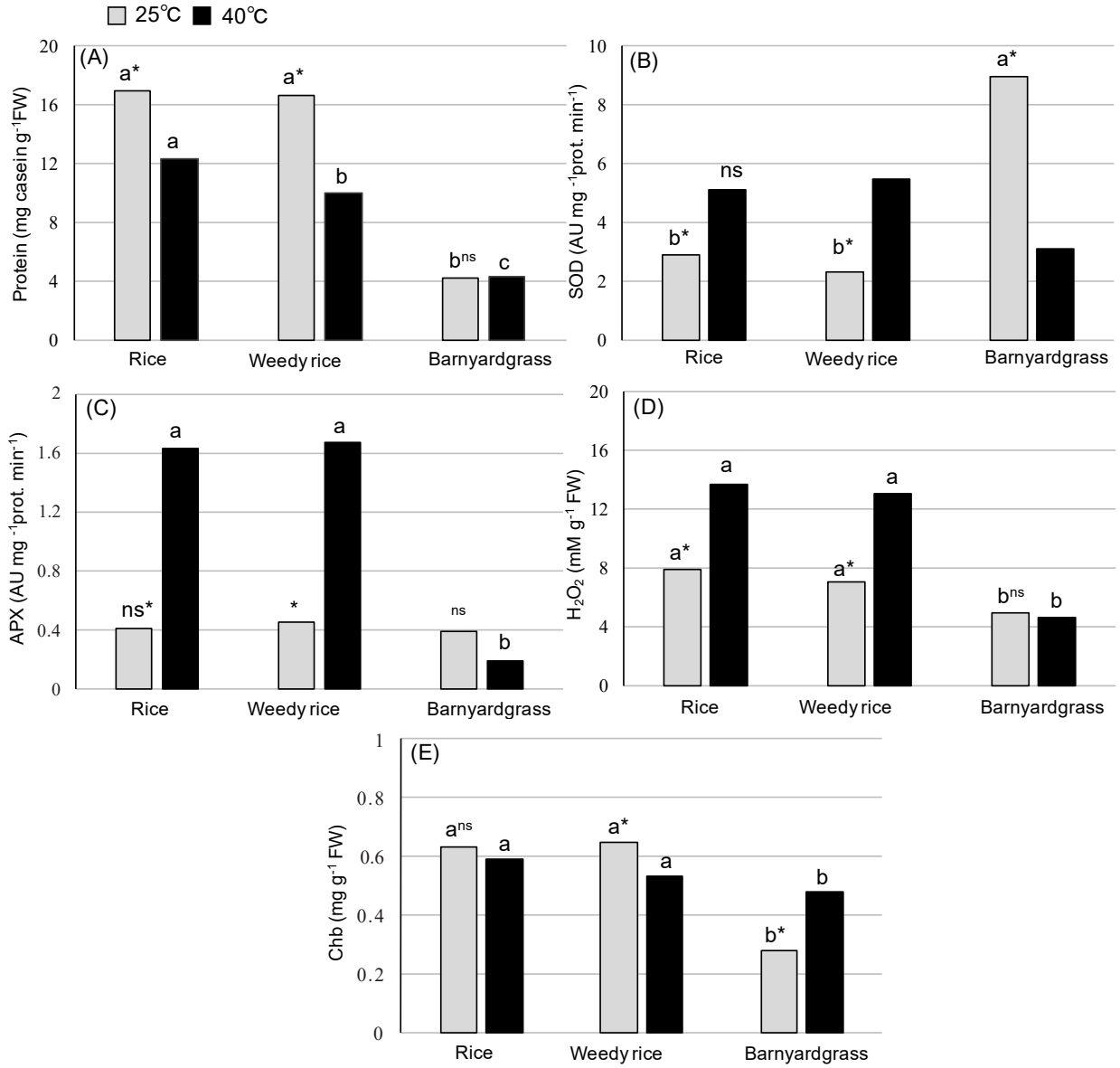
Rice and weedy rice plants reduced the protein concentration in the tissue when subjected to high temperatured, but the barnyardgrass did not present changes in the protein concentration between temperatures (Figure 2A). At both temperatures, the rice plants showed the highest protein levels, and the barnyardgrass the lowest (Figure 2A). When comparing the temperatures, the reduction of the total proteins teor in weedy rice at 40 °C was more pronounced than in rice, reaching 40%; in rice, the reduction reached 28%. Proteins are fundamental in every aspect of cellular structure and function; reduction in their concentration may represent a great damage to plant growth and development.

