#### Accepted Manuscript / Manuscrito aceptado

Vegetative Growth of Sorghum Cultivars under Increasing Air Temperature Doi: https://doi.org/10.15446/abc.v30n2.109430

To appear in / Para aparecer en: Acta Biológica Colombiana

Received Date / Fecha de recibido: 27<sup>th</sup> March 2023 / 27 de marzo de 2023

Revised Date / Fecha de revisado: 17th January 2025 / 17 de enero de 2025

Accepted Date / Fecha de aceptado: 13th March 2025 / 13 de marzo de 2025

Please cite this article as / Cite así: Nascimento, G. S. G. do., Guimarães, M. J. M., Falcão, H. M., Silva, E. G. F., Barros, J. R. A., Oliveira, A. R. de., Angelotti, F. (2025). Vegetative Growth of Sorghum Cultivars under Increasing Air Temperature. *Acta Biol Colomb.*, *30*(2), XX-XX. https://doi.org/10.15446/abc.v30n2.109430

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# ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

# VEGETATIVE GROWTH OF SORGHUM CULTIVARS UNDER INCREASING AIR TEMPERATURE

# Crecimiento vegetativo de cultivares de sorgo en condiciones de aumento de la

# temperatura del aire

# Running head: Sorghum growth under heat stress

Glaucia Suêrda Gomes do Nascimento<sup>1a</sup>, Miguel Júlio Machado Guimarães<sup>2b</sup>, Hiram Marinho

Falcão<sup>3c</sup>, Elioenai Gomes Freire Silva<sup>4d</sup>, Juliane Rafaele Alves Barros<sup>5e</sup>, Anderson Ramos de

Oliveira<sup>6f</sup>, Francislene Angelotti<sup>7g\*</sup>

<sup>1.</sup> Postgraduate Program in Environmental Science and Technology, University of Pernambuco – *Campus* Petrolina, BR 203, KM 2 – Vila Eduardo, Petrolina, 56328900, Brazil, <u>glauciasuerda@hotmail.com</u>.

<sup>2.</sup> Federal Institute of Education, Science and Technology of Baiano – *campus* Santa Inês, BR 420, Zona Rural, 45320000, Santa Inês, BA - Brasil, Brazil, <u>miguel.guimaraes@ifbaiano.edu.br</u>.

<sup>3.</sup> Program in Environmental Science and Technology, University of Pernambuco – *Campus* Petrolina, BR 203, KM 2 – Vila Eduardo, Petrolina, 56328900, Brazil, <u>hiram.falcao@upe.br</u>.

<sup>4.</sup> Postgraduate Program in Environmental Science and Technology, University of Pernambuco – *Campus* Petrolina, BR 203, Brazil, <u>elioenai.gomes@upe.br</u>.

<sup>5.</sup> Postgraduate Program in Plant Genetic Resources, Av. Transnordestina, s/n - Feira de Santana, Novo Horizonte - BA, 44036900, Brazil. <u>juliane-ab@hotmail.com</u>.

<sup>6.</sup> Embrapa Semi-Arid, BR-428, Km 152, s/n - Zona Rural, Petrolina -PE, 56302970, Brazil. <u>anderson.oliveira@embrapa.br</u>.

<sup>5.</sup> Embrapa Semi-Arid, BR-428, Km 152, s/n - Zona Rural, Petrolina -PE, 56302970, Brazil. <u>francislene.angelotti@embrapa.br</u>.

<sup>a</sup> ORCID: <u>https://orcid.org/0000-0003-3706-8108</u>, email: <u>glauciasuerda@hotmail.com</u>

<sup>b</sup> ORCID: <u>https://orcid.org/0000-0002-5497-6442</u>, email: <u>miguel.guimaraes@ifbaiano.edu.br</u>

<sup>c</sup> ORCID: <u>https://orcid.org/0000-0003-3198-1801</u>, email: <u>hiram.falcao@upe.br</u>

<sup>d</sup> ORCID: <u>https://orcid.org/0000-0002-9246-5336</u>, email: <u>elioenai.gomes@upe.br</u>

<sup>e</sup> ORCID: <u>https://orcid.org/0000-0002-0408-0904</u>, email: <u>juliane-ab@hotmail.com</u>

<sup>f</sup> ORCID: <u>http://orcid.org/0000-0003-4089-0995</u>, email: <u>anderson.oliveira@embrapa.br</u>

<sup>g</sup> ORCID: <u>https://orcid.org/0000-0001-7869-7264</u>, email: <u>francislene.angelotti@embrapa.br</u>

\* For correspondence: <u>francislene.angelotti@embrapa.br</u>.

