

Heat stress in sugarcane: physiological changes and gene expression

Estresse térmico em cana-de-açúcar: mudanças fisiológicas e expressão gênica

Estrés calórico en caña de azúcar: cambios fisiológicos y expresión génica

Received: 01/27/2022 | Reviewed: 02/01/2022 | Accept: 02/11/2022 | Published: 02/16/2022

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Abstract

Facing climate change is necessary to know the effects of temperature on plant development and identify which physiological factors are related to the reduction and/or increase in photosynthesis. This identification can be made in the first stages of development. Thus, the objective was to evaluate the physiological parameters of the sugarcane cultivar RB 86 7515 at 15°C, 28°C, and 40°C at different exposure times (0, 4, 8, 12, and 24 hours) and to evaluate 18S gene expression. The experiment was established in a completely randomized design, with six replications. Chlorophyll a and b were measured, and the a/b ratio, total chlorophyll in leaves, and variables related to photosystems. Initial (F₀) and maximum (F_m) fluorescence values were measured. The ratio F_v/F₀ and the potential quantum efficiency of photosystem II (PSII) F_v/F_m were estimated. The mRNA was extracted using the same types of leaves used in the photosynthesis assessment. High temperatures with exposure for 24 hours affect the photosynthetic efficiency of sugarcane plants. Exposure of sugarcane plants with 65 days of development to 40 °C for 24 hours is recommended to assess tolerance to temperature stress, aiming at early selection of genotypes.

Keywords: OJIP; Chlorophyll; Fluorescence; RNA; Photosystem.

Resumo

Frente às mudanças climáticas faz-se necessário conhecer os efeitos da temperatura no desenvolvimento das plantas, e identificar quais fatores fisiológicos estão relacionados à redução e/ou aumento da fotossíntese. Esta identificação pode ser feita nos primeiros estádios de desenvolvimento. Assim, objetivou-se avaliar parâmetros fisiológicos da cultivar de cana-de-açúcar RB 86 7515 a 15°C, 28°C e 40°C em diferentes tempos de exposição (0, 4, 8, 12 e 24 horas) e avaliar a expressão gênica 18S. O experimento foi estabelecido em delineamento inteiramente casualizado, com seis repetições. Foram feitas as medidas de clorofila a e b, e estimada a relação a/b, e o total de clorofila nas folhas, e variáveis relacionadas aos fotossistemas. Foram medidos os valores de fluorescência inicial (F₀) e máxima (F_m). De posse dessas variáveis foram estimadas as relações: F_v/F₀ e a eficiência quântica potencial do fotossistema II (FSII) F_v/F_m. O RNAm foi extraído usando os mesmos tipos de folhas usadas na avaliação da fotossíntese. Altas temperaturas com exposição por 24 horas afetam a eficiência fotossintética das plantas de cana-de-açúcar. Exposição

de plantas de cana-de-açúcar com 65 dias de desenvolvimento a 40 °C por 24 horas é recomendada para avaliação da tolerância ao estresse por temperatura, visando a seleção precoce de genótipos.

Palavras-chave: OJIP; Clorofila; Fluorescência; RNA; Fotosistema.

Resumen

Ante el cambio climático, es necesario conocer los efectos de la temperatura en el desarrollo de las plantas, e identificar qué factores fisiológicos están relacionados con la reducción y/o aumento de la fotosíntesis. Esta identificación puede hacerse en las primeras etapas de desarrollo. Así, el objetivo fue evaluar parámetros fisiológicos del cultivar de caña de azúcar RB 86 7515 a 15°C, 28°C y 40°C a diferentes tiempos de exposición (0, 4, 8, 12 y 24 horas) y evaluar la expresión del gen 18S. El experimento se estableció en un diseño completamente al azar, con seis repeticiones. Se midieron clorofila a y b, y se estimó la relación a/b, clorofila total en hojas y variables relacionadas con los fotosistemas. Se midieron los valores de fluorescencia inicial (F0) y máxima (Fm). Con estas variables en mente, se estimaron las relaciones Fv/Fo y la eficiencia cuántica potencial del fotosistema II (PSII) Fv/Fm. El ARNm se extrajo usando los mismos tipos de hojas que se usaron en la evaluación de la fotosíntesis. Las altas temperaturas con exposición durante 24 horas afectan la eficiencia fotosintética de las plantas de caña de azúcar. Se recomienda la exposición de plantas de caña de azúcar con 65 días de desarrollo a 40°C por 24 horas para evaluar la tolerancia al estrés por temperatura, visando la selección temprana de genotipos.

Palabras clave: OJIP; Clorofila; Fluorescencia; ARN; Fotosistema.

1. Introduction

The relationships of interactions in physiological processes are important in genotype selection and cultivar performance evaluation. Understanding how a cultivar responds to stress conditions at the first stadium of development contributes to saving time in plant breeding programs. Abiotic stress affects physiological processes in plants that are responsible for adaptation and crop production (Kock and Ba, 2018). There is multiple stress such as water deficit, salinity, high temperatures, and/or excess light. The search for genotypes with production under these conditions is a great challenge considering the stress can delay development, reduce productivity, and lead to plant death (Shu et al., 2013). The temperature stress is one factor that affects the photosynthetic efficiency and can be considered both a limiting factor and a stimulant of physiological processes (Sato et al., 2014; Shen et al., 2004).

Sugarcane (*Saccharum* spp.) is one of the most important industrial cash crops, contributing to the world sugar industry and biofuel production. It has been cultivated and improved from prehistoric times through natural selection and conventional breeding and, more recently, using the modern tools of genetic engineering and biotechnology. However, the heterogenicity, complex poly-aneuploid genome and susceptibility of sugarcane to different biotic and abiotic stresses represent impediments that require to pay greater attention to the improvement of the sugarcane crop. Compared to traditional breeding, recent advances in breeding technologies (molecular marker-assisted breeding, sugarcane transformation, genome-editing and multiple omics technologies) can potentially improve sugarcane, especially against environmental stressors (Shabbir et al., 2021).