C3 plants when exposed to supra-optimal temperatures resulted in higher SOD activity: around 43 and 58% in rice and weedy rice, respectively (Figure 2B). Contrary to what was observed in C3 species, SOD activity decreased in barnyardgrass exposed to a high temperature (Figure 2B); however, a reduction in SOD activity in response to supra-optimal temperatures has already been observed in corn, another species with a C4 photosynthetic route (Hu et al., 2010). On the other hand, studies show that the exposure of C3 plants, such as tomato, wheat and rice (Kumar et al., 2014) to supra-optimal temperatures stimulated SOD activity, data in consonance with those found in the present study.

The rise in SOD activity in C3 plants was accompanied by an increment in the APX activity. It was observed an increase of 75 and 73% in rice and weedy rice, respectively (Figure 2C). APX acts to remove the H_2O_2 resulting from SOD activity transforming it into water. APX enzyme has a high affinity for its substrate, and normally acts when H_2O_2 in medium or low concentrations; by contrast, CAT enzyme acts when H_2O_2 is found at high concentrations in the tissues (Gill and Tuteja, 2010). A temperature of 40 °C also stimulated APX activity in rice, corn and wheat, but a temperature of 45 °C reduced the activity of this enzyme in these plants (Kumar et al., 2014).

An increase of 42 and 46% in the H_2O_2 concentration was observed in leaf tissues of rice and weedy rice when exposed to a 40 °C temperature, respectively. This increase, besides being caused by the higher SOD activity, can be explained by the fact that photorespiration is one of the main sources of H_2O_2 in photosynthetic cells, particularly the glycolate oxidation. This also explains a higher concentration of H_2O_2 in C3 plant tissues in comparison with barnyardgrass. It was found that, despite the increase in the activity of the APX enzyme in the C3 plants, this was not enough to reduce the H_2O_2 in the tissues of these plants.

Environment temperature did not change the concentration of the Chb in rice plants; in the case of weedy rice, a reduction caused by an increase in temperature was observed; and for

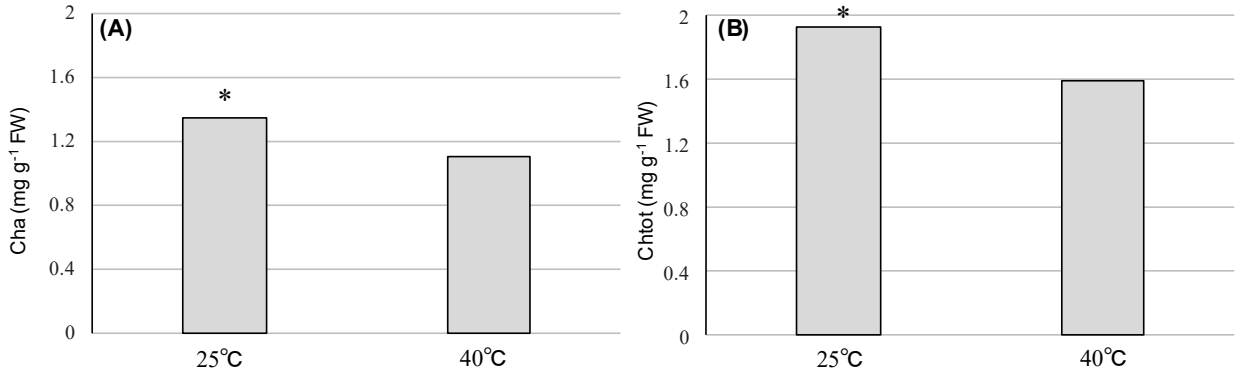


* Or ns indicate that means may differ or not between temperatures for the same plant according to the t test (p≤0.05). Letters compare different plants regarding temperatures according to Duncan's test (p≤0.05). FW = fresh weight; UA = active unit.