Received: 27th March 2023. Revised: 17th January 2025. Accepted: 13th March 2025

# Associate Editor: Susana Feldman

**Citation/ citar este artículo como:** Nascimento, G. S. G. do., Guimarães, M. J. M., Falcão, H. M., Silva, E. G. F., Barros, J. R. A., Oliveira, A. R. de., Angelotti, F. (2025). Vegetative Growth of Sorghum Cultivars under Increasing Air Temperature. *Acta Biol Colomb.*, *30*(2), XX-XX. https://doi.org/10.15446/abc.v30n2.109430

# ABSTRACT

The impact of climate on sorghum cultivation is generated mainly by scenarios of increased temperature which can affect plant development and cause losses in crop yield. Thus, this work aimed to evaluate sorghum cultivars tolerant to high temperatures through

the evaluation of biometric, enzymatic, and productive parameters. The experiment was carried out in growth chambers, with seven sorghum cultivars (AGRI-002E, BRS 506, BRS 716, SF 15, IAC Santa Elisa, BRS Ponta Negra, and Volumax) and four temperature regimes (20.0-26.0-33.0 °C; 24.8-30.8-37.8 °C; 26.3-32.3-39.3 °C and 27.8-33.8-40.8 °C), in a completely randomized design, with four replications. The biometric, biochemical, and productive parameters were evaluated. The cultivars AGRI-002E, BRS 506, BRS Ponta Negra, and Volumax showed better defense of the antioxidant system with increasing air temperature, with less accumulation of reactive oxygen species and greater biomass production. These cultivars can be classified as tolerant to an increase of up to 6.3 °C in air temperature, with emphasis on cultivar BRS 506, which showed higher production of stem dry mass.

Keywords: Enzymatic activity, Heat stress, Production, Sorghum bicolor.

#### RESUMEN

El impacto del clima en el cultivo del sorgo, generado principalmente por escenarios de aumento de temperatura, puede afectar el desarrollo de las plantas y ocasionar pérdidas en el rendimiento de los cultivos. Así, el objetivo de este trabajo fue seleccionar cultivares de sorgo tolerantes a altas temperaturas, mediante la evaluación de parámetros biométricos, enzimáticos y productivos. El experimento se realizó en cámaras de crecimiento, con siete cultivares de sorgo (AGRI-002E, BRS 506, BRS 716, SF 15, IAC Santa Elisa, BRS Ponta Negra y Volumax) y cuatro regímenes de temperatura (20.0-26.0-33.0 °C; 24.8-30.8-37.8 °C; 26.3-32.3-39.3 °C y 27.8-33.8-40.8 °C), en un diseño completamente al azar, con cuatro repeticiones. Se evaluaron los parámetros biométricos, bioquímicos y productivos. Los

cultivares AGRI-002E, BRS 506, BRS Ponta Negra y Volumax mostraron una mejor defensa del sistema antioxidante con el aumento de la temperatura del aire, con menor acumulación de especies reactivas de oxígeno y mayor producción de biomasa. Estos cultivares pueden clasificarse como tolerantes a un aumento de hasta 6.3 °C en la temperatura del aire, con énfasis en el cultivar BRS 506, que mostró mayor producción de masa seca de tallo. **Palabras clave:** Actividad enzimática, Estrés por calor, Producción, *Sorghum bicolor*.

# **INTRODUCTION**

Agricultural productivity is directly linked to environmental conditions, hence the increase in air temperature and variations in precipitation interfere with the growth and development of crops (Angelotti and Giongo, 2019). According to the IPCC (2021), it is predicted that by the end of the century, there will be an average increase of 4.3 °C in the average global temperature if mitigating actions for the emission of greenhouse gases are not implemented. In addition, the occurrence of extreme and more frequent weather events, such as heat waves, can compromise food security.