Osmotic adjustment is the most important physiological mechanism to enable plants to resist abiotic stresses (Peixoto & Sage, 2017). This pathway is mediated by the activation of gene expression, which is associated with traditional selection methods, constituting alternatives for identifying genotypes tolerant to the stress of temperature (Souza et al., 2011). Heat stress limits the growth, development, and yield of crop plants when it occurs during short or long periods of time in sugarcane genotypes, is a suitable useful parameter for selecting the genotypes tolerant to heat stress in a breeding program. This procedure, combined with other characters, helps to identify sugarcane plants with the ability to maintain a high yield photosynthetic rate under stressful field conditions. Furthermore, it offers an opportunity to improve selection efficiency over that of field testing, since high temperature stresses do not occur consistently under field conditions (Castro-Nava et al., 2019).

One of the physiological markers used in this evaluation is the fluorescence emission of Chlorophyll (Chl) (Bustin, 2002; Gutierrez et al., 2008; Dundas & Ling, 2012; Kozera & Rapacz, 2013; Chapman & Waldenström, 2015). The general

circulation models estimated an average increase in global surface temperature about 4 °C (2.9 to 5.5 °C). Escalating temperature and extreme weather events are causing higher variations in substantial yield losses leading to food insecurity. Temperature flux, more notably high temperature, affects plants' physio-biochemical processes, resulting in serious yield reduction and quality due to poor agronomic management in plants. Despite the impact of global insecurity on world food production, limited success has been achieved to heat stress adaptation. Extensive research has been made to assess the consequences of abiotic stresses in early growth stages and endurance under subsequent intensity. However, the consequences of abiotic, especially heat/high-temperature stress on reproductive growth, development, and phenology, received comparatively less consideration (Ul Hassan et al., 2021). The fluorescence test (OJIP) reflects the reduction kinetics of photosystem II (PSII), quinone A (QA), plastoquinone (PQ), and other intersystem acceptors; and photosystem I (PSI) (Malaspina et al., 2018).

In addition to the temperature playing an essential role in the development of plants, it tends to increase plant respiration and carbohydrate consumption (Loka & Oosterhuis, 2010; Sanghera et al., 2019). Temperature variations modify the photosynthetic rate of sugarcane plants. At 10°C, a reduction of 20% was obtained, compared to plants at 30°C, in photosynthetic rates (Peixoto & Sage, 2017). In another Poaceae, corn, photosynthetic rates at six temperatures contributed to increasing rates from 15°C to 35°C, and from there, there was a reduction (Massad et al., 2007; De Silva et al., 2021).

In general, this condition increases leaf diffusive resistance to water vapor by closing the stomata, reducing transpiration and the supply of CO₂ for photosynthesis. Osmotic adjustment is the most important physiological mechanism to enable plants to resist abiotic stresses (Singh et al., 2018). However, the evaluation cannot continually be assessed without using tissue-destructive processes at various stages of development.

Another way of understanding stress responses is to relate genes expressed during stress, which may remain stable under various treatments and/or have relative changes at different phases of development (Pfaffl, 2004; Yan et al., 2021). As a result, proteins are synthesized and involved in stress tolerance (Rocha et al., 2015; Singh et al., 2018). Some proteins with catalytic activity are essential enzymes for the antioxidant defense against oxidative stress damage (Singh et al., 2018), such as chaperones or heat shock proteins (HSPs); like the HSPs studied under temperature stress (HSP70, HSP90, and sHSPs) (Yan et al., 2006; Timperio et al., 2008; Pinheiro & Ramos, 2018).

The relative resilience of the plant in a short period can help monitor the plant's response to these stresses. Thus, this work aims to investigate physiological and genetic changes in a short time as a way of evaluating the response speed of seedlings of cultivar RB867515 to temperature.

2. Methodology

Sugarcane seedlings production

The material was obtained by adopting the sugarcane cultivar RB867515 by multiplication system with pre-sprouted seedlings from individual buds in a nursery with 50% shade and daily irrigation. The substrate used was a mixture of Plantmax® and coconut powder in a 1:1 ratio (v/v).

At 15 days after planting (DAP), seedlings were transplanted into tubes with a volume of 53 cm³. With 40 DAP, the plants were pruned and placed for acclimatization in full sun and daily irrigation. The plants with 65 DAP were placed in an acclimatized chamber, with constant temperature control, irradiance (luminous flux density 3,000 lm m⁻² s⁻¹ = 60,000 Lux), and a photoperiod of 12 hours.

A completely randomized design (DIC) was adopted, with six replications, and the experimental plot consisted of a tube containing a single plant. The seedlings were submitted to three temperature regimes 15°C, 28°C, and 40°C during nine

periods (0; 1; 2; 4; 6; 8; 10; 12 and 24 hours).

Physiological determinations

Chlorophyll a and b

For the evaluation of chlorophyll, a and b, we used a chlorophyll meter (ClorofiLOG CFL1030 Model) (Falker, 2008). The total chlorophyll was obtained through the sum of the chlorophyll, a and b, and a/b ratio. This device provides non-destructive measurements and is strongly correlated to destructive methods. The reading is obtained from photodiodes after light emission at wavelengths 635, 660, and 880 nm (Krenchinski et al., 2000; Brito et al., 2011; Barbieri et al., 2012; Olivoto et al., 2016; Siqueira-Silva et al., 2019).

At the momentum of the evaluations, the second leaf, fully expanded (visible ligule), was chosen. In the third middle part of each leaf were performed the readings.

Transient fluorescence of chlorophyll a

The transient fluorescence states of chlorophyll *a* were measured with portable fluorimeter model OS-30p (Opti-Sciences Inc., USA) to evaluate the photosystems and the electron transport chain. Measurements were performed on the same sheets as the previous analysis, adapted to the dark condition for 30 minutes. The transient chlorophyll fluorescence states were produced by the emission of actinic light ($\lambda = 660$ nm) at an intensity of $3,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (photons) for 1 second. Irradiation was applied homogeneously on a leaf area with a diameter of 4 mm (Dinis et al., 2016).

The rapid kinetics of fluorescence emission from initial to maximum fluorescence (F_0 to F_m) was measured by the OJIP steps: $O \cong F_0$ (50 μs), J (2 ms), I (30 ms), $P \cong F_m$ and, also the time values for maximum fluorescence $t(F_m)$ and the area above the OJIP curve (A).

Falker chlorophyll index (FCI) measurements were performed for chlorophyll a (Clo a) and chlorophyll b (Clo b) using a non-destructive Falker chlorophyll index device, model CFL1030 (Falker et al., 2008). The device's operation is based on the absorbance of light emitted by diodes at three wavelengths (λ), 635 and 660 nm (red), and 880 nm (infrared). After passing through the sheet, the light is captured by silicon photodiodes, transmitting the signals analogously. The equipment provides absorbance readings that allow estimating the pool of chlorophylls a and b (Barbieri et al., 2012; Cancellier et al., 2013; Schlichting et al., 2015). Total chlorophyll was calculated and estimated as Clo a and Clo b. The ratio of chlorophyll a and b was also determined.