Figure 2 - Total protein content (A), activity of superoxide dismutase enzyme (SOD) (B), ascorbate peroxidase enzyme (APX) (C), hydrogen peroxide (H_2O_2) (D) and chlorophyll b content (Chb) (E) in rice, weedy rice and barnyardgrass subjected to temperatures of 25 and 40 °C.

barnyardgrass, an increase was observed when the temperature rose (Figure 2E). Rice and weedy rice plants displayed a higher Chb concentration than barnyardgrass in both temperatures. In general, the elevated temperature led to a reduction in Cha and Chtot in leaf tissues of plants exposed at 40 °C (Figure 3). The reduction in the concentration of photosynthetic pigments results in a decrease in photosynthesis (Wahid et al., 2007). Chlorophyll degradation during high temperature stress has been reported in wheat, barley and corn (Reda and Mandoura, 2011). In rice, heat-tolerant genotypes kept the chlorophyll content for longer than in susceptible genotypes (Jagadish et al., 2010).

Rice and weedy rice revealed a higher activity of CAT and TBARS and increased levels of proline, Cha and Chtot than barnyardgrass, regardless of the temperatures, with no difference between them (Table 2). High concentrations of these biomolecules are related to the inherent features of each species and cannot be related to the higher oxidative stress observed in these plants, considering that even at 25 °C C3 plants have a higher concentration of these molecules.



* Or ns indicate that means may differ or not between temperatures according to the t test (p≤0.05). FW = fresh weight.

Figure 3 - Average chlorophyll a concentration (Cha) (A) and total chlorophylls (Chtot) (B) in rice, weedy rice and barnyardgrass subjected to temperatures of 25 and 40 °C.

Table 2 - Catalase enzyme activity (CAT), thiobarbituric acid reactive species (TBARS), proline, chlorophyll a (Cha) and total chlorophylls (Chtot) in rice, weedy rice and barnyardgrass, irrespective to the temperature evaluated

	Rice	Weedy rice	Barnyardgrass	CV (%)
CAT (AU mg^{-1} prot. min^{-1})	0.13 a	0.12 a	0.06 b	21.74
TBARS (nM MDA2 g^{-1} de FW)	17.55 a	17.65 a	14.65 b	18.01
Proline (mg proline g^{-1})	0.06 a	0.06 a	0.04 b	23.45
Cha (mg g^{-1} FW)	1.64 a	1.24 a	1.10 b	17.98
Chtot (mg g^{-1} FW)	2.25 a	1.87 a	1.45 b	17.41

Means followed by the same lower case letters do not differ according to the Duncan's test (p≤0.05). FW = fresh weight; UA = active unit.

In thermal stress situations, there was no difference between the photosynthetic capacity and the oxidative damage of the C3 plants tested, although some studied reported that weedy rice shows a greater efficiency in the use of CO_2 and nitrogen, important components related to photosynthesis, in comparison to cultivated rice (Gealy et al., 2012). Barnyardgrass plants were not adversely affected by the temperature of 40 °C. C4 plants usually have a higher photosynthetic efficiency in comparison with C3 plants, especially in hot arid environments and under high incidence of light.

Regarding the analysis of gene expression, there was increase by approximately 2.5 and 2 folds in the OsAPX2 gene expression for rice and weedy rice plants, respectively, when exposed to a 40 °C temperature in comparison than exposure to 25 °C, a finding that was not observed in barnyardgrass (Figure 4A). The highest OsAPX2 gene expression occurred in weedy rice and the lowest in barnyardgrass in both temperatures (Figure 4A). The expression in weedy rice plants stands out, as it was 30 and 64-folds higher than in the control treatment (rice at 25 °C), when exposed to temperatures of 25 and 40 °C, respectively.

The higher expression of the OsAPX2 gene reflected in the greater activity of the APX enzyme in rice and weedy rice plants (Figure 2C). This increase occurs due to a higher concentration of H_2O_2 in the leaf tissues of C3 plants (Figure 2D), as the the expression of *APX* genes in several species is positively regulated by the concentration of H_2O_2 (Said et al., 2011). In a similar study, it was observed that the high temperature induced APX2 gene expression in *Arabidopsis thaliana*, and the increase in the expression of this gene was related to the tolerance to thermal stress in this species (Matsuura et al., 2010).