Sorghum (*Sorghum bicolor* L. Moench) is a cereal used for multiple purposes, such as human food, fodder, animal feed, in addition to being a promising source of liquid biofuel (Mundia et al., 2019) and biomass burning (Silva et al., 2018). In recent years, crop production has grown significantly (Tabosa, 2020) due to adaptation to adverse environmental conditions, such as salinity, different soil fertility conditions, and tolerance to water deficit. Sorghum presents optimal development in the temperature range from 29 °C to 33 °C (Tabosa, 2020). The exposure of plants to temperatures above 33 °C during the

vegetative stage can reduce the photosynthetic rate, with a negative effect on crop yield (Tack et al., 2017). In an arid region of Mali, a reduction of 11 % to 17 % in sorghum production by 2030 is projected due to temperature changes (Butt et al., 2005). Similar results were observed for West Africa, with yield reduction of 41 % as a function of a 6 °C increase (Sultan et al., 2013). In Brazil, studies for the selection of sorghum cultivars tolerant to heat stress have not yet been carried out. Understanding the response of cultivars to the increase in air temperature studied can contribute to the reduction of losses and the maintenance of the productive capacity of the culture.

One of the responses of plants to the increase in temperature is related to physiological and biochemical changes in plants that affect their growth and development. Furthermore, to the generation of harmful by-products to plants such as reactive oxygen species (ROS). ROS are strongly reactive forms of oxygen, capable of oxidizing various cellular components and causing damage to cells (Thorpe et al., 2013; Nouman et al., 2014). As a defense mechanism, plants produce antioxidant enzymes capable of controlling the overproduction of ROS (Das and Roychoudhury 2014; Ding, 2016). The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which act in sync to defend plant cells against oxidative damage (Wang et al., 2018). Thus, interpreting the form of production, signaling, and elimination of ROS through enzymatic antioxidant processes represents an important means of defense against damage caused by heat stress (Cheah, 2017).

The response of plants to the increase in temperature is a complex mechanism that includes the interaction of different production components, including biometric, biochemical and productive parameters (Barros et al., 2021). To understand these

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complicated interactions, multivariate analysis tools such as the Principal Components Analysis (PCA), correlation analysis (e.g., Pearson), multiple regression models, and network analysis are essential (Hongyu et al., 2015; Sena et al., 2021). They enable us to identify patterns, causal and effect relationships, and priority factors that influence plant adaptation to heat, providing a more comprehensive and robust system view. For example, while Pearson's correlation is perfect for investigating basic relationships between two variables, network analysis is crucial for comprehending interconnected systems and their emergent properties (Schober et al., 2018; Chalmers et al., 2022). On the other hand, PCA reduces the dimensionality of a dataset by transforming several correlated variables into a smaller number of uncorrelated principal components, being more for analyzing multidimensional interactions (Jolliffe and Cadima, 2016).

Thus, the objective of this study was to select sorghum cultivars tolerant to high temperatures, through the evaluation of biometric, productive, enzymatic parameters and analysis of principal components.

#### MATERIAL AND METHODS

The experiment was carried out in growth chambers, Fitotron type, with control of humidity, photoperiod, luminosity, and temperature (Fig. 1). The study was a two-factorial laid out in a completely randomized design, with seven sorghum cultivars, three of the biomass type (AGRI-002E, BRS 716, IAC Santa Elisa), three of the silage type (SF 15, BRS Ponta Negra and Volumax) and one of saccharine type (BRS 506), in four temperature regimes, with four replications (Table 1).

The temperature regimes were determined from the daily minimum, average, and maximum temperature of the São Francisco Valley, in the Brazilian semiarid region.

From the average of the minimum, average, and maximum daily temperature of the last 30 years, which vary from 18-22 °C, 25-27 °C and 32-34 °C, respectively, simulations of temperature increase were performed, based on the future scenario recorded by the Intergovernmental Panel on Climate Change (IPCC, 2013). The relative humidity in the chambers ranged from 50 % to 70 % and a photoperiod of 12 h. For sowing, eight-liter pots were filled with a layer of gravel at the base (2 cm) and filled with soil (collected from the 0-30 cm layer of Eutrophic Yellow Argisol). Ten seeds were sown per pot at a depth of 2 cm. Irrigation control was performed with the aid of a TDR (Time Domain Reflectometer). Irrigations were carried out every two days from the data generated by the TDR, with the replacement of the volume of evapotranspired water, maintaining the availability of water in the soil. Thirteen days after sowing, thinning was performed, leaving only one plant per pot.