Quantitative PCR - RT-qPCR

All PCR products were performed in an Applied Biosystems® 7500 Real-Time PCR System, using Power SYBR® Green PCR Master Mix. Reactions were performed in 20 μl containing 5 μl of 40 uM μl^{-1} cDNA, 12.5 μl SYBR Green, 6.5 μl of water, and 0.5 μl of each primer (forward and reverse). All samples were analyzed in technical triplicates.

The primers used were those obtained from sequences of *Arabidopsis thaliana* L. 18S. The amplification conditions were: 95 °C for 10 min, 40 cycles of 95 °C for 30 seconds, indicated temperature for the primer for 60 seconds, 72 °C for 10 seconds, and the melting curves were performed immediately after the end of RT-qPCR and fluorescence Measured from 65 to 99°C.

Determination of the amplification efficiency of primers

The amplification efficiency for the primers used was estimated utilizing qPCR reactions containing cDNA subjected

to serial 4-fold dilutions (1:10). Three replicates were prepared for each of the five dilutions of the cDNA samples. The Cts (Cycle Threshold) obtained for each gene were submitted to linear regression, and the correlation coefficients between the points and the plotted line were calculated for regression validation. The 18S rRNA reference gene was used with SCFRRE06 18S adhesion referring to the ribosomal region of the RNA, F/R sequence CTACGTCCTGCCCTTTGTACA and ACACTTCACCGGACCATTCAA.

Data analysis

Data were submitted to multivariate analysis using Principal Component Analysis (PCA) (Cruz, 2013) using the Genes program. In both physiological and molecular assessments, data normality was assessed by the Shapiro-Wilk test. Analysis of variance of treatment effects, Tukey's mean comparison test ($P \leq 0.05$), and regression analysis were performed using the Sisvar program (Ferreira, 2011).

3. Results and Discussion

Falker Chlorophyll Indexes (FCI)

The Chl a, b, and total Chl of sugarcane indices were influenced ($p < 0.05$) by temperatures. The results showed that temperatures of 15° and 28°C promoted an increase in Chl a and total Chl indices. Regarding the Clh b index, plants exposed to 15°C presented the highest value. However, the Chl a Chl b⁻¹ ratio did not differ statistically between temperatures (Table 1).

Table 1. Chlorophyll Falker Indexes (ICF) of chlorophyll b (Cl) of sugarcane plants subjected to heat stress.

Stress	Chl <u>a</u>	Chl <u>b</u>	Chl Total	Chl <u>a</u> Chl <u>b</u> ⁻¹
15	25.74a	8.03a	33.90a	3.22a
28	25.93a	7.87ab	33.83a	3.31a
40	24.45 b	7.45 b	31.70 b	3.30a
Significance	***	**	***	ns

* Means followed by the same lowercase letters in the column do not differ statistically for the Tukey Test ($p \leq 0.05$) (n=6). Source: Authors.

When evaluating the influence of the exposure time of sugarcane plants to different temperatures, it was observed that the Chl b, total Chl, and Chl a Chl b⁻¹ ratios differed statistically ($p < 0.05$). A gradual decrease in the Chl b index was obtained, where at 0 hours it was identified the highest index and the lowest index at 24 hours of exposure. At 6 hours was obtained for total Clh, the highest value. Differently from the result found for the Clh b index, for the variable Chl a Chl b⁻¹ there was a gradual increase from 0 hours to 24 hours of exposure. The time factor did not statistically influence Chl a (Table 2).

Table 2. Falker Chlorophyll (FCI) indices of chlorophyll b (Chl) of sugarcane plants over time.

Tempo (h)	Chl <u>a</u>	Chl <u>b</u>	Chl Total	Chl <u>a</u> <u>b</u> ⁻¹
0	25.52a	8.31a	33.70ab	3.08 b
6	25.77a	7.95ab	34.00a	3.24ab
12	25.37a	7.47 bc	32.84ab	3.41a
24	24.83a	7.39 c	32.03 b	3.38a
Significance	ns	***	*	**

* Means followed by the same lowercase letters in the column do not differ statistically for the Tukey Test ($p \leq 0.05$) (n=6). Source: Authors.

Chlorophylls are photosynthetic pigments located in chloroplasts, which play a fundamental role in photosynthesis, as these pigments are responsible for capturing the light energy used to generate ATP and NADPH. Throughout the development of plants, the content of chlorophyll present in tissues varies. Additionally, as they are sensitive structures, external factors such as changes in temperature can accelerate the degradation of chlorophylls, reducing their amount in chloroplasts (Roca et al., 2016). The declination of plants' photosynthetic capacity is due to these stresses, consequently reducing yield. Therefore, detailed information of the plant responses and a better understanding of the photosynthetic machinery could help develop new crop plants with higher yields even under stressed environments. Interestingly, cracking of signaling and metabolic pathways, identification of some key regulatory elements, characterization of potential genes, and phytohormone responses to abiotic factors have advanced our knowledge related to photosynthesis. However, our understanding of the dynamic modulation of photosynthesis under dramatically fluctuating natural environments remains limited (Muhammad et al., 2021).

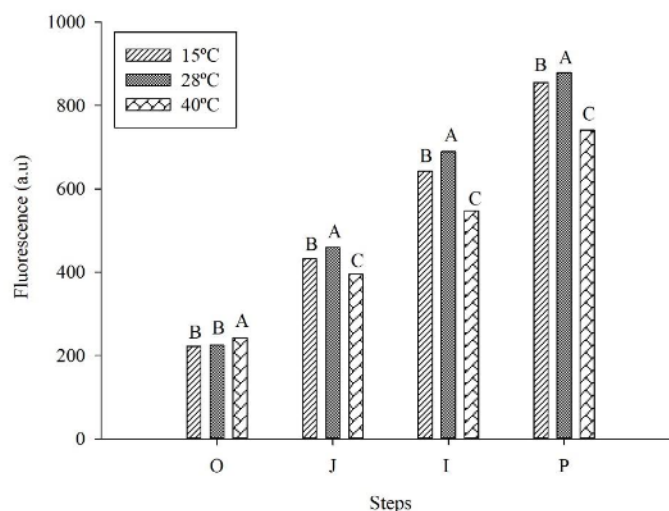
When submitted to a temperature of 40°C, the sugarcane plants reduced the indices of Chl *a*, Chl *b*, and, consequently, total Chl (Table 1). However, reducing the temperature to 15°C kept the chlorophyll content higher (Table 2). In addition to inducing quantitative and qualitative losses in production, excessive heat shortens the crop cycle's duration, reducing the leaf area accelerating metabolism, consequently causing early plant senescence. Photosynthesis is one of the physiological processes most sensitive to heat (Demirevska-Kepova et al., 2005; Flack-Prain et al., 2021) due to the sensitivity of the thylakoid membrane, the structure where chloroplasts are contained, damage caused by excess heat causes a reduction in chlorophyll production (Ristic et al., 2007; Sharma et al., 2022).