The expression of the *OsHSP24.15* gene in the three plants was affected by the difference in temperature, as it was found that rice and weedy rice increased the expression of the gene by 7 and 1.5-folds when exposed to high temperature, respectively; on the other hand, in barnyardgrass the gene expression under the same condition dropped by 80% (Figure 4B). At 25 °C, rice was the plant that least expressed the *OsHSP24.15* gene, rice expressed more this gene than weeds

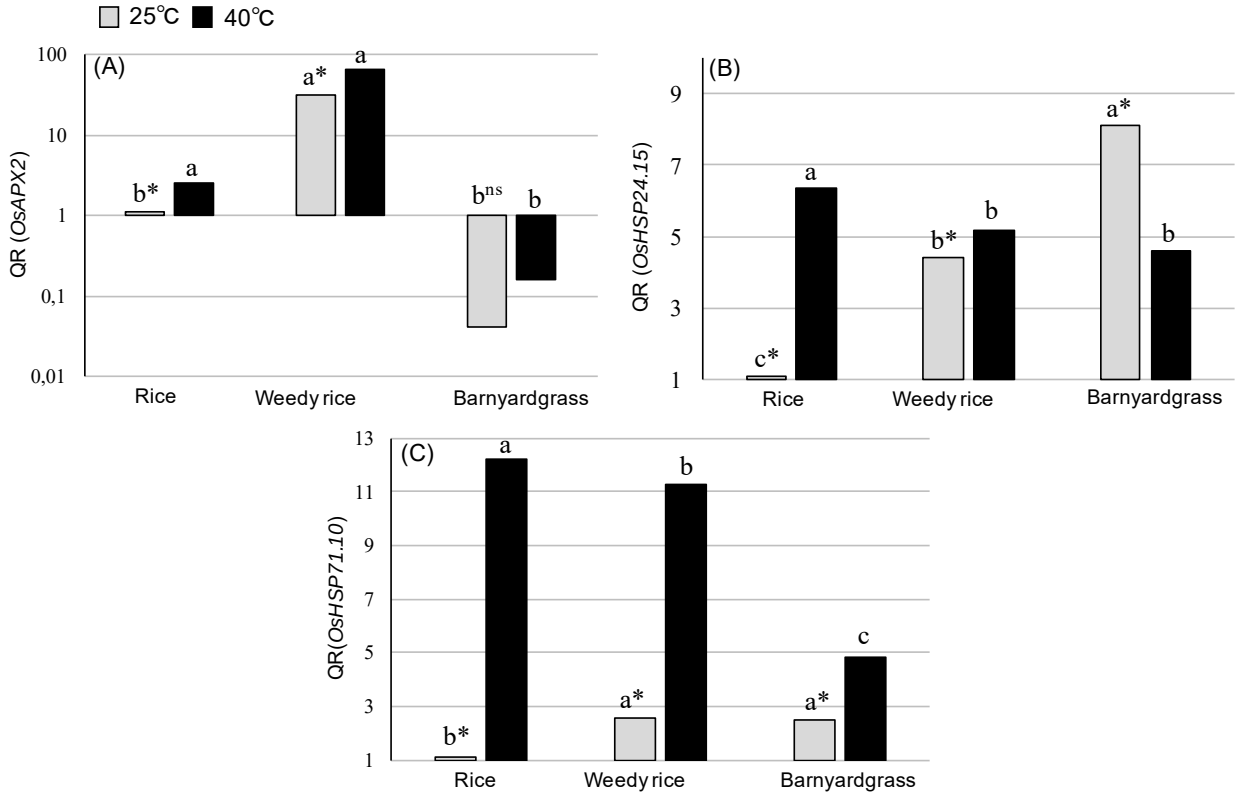


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Figure 4 - Relative quantification (QR) of the (A) gene OsAPX2, (B) gene OsHSP24.15 and (C) gene OsHSP71.10 expression in rice, weedy rice and barnyardgrass subjected to temperatures of 25 and 40 °C.