For the evaluation of enzymatic activity, 30 days after planting (DAP), fragments of healthy leaves, without lesions and fully expanded, were collected specifically in the middle third of the plant, in the morning. The samples were immediately stored in aluminum foil envelopes and immersed in liquid nitrogen. Plant extracts were prepared using one gram of plant material macerated in liquid nitrogen with the addition of 0.01 g of polyvinylpolypyrrolidone and three mL of extraction buffer (pH 7.5) at a concentration of 100 mM of potassium phosphate. Then, the extracts were centrifuged at 15.000 g for 15 minutes at 4 °C, and the supernatant obtained was used as a crude enzyme extract (Guimarães et al., 2020).

Catalase activity (CAT) was determined following the decomposition of  $H_2O_2$  for 60 seconds, and subsequent spectrophotometric reading at 240 nm, according to the method described by Havir and Mchale (1987). Ascorbate peroxidase (APX) activity was determined as described by Nakano and Asada (1981), by monitoring the rate of ascorbate oxidation for 60 seconds using a spectrophotometer with a wavelength at 290 nm. Superoxide dismutase (SOD) activity was determined according to the methodology by Giannopolitis and Ries (1977), with readings in a spectrophotometer at a wavelength of 560 nm and defining the unit of SOD as the amount of enzyme required to inhibit by 50 % the photoreduction of nitrotetrazolium blue (NBT). The total soluble protein content was determined by spectrophotometry according to the method by Bradford (1976) at 595 nm. At 67 DAP, the harvest was performed, cutting the plants close to the ground. The following biometric parameters were evaluated: number of leaves per plant; +3 leaf length and width (third leaf from apex to base), and stem diameter. In counting the number of leaves, green leaves were considered along the entire length of the stem, from the base of the plant close to the ground to the last leaf with a visible ligule.

To evaluate the height of the plant, it was measured from the neck to the insertion of the last leaf with a visible ligule, using graduated measuring tape. A digital vernier caliper was used to determine the stem diameter, calculating the average of three consecutive measurements between the first and second internode (leaf insertion).

After biometric evaluations, the plant material was separated into shoots and roots. The leaves and stems were immediately weighed on a scale to determine the fresh mass, in grams. Then, the samples were placed in paper bags and stored in an air circulation oven at 65 °C for drying. After reaching constant mass, the samples were weighed again on an analytical balance to obtain the dry mass. The roots were washed in running water to separate the soil and subjected to the same weighing and drying process used in the above-ground part to obtain the dry mass.

All data were tested for normality and homogeneity. Data was submitted to factorial analysis of variance (factorial ANOVA), with the different temperature treatments and cultivars as independent variables. The averages were compared using the Scott Knott test at 5 % significance. To verify a possible convergence of plant attributes according to temperature variation, a Principal Component Analysis (PCA) was performed. This technique reduces dimensionality, padronizes the variables, and finds adjacent patterns, converting the original variables into principal uncorrelated components that capture the majority of the variability. This enables a clearer interpretation of the data and the identification of trends or clusters that are not visible in univaried analyses. Additionally, by concentrating on the most important components, PCA helps to reduce noise, which improves the quality of the ensuing analyses. The analysis was performed in the Origin 8.0 software (Origin Lab Corp., Northampton, EUA).

## RESULTS

The temperature and cultivar interaction were significant for sorghum plant height, stem diameter and leaf length. For plant height, it is observed that in the regime of 27.8-33.8-40.8 °C, the cultivars showed no significant difference between them (Table 2). The plant height of cultivar BRS 506 was highlighted in the regimes of 24.8-30.8-37.8 °C and 26.3-32.3-39.3 °C, with values of 72.05 cm and 53.57 cm, at 67 days after planting, respectively.

It is noteworthy that the height of cultivars AGRI-002E, BRS 716, SF 15, IAC Santa Elisa and Volumax was not affected by the increase in air temperature. For cultivars BRS 506 and BRS Ponta Negra, plant height was favored by an increase of up to 4.8 °C in air temperature. In general, in the lower temperature regime (20.0-26.0-33.0 °C) the sorghum plants showed lower height values.

All sorghum cultivars submitted to the 27.8-33.8-40.8 °C regime showed a reduction in stem diameter, when compared to the 26.3-32.3-39.3 °C regime (Table 2). In the temperature regime of 20.0-26.0-33.0 °C the cultivars showed no difference in stem diameter. Cultivars AGRI-002E, BRS 506, BRS 716, SF 15, BRS Ponta Negra, and Volumax presented a larger stem diameter with an increase of 6.3 °C in the average temperature.