In the presence of high temperatures, plants develop defensive reactions such as the activation of several genes responsible for the synthesis of a set of substances related to the occurrence of damage or cell death. Among these substances are peroxidases, enzymes that favor the oxidation of cells, producing free radicals that act on cell membranes and proteins, damaging them or causing them to die (Essemine et al., 2010; Sellami et al., 2022). In addition, heat stress favors the emergence of active oxygen species (ROS) such as superoxide (O_2^-), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2) radicals that constitute oxidative stress and cause lipid peroxidation. As a result of their actions, these radicals cause damage to the membrane, degradation, inactivation of proteins, and disruption of the DNA structure (Sairam et al., 2000; Sharma et al., 2022).

Chlorophyll a fluorescence

The chlorophyll *a* (OJIP) fluorescence transient was influenced ($p < 0.05$) only by heat stress (Figures 1 and 2). Plants subjected to stress at 40°C showed higher initial fluorescence intensity (O) when compared to other temperatures. However, the values were higher for the J, I, and P fluorescence steps for sugarcane plants cultivated at 28°C (Figure 1).

Figure 1. Chlorophyll fluorescence transients in sugarcane plants subjected to different temperatures. Means followed by equal capital letters do not differ statistically by Tukey's test ($p \leq 0.05$) ($n=4$).



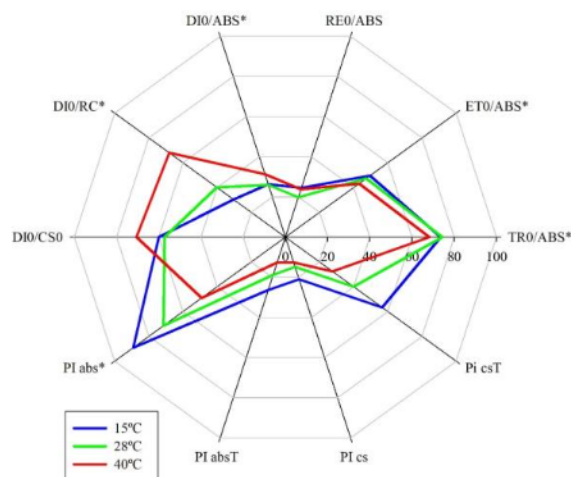
O: initial value of fluorescence intensity; J: fluorescence value at 2 milliseconds; I: fluorescence value at 30 milliseconds; Q: or Fm, maximal fluorescence intensity. Source: Authors.

Temperature is one of the main abiotic factors that directly interfere with plant photosynthesis. Occurs the interference in results with heat stress in sugarcane plants. The temperature of 40°C increased the value of step O (Figure 1), directly related to the process of opening and closing of stomata (Mathur et al., 2019). In turn, stomatal opening and closing is a physiological plant protection mechanism directly related to photosynthetic efficiency. Since the high temperature causes the stomata to close, it reduces the passage of CO₂ photosynthesis and delays the development of the plant. On the other hand, the increase in the initial fluorescence (O), as well as the fluorescence at 2 milliseconds (J), in plants cultivated at a temperature of 28°C, show that, possibly, there was a favoring for the chlorophyll reaction centers to keep open, generating the capture of electrons (Mathur et al., 2019), favoring the photosynthetic process.

As for step J, in steps I-P, plants showed higher values when cultivated at 28°C (Figure 2). Called the thermal phase of photosynthesis, the I-J phase is related to the reduction of the electron transport chain, in addition to the number of reaction centers present in the PSI (Kalaji et al., 2014, Zivcak et al., 2014). Chlorophyll fluorescence is a tool that helps identify possible changes in the photosynthetic apparatus of plants when subjected to abiotic stresses in their developmental phase. These changes are mainly in the PSII (Kalaji et al., 2014). Thus, it is possible to infer that the cultivation of plants at 28°C allowed the plants to keep the photosystems in good working order.

The phenomenological energy flux parameters of TR0/ABS, ET0/ABS, DI0/ABS DI0/RC, and PIabs differed statistically (Figure 2). The use of temperatures of 15° and 28°C favored the increase of TR0/ABS, ET0/ABS, and PIabs, and consequently reduced the energy dissipation (DI0/ABS and DI0/RC). In turn, the use of a temperature of 40°C reduced the parameters of TR0/ABS, ET0/ABS, and PIabs, causing an increase in DI0/ABS and DI0/RC (Figure 2).

Figure 2. Radar graph showing the parameters of the OJIP Test in sugarcane plants subjected to heat stress. Asterisks correspond to significant differences between treatments by Tukey's test ($p \leq 0.05$). Values were standardized on a scale of 0 to 100.



Abbreviations of parameters and biological meanings are described in the abbreviations.

TR0/ABS: The maximum quantum yield of PSII primary photochemistry

ET0/ABS: The quantum yield of electron transport from QA to PQ

DI0/ABS: The quantum yield of energy dissipated in the PSII antenna, related to non-photochemical processes (heat)

ABS/RC: Determination of the average of absorbed photon fluxes (ABS) by the total of PSII reaction centers (RC), also related to the measurement of the apparent size of the PSII antenna (ABS/RC)

DI0/RC: Flow of energy dissipated in other non-photochemical processes by active reaction centers in PSII

PIcs: Performance index over the base cross-section

PIabs: Base Absorption Performance Index.

Source: Authors.