(Figure 4B). Similar results were observed for rice subjected to 42 °C, where the expression of the *OsHSP24.15* gene increased significantly three hours after treatment (Ye et al., 2012).

HSP24.15 protein belongs to the family known as *small heat shock proteins* (sHSPs). The induction of sHSP gene expression and accumulation of these proteins in plants subjected to environmental stress indicates that they play an important role in stress tolerance, especially in thermal stress. Hence, the increased expression of the *OsHSP24.15* gene in rice and weedy rice plants can be considered a physiological defense mechanism. The induction of *sHSPs* in carrot and potato increased thermotolerance by enhancing cell membrane stability (Hu et al., 2010); since the evaluation of three rice genotypes with a different tolerance to high temperatures revealed a greater accumulation of *sHSPs* in the tolerant genotypes in comparison with the sensitive genotype, and the genotype showing moderate tolerance revealed an intermediate concentration of sHSPs (Jagadish et al., 2010).

The three plants increased the expression of the *OsHSP71.10* gene when exposed to the increased temperature, and the gene expression increased by 12, 6 and 2-folds for rice, weedy rice and barnyardgrass, respectively (Figure 4C). In the treatment at 25 °C, rice exhibited a lower expression of the OsHSP71.10 gene than weeds; however, at 40 °C, rice showed a higher expression, and the barnyardgrass a lower (Figure 4C).

HSP70-family proteins are the first HSP to accumulate in plant tissue in response to high temperatures (Fragkostefanakis et al., 2015). It has been reported that the accumulation of HSP70 enzymes assists in denatured proteins translocation, proteolysis, translation, folding, aggregation and refolding. Besides protecting PSII and consequently in the maintenance of the electron transport chain during stress, it plays an important role in the positive regulation of enzymatic antioxidant defense, indirectly helping to control ROS (Hu et al., 2010). The increase in the expression of the *HSP71.10* gene, as it was observed in the three species evaluated, was

reported in tomato species and in different grape varieties in high temperature conditions, where the most stress tolerant variety showed a greater expression than the others (Bita et al., 2011).

Molecular analyses have shown that some plants, especially rice and weedy rice, have increased the expression of genes responsible for their defense to oxidative stress and structural damage in cells. This molecular response may be related to the increase of the oxidative stress observed in these plants, since there is a close relationship between oxidative stress and the response of plants subjected to supra-optimal temperatures. It has been suggested that H_2O_2 is necessary to trigger a response to heat stress, and in *Arabidopsis* the expression of the *APX2*, *HSP17.6* and *HSP18.2* genes was induced by increasing the concentration of H_2O_2 in the tissues (Matsuura et al., 2010). In barnyardgrass case, in which no changes were observed in the physiological parameters, there was an increase in the expression of the *OsHSP70.10* gene, demonstrating that this gene is responsive to the temperature increase in the three plants tested and its increased expression can be mediated by other markers than H_2O_2.

Analyzing Pearson's correlation, of the 160 possible correlations, 65% were significant, of which 60% were positive and 40% negative (data not shown). A correlation between liquid photosynthesis and total protein content (0.89*), SOD activity (-0.89*), H_2O_2 content (-0.84*) and TBARS (-0.84* 79*) was found, and these results indicate that increased oxidative stress led to a reduced carbon uptake in the plants assessed. Furthermore, a positive correlation between H_2O_2 content and the Os*APX2* (0,91*) and Os*HSP24.15* (0,88*) genes expression was verified, demonstrating that the expression of these genes is positively regulated by this ROS in these species.

From the results of the present study, it may be concluded that the 40 °C temperature causes reduction in photosynthesis, gas exchange and protein content, and that it increases oxidative stress in the C3 plants tested, with no negative effect on the C4 plant subjected to this temperature. In response to high temperature, rice and weedyrice increased the activity of the APX and SOD enzymes and the expression of *OsAPX2*, *OsHSP24.15* and *OsHSP71.10* genes; in barnyardgrass, it was only observed an increase in the expression of the *HSP71.10* gene.

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