Cultivars that grew under the temperature regime of 24.8-30.8-37.8 °C showed no significant difference in leaf length +3 (Table 2). Cultivars BRS 716, SF 15, and BRS Ponta Negra had greater leaf length with an increase of 6.3 °C in air temperature.

For the yield components, the interaction between temperature and cultivar was significant for leaf and stem fresh and dry mass (Table 3). In comparison with the temperature regime of 20.0-26.0-33.0 °C, the increment of up to 7.8 °C maintained or increased all the productive parameters evaluated in (Table 3). Thus, none of the cultivars tested was jeopardized by the increase in temperature, although some materials show better performance when grown under increases of 4.8 and 6.3 °C (Table 3).

The increase of up to 7.8 °C (referring to the 27.8-33.8-40.8 °C regime) compared to the temperature regime of 20.0-26.0-33.0 °C promoted an increase in the fresh mass of leaves of cultivars AGRI-002E, BRS 506 and IAC Santa Elisa (Table 3). However, for stem fresh

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mass, this increase was observed only as a response of cultivars AGRI-002E and IAC Santa Elisa. Cultivars BRS 716, SF 15, BRS Ponta Negra and Volumax presented higher fresh mass of leaves and stem in the temperature regimes of 24.8-30.8-37.8 °C and 26.3-32.3-39 .3 °C (Table 3).

For the dry mass of leaves, the performance of cultivars AGRI-002E, BRS 506, BRS Ponta Negra and Volumax was positive with the increase of 4.8 and 6.3 °C in air temperature (Table 3). It is noteworthy that, when compared to the average temperature of the Submedium region of the São Francisco Valley (20.0-26.0-33.0 °C), the increase of 4.8 °C had a positive effect on the production of leaf and stem dry mass for all cultivars, while an increase of 7.8 °C had a positive or null effect. Similarly, fresh and dry root mass were favored by increases of 4.8 and 6.3 °C in air temperature (Fig. 2). On the other hand, the 7.8 °C increase in air temperature was statistically equal to the 20.0-26.0-33.0 °C regime.

The interaction of temperature regime and cultivars was significant for the activity of SOD, CAT and APX enzymes. In the temperature regime with an increase of 7.8 °C, cultivar AGRI-002E reduced the production of dry mass about increases of 4.8 °C and 6.3 °C, despite the increase in SOD activity (Table 3, Fig. 2).

When compared to the temperature regime of 20.0-26.0-33.0 °C, the increase of 7.8 °C in air temperature was not harmful to cultivar AGRI-002E, since the production of dry mass remained statistically superior. The increase in dry mass production of cultivar AGRI-002E in the 24.8-30.8-37.8 °C regime coincided with greater activity of APX, which grew 84 % about the 20.0-26.0-33.0 °C regime (Fig. 3). In the 26.3-32.3-39.3 °C regime, the dry

mass production remained high, despite the reduction in the APX enzyme compared to the 24.8-30.8-37.8 °C regime (Fig. 3).

Cultivar BRS 506 showed higher SOD activity when submitted to the 26.3-32.3-39.3 °C regime, with an increase of 110 % when compared to the 20.0-26.0-33.0 °C regime (Fig. 2). As for APX activity, which eliminates  $H_2O_2$  generated by SOD, increasing air temperature by 6.3 °C (26.3-32.3-39.3 °C) increased its activity by 211 % (Fig. 3). This increase contributes to the reduction of reactive oxygen species, reducing the damage caused by the increase in temperature to plant metabolism and, consequently, maintaining the production of plant dry mass, as observed in (Table 3). Likewise, for the thermal increase of 7.8 °C, there was a reduction in CAT activity (Fig. 4) and an increase in APX activity (Fig. 3), compared to the temperature regime 20.0-26.0-33.0 °C (Table 3).

For cultivar, BRS 716, an increase of 6.3 °C in air temperature promoted an increase of 294 % in APX activity and 66 % in CAT activity (Figs. 3 and 4). This increase favored the production of stem dry mass (Table 3). This can be explained, since the cultivar may be tolerant to superoxide ( $O_2^-$ ), and the activity of H<sub>2</sub>O<sub>2</sub>-scavenging enzymes (CAT and APX) is sufficient to reduce oxidative stress, reflecting the maintenance of production even at high temperatures. The increase of 7.8 °C in the air temperature allowed the maintenance of the dry mass of the plants of cultivar BRS 716, despite the significant reduction in the activity of the ROS scavenging enzymes, with the increase in temperature (Table 3).