The results demonstrate that the use of temperatures of 15° and 28° promoted an increase in the efficiency in capturing energy absorbed from the photosynthetic apparatus (TR0/ABS) and in the quantum yield of electron transport from Qa to PQ (ET0/ABS) of sugarcane plants of sugar (Figure 2). Furthermore, the use of these temperatures allowed a reduction in the non-photochemical dissipation (DI0/ABS) of the antenna complex, causing an increase in the base absorption performance index (PIabs) (Figure 2). The quantum yield of energy dissipated in the PSII antenna (DI0/ABS) is directly related to the size of the LCHII antenna complex and the proportion between photosystems I and II (Cascio et al., 2010; Da Silva et al., 2019). Thus, the temperatures of 15° and 28°C reduced energy losses by heat, preventing the inactivation of the photosystem reaction centers, helping to increase the PIabs, mainly for the temperature of 15°C.

However, seedlings cultivated at 40°C showed the opposite result to that found for temperatures of 15° and 28°C (Figure 3). With the excessive increase in ambient temperature, the sugarcane plants had reduced chlorophyll levels (Table 1), increased initial fluorescence (O) (Figure 3), and decreasing parameters of TR0/ABS, ET0/ABS, and PIabs (Figure 3). Additionally, energy dissipation losses in the photosystem were also higher. These results demonstrate the high sensitivity of photosystems to abiotic stress. Sugarcane is a long-term, high-demand commercial crop grown under different agroecological conditions. In addition to experiencing year-round variations in climate, climate change further exacerbates the effects of these abiotic stresses, affecting growth, development, sugar synthesis, accumulation and recovery, and cane availability for subsequent planting (Shrivastava, 2017).

In sugarcane, abiotic stresses seem to occur due to the good compensatory capacity, as they are C4 plants. Plants that have the C4 structure, as a rule, are more resistant to higher temperatures, which can be positive for most physiological activities, such as sugar accumulation. Additionally, *S. spontaneum* genetic complements confer tolerance to various abiotic

stresses and enable carbon management capacity. In turn, some physiological interventions such as induction of drought resistance, root system depth, age, rhizospheric salinity/alkalinity, and many genes, micro RNAs (miRNAs), are also involved in epigenetic responses (Zandalinas et al., 2018).

The reduction of Fv/Fm may be associated with excess electrons since low temperatures reduce the efficiency of the Calvin Cycle enzymes, reducing the consumption of photo chemicals generating an excess of electrons. The first damage caused by this excess of electrons is the loss of stability and the physical-chemical disruption of thylakoid biomembranes, affecting photosystem II. After simulating the functional integrity of chloroplast membranes and mitochondria, processes such as photosynthesis and respiration can also be impaired as they depend on the activity of electron transport and membrane-associated enzymes (Havaux, 1993; Mamedov et al., 1993). Therefore, sugarcane photosynthesis at high temperatures may be limited, mainly by reducing the biochemical activity that leads to photochemical damage.

The quantum yield of energy dissipated in the PSII antenna related to non-photochemical processes (heat) is given by F0/FM. Under stress, plants' mechanisms are the dissipation of excess energy absorbed that cannot be stored or transported from the PSII. The triggering of pathways that can result from a series of damages, such as the PSII structures or the thylakoid membrane subjected to the effect of hydrocarbons (Kreslavski et al., 2017). In addition to inducing quantitative and qualitative losses in production, excessive heat shortens the cycle duration, reduces leaf area, height, and frequency of fertilization accelerates the cycle and senescence. Photosynthesis is one of the most heat-sensitive physiological processes (Demirevska-Kepova et al., 2005), mainly because of the sensitivity of the thylakoid membrane, with reduced production of chlorophyll (Ristic et al., 2007).

In the presence of high temperatures, plants develop defensive reactions such as the activation of several genes responsible for the synthesis of a set of substances, including peroxidases. These enzymes favor cell oxidation, producing free radicals that act on cell membranes and proteins, causing damage or cell death (Essemine et al., 2010). Thermal stress favors the emergence of active oxygen species (ROS) such as the superoxide radical (O_2^-), the hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2) that constitute oxidative stress, causing lipid peroxidation, consequently causing membrane injuries, protein degradation and inactivation, and disruption of DNA structure (Sairam and Srivastava, 2000).

In sugarcane, abiotic stresses seem to be due to good compensatory capacity, C4 photosynthesis, the optimal temperature for most physiological activities (but for sugar accumulation), greater efficiency in water use, use of genetic complements of *S. spontaneum* conferring tolerance to various abiotic stresses and carbon management capacity. In addition, some of the physiological interventions include induction of drought resistance, root system depth, age, salinity/rhizospheric alkalinity, etc. In addition to many genes, micro RNAs (miRNAs) are also involved in epigenetic responses (Zandalinas et al., 2018).

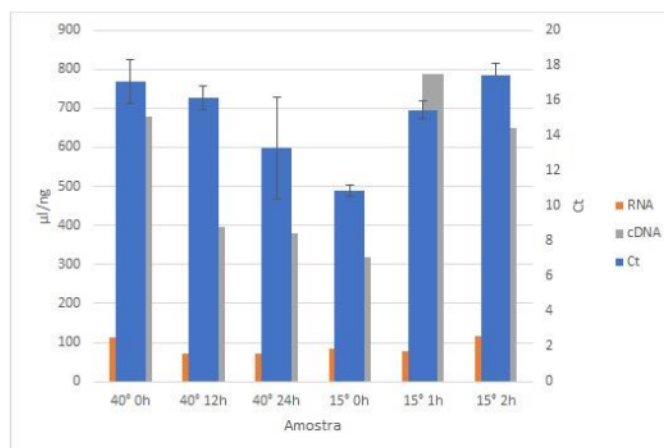
Sugarcane is a long-term, high-demand commercial crop grown under different agroecological conditions. In addition to experiencing year-round variations in climate, climate change further exacerbates the effects of these abiotic stresses, affecting growth, development, sugar synthesis, its accumulation and recovery, and the availability of cane for subsequent planting (Zandalinas et al., 2018).

Gene expression in sugarcane

18S rRNA is the active center of protein synthesis. The increase in the number of ribosomes, which leads to increases in RNA transcription and protein synthesis, is proportional to increases in 18S rRNA. Nucleic acids and proteins are damaged by oxidation. The level of 18S gene expression is an ideal indicator for measuring protein synthesis, including antioxidant enzymes, and for investigating the effects of antioxidants. Thus, the synthesis of 18S genes was evaluated for inferences on the

gene expression of proteins. The total RNA results for the samples' A260/A280 nm ratio ranged between 2.09 and 2.33. There was detectable gene activity for 18S at 15 °C and 40 °C. For the other treatments, there was no detectable expression.

Figure 3. Gene expression for the 18S ribosomal subunit in sugarcane cv. RB 86 7515 was subjected to temperatures of 15 °C and 40 °C at different evaluation times.



Source: Authors.

The variation in gene expression depends on the type of environment and abiotic conditions (Thellin et al., 1999). In plants, some conditions related to semiarid conditions have been shown to have an extremely complex abiotic effect, a condition characterized by interactions associated with cell maintenance, protein production and degradation, and changes in metabolism (Shanker et al., 2014).

LEA (Late Embryogenesis Abundant) proteins are found in many organisms, such as bacteria, insects, and plants. These proteins prevent cellular damage caused by abiotic stresses (Wang et al., 2014). Heat shock protein (HSP) biosynthesis is induced to avoid macroparticle denaturation. These proteins remain stable for some time and are probably the primary survival mechanism of plants to increase temperature. HSPs are found in the cytoplasm and organelles such as the nucleus, mitochondria, chloroplasts, and endoplasmic reticulum. The tolerance conferred by HSP results in better functioning of physiological phenomena such as photosynthesis, water, and nutrient use efficiency and membrane stability (Al-Whaibi, 2011; Zandalinas et al., 2018; Wahid, 2017).

HPS and chaperones may be related to stress signaling, gene activation, and regulation of cellular redox state (Al-Whaibi, 2011; Wahid, 2007). These proteins also interact with other stress response mechanisms, such as the production of osmolytes and antioxidants. HSP minimizes cell damage at excessively high temperatures by protecting them from denaturation and creating chelating bonds with ions leaking from the vacuole into the cytosol (Kotak et al., 2007; Al-Whaibi, 2011). Enzymes of the antioxidant defense system in plants are such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidases (APX), glutathione peroxidase (GPX), type III peroxidases, and peroxiredoxins (PRX) (Bhatt et al., 2011).

In plants, 1-Cys Prx is encoded by single-copy genes. So far, the only known case with two genes for this enzyme is in rice, with the genes arranged in tandem (Pulido et al., 2011; Bhatt et al., 2011). High temperatures with exposure for 24 hours affect the photosynthetic efficiency of sugarcane plants. Exposure of sugarcane plants with 65 days of development to 40°C for 24 hours is recommended to assess tolerance to temperature stress.

4. Conclusion

For the cultivar RB867515, high temperatures (40°C) affect the photosynthetic efficiency of plants. However, it is essential to emphasize the importance of this study that validates the use of seedlings, i.e., plants in the first stages of development, to identify tolerance to high temperatures. However, the need to test the response for different cultivars is worth mentioning as a perspective for future work.

We state suggestions for future with the evaluation of mRNA gene expression, the gene sequences used for the 18S ribosomal subunit are an indicator for the evaluation of expression and use in the selection of genotypes. In the current political and economic context of science promotion, this genomic sequence can be used to evaluate temperature stress instead of using countless sequences, which would require inputs and more time in this evaluation.

Acknowledgments

This study was financed in part by the National for Scientific and Technological Development - Brazil (CNPq), by the Support Foundation for Research and Technological Innovation of the State of Sergipe (Fapitec/SE) - Brazil, by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES - Financial Code 001) and Financier of Studies and Projects - Brazil (FINEP).

References

- Al-Wahaibi, M. H. (2011). Plant heat-shock proteins: a mini review. *Journal of King Saud University-Science*, 23(2), 139-150.
- Barbieri Junior, É., Rossiello, R. O. P., Silva, R. V. M. M., Ribeiro, R. C., & Morenz, M. J. F. (2012). Um novo clorofilômetro para estimar os teores de clorofila em folhas do capim Tifton 85. *Ciência Rural*, 42, 2242-2245.
- Bhatt, D., Negi, M., Sharma, P., Saxena, S. C., Dobriyal, A. K., & Arora, S. (2011). Responses to drought induced oxidative stress in five finger millet varieties differing in their geographical distribution. *Physiology and Molecular Biology of Plants*, 17(4), 347-353.
- Brito, G. G., Sofiatti, V., Brandão, Z. N., Silva, V. B., Silva, F. M., & Silva, D. A. (2011). Non-destructive analysis of photosynthetic pigments in cotton plants. *Acta Scientiarum. Agronomy*, 33(4), 671-678.
- Bustin, S. A. (2002). INVITED REVIEW Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *Journal of molecular endocrinology*, 29, 23-39.
- Cancellier, E. L., Cruz, J. S., dos Santos, M. M., Siebeneichler, S. C., & Fidelis, R. R. (2013). Índices de clorofila em partes da planta de arroz de terras altas. *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, 8(1), 29.
- Castro-Nava, S., & López-Rubio, E. (2019). Thermotolerance and Physiological Traits as Fast Tools to Heat Tolerance Selection in Experimental Sugarcane Genotypes. *Agriculture*, 9(12), 251.
- Chapman, J. R., & Waldenström, J. (2015). With reference to reference genes: a systematic review of endogenous controls in gene expression studies. *PloS one*, 10(11), e0141853.
- Cruz, C. D. (2013). Genes: a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum. Agronomy*, 35(3), 271-276.
- de Melo Peixoto, M., & Sage, R. F. (2017). Comparative photosynthetic responses in upland and lowland sugarcane cultivars grown in cool and warm conditions. *Brazilian Journal of Botany*, 40(4), 829-839.
- Demirevska-Kepova, K., Holzer, R., Simova-Stoilova, L., & Feller, U. (2005). Heat stress effects on ribulose-1, 5-bisphosphate carboxylase/oxygenase, Rubisco binding protein and Rubisco activase in wheat leaves. *Biologia Plantarum*, 49(4), 521-525.
- De Silva, A. C., Senarathna, H. N., & De Costa, W. J. M. (2021). Genotypic variation of the interactive effects of elevated temperature and CO₂ on leaf gas exchange and early growth of sugarcane. *Physiologia Plantarum*, 173(4), 2276-2290.
- Deveci, M., & Pitir, M. (2016). Effect of water deficiency on physiological and chemical properties of pepper grown in greenhouse. *Appl. Ecol. Envir. Res*, 14, 587-596.
- Deveci, M., Cabi, E., Arin, L., & Yavas, Ö. (2017). The Effect of Water Deficit on some Physiological Properties of *Abelmoschus esculentus* (L.) Moench cv." Sultani".
- Dinis, L. T., Ferreira, H., Pinto, G., Bernardo, S., Correia, C. M., & Moutinho-Pereira, J. (2016). Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. *Photosynthetica*, 54(1), 47-55.