For cultivar SF 15, the 4.8 °C increase in air temperature promoted a 28 % increase in CAT activity (Fig. 4), which was sufficient to allow an increase in plant dry mass (Table 3). Considering the 6.3 °C increase in air temperature, compared to the 24.8-30.8-37.8 °C

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regime, there was a reduction in dry mass production and low enzymatic activity of APX and CAT (Table 3, Figs. 3 and 4).

For cultivar BRS Ponta Negra, SOD activity did not change statistically with the increase of 6.3 °C about the regime 20.0-26.0-33.0 °C, however, there was a reduction of 46 and 26 %, compared to increases of 4.8 and 7.8 °C, respectively (Fig. 2). CAT activity did not change with increases of 4.8 and 6.3 °C, showing that in response to the increase in temperature this cultivar was able to regulate the levels of  $H_2O_2$  in the plants and guarantee the production of dry mass (Figs. 4 and 3). However, a reduction in APX-specific activity is observed in all evaluated temperature increases (Fig. 3).

Significant reductions in SOD and APX activity were observed for cultivar IAC Santa Elisa at increases of 4.8 °C, 6.3 °C, and 7.8 °C (Figs. 3 and 4). CAT activity was also reduced by 64 % with a 7.8 °C increase in air temperature (Fig. 5). Despite the enzymatic changes without synchrony, cultivar IAC Santa Elisa managed to maintain the production of dry mass of the leaf and stem in the temperature regime of 27.8-33.8-40.8 °C, with averages greater or equal, statistically, compared to the temperature regime of 20.0-26.0-33.0 °C. However, compared to the temperature regime 24.8-30.8-37.8 °C, the production of dry mass was lower (Table 3).

For cultivar Volumax, there was no change in SOD enzymatic activity with increasing temperature (Fig. 3). As for APX and CAT activity, reductions of 96 % and 70 % were observed, respectively, with an increase of 7.8 °C (Figs. 4 and 5). Dry mass production was positively affected by increases of 4.8 and 6.3 °C in air temperature, with no change in SOD and APX activity in these temperature regimes.

Cultivars SF 15 and IAC Santa Elisa can be classified as sensitive to the thermal increase evaluated, as they presented reductions in the production of dry mass when compared to the regime of 24.8-30.8-37.8 °C, associated with the lack of synchrony on the activities of the enzymes evaluated. Cultivars AGRI-002E, BRS 506, BRS 716, BRS Ponta Negra and Volumax, on the other hand, were tolerant to the applied heat stress, as they showed synchronic changes in the activities of ROS scavenging enzymes and in the production of dry mass (Figs. 3, 4 and 5; Table 3).

In general, the results obtained in this study showed that an increase of 7.8  $^{\circ}$ C had a positive or null effect on the production of sorghum plants when compared to the 20.0-26.0-33.0  $^{\circ}$ C regime (Table 3).

The principal component analysis performed for the variables studied showed that the functional traits analyzed respond to variations in air temperature and that these traits can be used as good descriptors of the biometric and reproductive responses of the sorghum cultivars used in this study. An overview of all the data and their influence across the four temperature regimes can be seen through the principal component analysis of (Fig. 6).

In (Fig. 6), referring to the temperature regime 20.0-26.0-33.0 °C, it is noted that the variation of functional traits explains 62.16 % of the response presented by the cultivars. Among the cultivars, it is possible to observe a separation of cultivar BRS 506 from the others, mainly influenced by primary productivity parameters, such as fresh and dry weight of leaves and stems, and by biometric parameters. The other cultivars formed a cluster according to the PC2 axis, with emphasis on cultivar SF15, which had the greatest negative impact in this temperature regime, with high levels of enzymes in the antioxidant system, such as CAT and APX, evidencing possible damage to the photosystems. Cultivars IAC

Santa Elisa, BRS Ponta Negra and Volumax were close to the zero value of Blipot and, therefore, present intermediate values, with adaptive behavior to the temperature regime submitted.