- Dundas, J., & Ling, M. (2012). Reference genes for measuring mRNA expression. *Theory in Biosciences*, 131(4), 215-223.
- Essemine, J., Ammar, S., & Bouzid, S. (2010). Impact of heat stress on germination and growth in higher plants: Physiological, biochemical and molecular repercussions and mechanisms of defence. *Journal of Biological Sciences*, 10(6), 565-572.
- Falker Automação Agrícola Ltda. (2008). *Manual do medidor eletrônico de teor clorofila* (ClorofiLOG/CFL 1030).
- Ferreira, D. F. (2011). Sisvar: um sistema computacional de análise estatística. *Ciênc. Agrotec.* [online] 35: 1039-1042.
- Flack-Prain, S., Shi, L., Zhu, P., da Rocha, H. R., Cabral, O., Hu, S., & Williams, M. (2021). The impact of climate change and climate extremes on sugarcane production. *GCB Bioenergy*, 13(3), 408-424.
- Goltsev, V. N., Kalaji, H. M., Paunov, M., Băba, W., Horacek, T., Mojski, J., & Allakhverdiev, S. I. (2016). Variable chlorophyll fluorescence and its use for assessing physiological condition of plant photosynthetic apparatus. *Russian Journal of Plant Physiology*, 63(6), 869-893.
- Gutierrez, L., Mauriat, M., Guénin, S., Pelloux, J., Lefebvre, J. F., Louvet, R., & Van Wuytswinkel, O. (2008). The lack of a systematic validation of reference genes: a serious pitfall undervalued in reverse transcription-polymerase chain reaction (RT-PCR) analysis in plants. *Plant biotechnology journal*, 6(6), 609-618.
- Havaux, M. (1993). Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant, Cell & Environment*, 16(4), 461-467.
- Kalaji, H. M., Bosa, K., Kościelniak, J., & Hossain, Z. (2011). Chlorophyll a fluorescence—a useful tool for the early detection of temperature stress in spring barley (*Hordeum vulgare* L.). *Omic: a journal of integrative biology*, 15(12), 925-934.
- Kalaji, H. M., Schansker, G., Ladle, R. J., Goltsev, V., Bosa, K., Allakhverdiev, S. I., & Zivcak, M. (2014). Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. *Photosynthesis Research*, 122(2), 121-158.
- Kok, D., & Bal, E. (2018). Enhancing skin color and phenolic compounds of cv. Red Globe table grape (*V. vinifera* L.) utilizing of different preharvest treatments. *Erwerbs-Obstbau*, 60(1), 75-81.
- Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., & Scharf, K. D. (2007). Complexity of the heat stress response in plants. *Current Opinion in Plant Biology*, 10(3), 310-316.
- Kozera, B., & Rapacz, M. (2013). Reference genes in real-time PCR. *Journal of Applied Genetics*, 54(4), 391-406.
- Kreslavski, V. D., Brestic, M., Zharmukhamedov, S. K., Lyubimov, V. Y., Lankin, A. V., Jajoo, A., & Allakhverdiev, S. I. (2017). Mechanisms of inhibitory effects of polycyclic aromatic hydrocarbons in photosynthetic primary processes in pea leaves and thylakoid preparations. *Plant Biology*, 19(5), 683-688.
- Lichtenthaler, H. K., Wenzel, O., Buschmann, C., & Gitelson, A. (1998). Plant Stress Detection by Reflectance and Fluorescence a. *Annals of the New York Academy of Sciences*, 851(1), 271-285.
- Loka, D. A., & Oosterhuis, D. M. (2010). Effect of high night temperatures on cotton respiration, ATP levels and carbohydrate content. *Environmental and Experimental Botany*, 68(3), 258-263.
- Malaspina, P., Modenesi, P., & Giordani, P. (2018). Physiological response of two varieties of the lichen *Pseudevernia furfuracea* to atmospheric pollution. *Ecological Indicators*, 86, 27-34.
- Mamedov, M., Hayashi, H., & Murata, N. (1993). Effects of glycinebetaine and unsaturation of membrane lipids on heat stability of photosynthetic electron-transport and phosphorylation reactions in *Synechocystis* PCC6803. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1142(1-2), 1-5.
- Massad, R. S., Tuzet, A., & Bethenod, O. (2007). The effect of temperature on C4-type leaf photosynthesis parameters. *Plant, cell & environment*, 30(9), 1191-1204.
- Mehta, P., Jajoo, A., Mathur, S., & Bharti, S. (2010). Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. *Plant Physiology and Biochemistry*, 48(1), 16-20.
- Olivoto, T., Nardino, M., Carvalho, I. R. C., Follmann, D. N., Szareski, V. J., Ferrari, M., & Souza, V. Q. (2016). Pearson correlation coefficient and accuracy of path analysis used in maize breeding: a critical review. *International Journal of Current Research*, 8(9), 37787-37795.
- Oukarroum, A., Schansker, G., & Strasser, R. J. (2009). Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl a fluorescence kinetics in barley varieties differing in their drought tolerance. *Physiologia Plantarum*, 137(2), 188-199.
- Pfaffl, M. W. (2004). *Quantification strategies in real-time PCR*. *AZ of quantitative PCR*, 1, 89-113.
- Pinheiro, G. M., & Ramos, C. H. (2018). Initial characterization of newly identified mitochondrial and chloroplast small HSPs from sugarcane shows that these chaperones have different oligomerization states and substrate specificities. *Plant Physiology and Biochemistry*, 129, 285-294.
- Pulido, P., Cazalis, R., & Cejudo, F. J. (2009). An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. *The Plant Journal*, 57(1), 132-145.
- Ristic, Z., Bukovnik, U., & Prasad, P. V. (2007). Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Science*, 47(5), 2067-2073.

- Rocha, A. J., Monteiro-Júnior, J. E., Freire, J. E., Sousa, A. J., & Fonteles, C. S. (2015). Real time PCR: the use of reference genes and essential rules required to obtain normalisation data reliable to quantitative gene expression. *Journal of Molecular Biology Research*, 5(1), 45.
- Sairam, R. K., & Srivastava, G. C. (2000). Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. *Biologia Plantarum*, 43(3), 381-386.
- Sairam, R. K., & Srivastava, G. C. (2000). Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible wheat genotypes. *Biologia Plantarum*, 43(3), 381-386.
- Sanghera, G. S., Malhotra, P. K., Singh, H., & Bhatt, R. (2019). Climate change impact in sugarcane agriculture and mitigation strategies. *Harnessing Plant Biotechnology and Physiology to Stimulate Agricultural Growth*, 99-115.
- Sato, S. T. A., Ribeiro, S. D. C. A., Sato, M. K., & Souza, J. N. S. (2014). *Caracterização física e físico-química de pitayas vermelhas (Hylocereus costaricensis) produzidas em três municípios paraenses*.
- Sellami, K., Couvert, A., Nasrallah, N., Maachi, R., Abouseoud, M., & Amrane, A. (2022). Peroxidase enzymes as green catalysts for bioremediation and biotechnological applications: A review. *Science of the Total Environment*, 806, 150500.
- Shabbir, R., Javed, T., Afzal, I., Sabagh, A. E., Ali, A., Vicente, O., & Chen, P. (2021). Modern Biotechnologies: Innovative and Sustainable Approaches for the Improvement of Sugarcane Tolerance to Environmental Stresses. *Agronomy*, 11(6), 1042.
- Shanker, A. K., Maheswari, M., Yadav, S. K., Desai, S., Bhanu, D., Attal, N. B., & Venkateswarlu, B. (2014). Drought stress responses in crops. *Functional & integrative genomics*, 14(1), 11-22.
- Sharma, K., Salik, F., & Rather, S. A. (2022). Responses and Adaptation of Photosynthesis and Respiration under Challenging Environments. *Plant Abiotic Stress Physiology: Volume 1: Responses and Adaptations*, 119.
- Shen, Y., Zhang, Y., Kondoh, A., Tang, C., Chen, J., Xiao, J., & Sun, H. (2004). Seasonal variation of energy partitioning in irrigated lands. *Hydrological Processes*, 18(12), 2223-2234.
- Shu, S., Yuan, L. Y., Guo, S. R., Sun, J., & Yuan, Y. H. (2013). Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in *Cucumis sativus* L. under salt stress. *Plant Physiology and Biochemistry*, 63, 209-216.
- Silva, H. W. D., Costa, L. M., Resende, O., de Oliveira, D. E., Soares, R. S., & Vale, L. S. (2015). Higroscopicidade das sementes de pimenta (*Capsicum chinense* L.). *Revista Brasileira de Engenharia Agrícola e Ambiental*, 19, 780-784.
- Singh, R., Upadhyay, A. K., Chandra, P., & Singh, D. P. (2018). Sodium chloride incites reactive oxygen species in green algae *Chlorococcum humicola* and *Chlorella vulgaris*: implication on lipid synthesis, mineral nutrients and antioxidant system. *Bioresource Technology*, 270, 489-497.
- Singh, A., Sengar, K., Sharma, M. K., Sengar, R. S., & Garg, S. K. (2018). Proline metabolism as sensors of abiotic stress in sugarcane. *Biotechnology to enhance sugarcane productivity and stress tolerance*, 265-284.
- Siqueira-Silva, A. I., Rios, C. O., & Pereira, E. G. (2019). Iron toxicity resistance strategies in tropical grasses: the role of apoplastic radicular barriers. *Journal of Environmental Sciences*, 78, 257-266.
- Sitko, K., Rusinowski, S., Kalaji, H. M., Szopiński, M., & Małkowski, E. (2017). Photosynthetic efficiency as bioindicator of environmental pressure in *A. halleri*. *Plant Physiology*, 175(1), 290-302.
- Souza MA, Pimentel AJB, Ribeiro G. Melhoramento para tolerância ao calor. Melhoramento de plantas para condições de estresses abióticos. Viçosa: Suprema, p. 199-226, 2011.
- Strasser, R. J., Tsimilli-Michael, M., & Srivastava, A. (2004). Analysis of the chlorophyll a fluorescence transient. In *Chlorophyll a fluorescence* (pp. 321-362). Springer, Dordrecht.
- Strasser, R. J., Tsimilli-Michael, M., Qiang, S., & Goltsev, V. (2010). Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1797(6-7), 1313-1326.
- Thellin, O., Zorzi, W., Lakaye, B., De Borman, B., Coumans, B., Hennen, G., & Heinen, E. (1999). Housekeeping genes as internal standards: use and limits. *Journal of biotechnology*, 75(2-3), 291-295.
- Timperio, A. M., Egidi, M. G., & Zolla, L. (2008). Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *Journal of proteomics*, 71(4), 391-411.
- Ul Hassan, M., Rasool, T., Iqbal, C., Arshad, A., Abrar, M., Abrar, M. M., & Fahad, S. (2021). Linking Plants Functioning to Adaptive Responses Under Heat Stress Conditions: A Mechanistic Review. *Journal of Plant Growth Regulation*, 1-18.
- Wahid, A. (2007). Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *Journal of plant Research*, 120(2), 219-228.
- Wang, D., Heckathorn, S. A., Wang, X., & Philpott, S. M. (2012). A meta-analysis of plant physiological and growth responses to temperature and elevated CO₂. *Oecologia*, 169(1), 1-13.
- Wang, L., Guo, Y., Jia, L., Chu, H., Zhou, S., Chen, K., & Zhao, L. (2014). Hydrogen peroxide acts upstream of nitric oxide in the heat shock pathway in *Arabidopsis* seedlings. *Plant Physiology*, 164(4), 2184-2196.

Yan, S. P., Zhang, Q. Y., Tang, Z. C., Su, W. A., & Sun, W. N. (2006). Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Molecular & cellular proteomics*, 5(3), 484-496.

Yan, M., Jin, X., Liu, Y., Chen, H., Ye, T., Hou, Z., & Niu, X. (2021). Identification and evaluation of the novel genes for transcript normalization during female gametophyte development in sugarcane. *PeerJ*, 9, e12298.

Yusuf, M. A., Kumar, D., Rajwanshi, R., Strasser, R. J., Tsimilli-Michael, M., & Sarin, N. B. (2010). Overexpression of γ -tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll a fluorescence measurements. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1797(8), 1428-1438.

Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., & Gómez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. *Physiologia plantarum*, 162(1), 2-12.