For the temperature regime 24.8-30.8-37.8 °C (Fig. 6b), the functional traits analyzed explained 58.73 % of the cultivars' distribution along PCA axes one and two. In this temperature regime, cultivar BRS 506 also remained isolated from the others. However, leaf and stem yield parameters begin to be affected. In addition, it is possible to verify the action of SOD and CAT enzymes, responsible for the elimination of reactive oxygen species (ROS). Thus, with the increase in air temperature by 4.8 °C, cultivar BRS 506 begins to have its ecophysiological performance negatively affected. Cultivars AGRI-002E, BRS 716, and Volumax showed adaptation to this temperature level, as they formed a cluster mainly influenced by production parameters and biometrics, opposite responses to cultivars IAC Santa Elisa and SF15, with lower performance.

In (Fig. 6c), represented by the increase of 6.3 °C, the analyzed variables explain 62.96 % of the cultivar distribution. It is possible to observe that cultivar BRS 506 remained isolated from the others, with a performance similar to that presented in the temperature regime 24.8-30.8-37.8 °C. This behavior may be associated with the ability of this cultivar to adapt to high temperatures. In this sense, we can still see productivity parameters being positively related to this cultivar, even though enzymes of the antioxidant system are acting. The performance of cultivars IAC Santa Elisa and AGRI-002E was more affected in this temperature regime. It is noted that cultivar SF 15 presented adjustment to high temperature, presenting responses like the other cultivars, such as Ponta Negra, BRS 716, and Volumax.

In (Fig. 5d), referring to the increase of 7.8 °C, the variables explain 76.19 % of the variation in the cultivars. This extreme increase in temperature negatively affects most cultivars that until now were stable with thermal variation, as is the case of BRS Ponta Negra and Volumax. Cultivars IAC Santa Elisa and SF 15 were the ones that presented the greatest impact against the increase in temperature, evidenced by the low productivity and high levels of CAT and APX enzymes. Cultivar BRS 506 continued to produce stem and leaf dry weight, demonstrating an expressive acclimatization capacity, and demonstrating that this cultivar invests in photosystem protection at the expense of productivity to adapt to new thermal conditions and energy production. Even so, there is investment in SOD, to keep safe regarding the formation of ROS. Also noteworthy is cultivar AGRI-002E, which in the regime of higher temperatures showed an increase in productivity, mainly in the root and leaf, and in the biometric parameters. Cultivar BRS 716, despite being efficient in mild temperatures, showed acclimatization capacity in the face of increased temperature, not translating this in terms of productivity.

#### DISCUSSION

Sorghum is a plant with C4 metabolism, which supports high temperatures, responding with high photosynthetic rates (Sage, 2004; Sage and Kubien, 2007), which explains the potential development of plants and their acclimatization to the increase in air temperature. For C4 plants in a tropical climate, such as sorghum, the optimal temperature for photosynthesis varies from 30 to 42 °C (Matsuoka et al., 2001). Thus, the temperature increases evaluated possibly did not interfere with the photosynthetic activity of sorghum plants, since photosynthesis is the main physiological process that drives plant growth (Singh

et al., 2014). In addition, the positive response of sorghum to the increase in temperature may also be related to the accumulation of degree days, which favors the growth and development of the crop (Lobell and Gourdji, 2012).

Temperature is one of the limiting climatic elements for agricultural cultivation (Hatfield and Prueger, 2015; Bergamashi and Bergonci, 2017). In the United States, the sorghum breeding program pointed to a negative impact on the production of cultivars currently used in the country at temperatures above 33 °C (Tack et al., 2017). However, the Brazilian cultivars studied performed well against the increase in air temperature. Confirming that within the same species the cultivars may present different responses regarding adaptability to the environment.

The enzymatic activity of sorghum cultivars responded differently to the temperature regimes applied, corroborating with several authors who reported different behaviors of the physiological metabolism of sorghum when subjected to different abiotic stresses (Guimarães et al., 2018; Guimarães et al., 2020). The changes in the activities of ROS-sequestering enzymes under high-temperature conditions, as observed in this study, may be associated with the adaptability of cultivars subjected to heat stress (Mansoor and Naqvi, 2013). The increase in SOD and APX activity as a function of the increase of 6.3 °C shows that the sorghum plants of Cultivar BRS 506 were under heat stress since these antioxidant enzymes increase their activities when the plants are subjected to heat stress as a form of protection (Naudts et al., 2014). The maintenance of enzymatic activity when subjected to abiotic stress is essential for plant development. The significant increase in enzymatic activity that eliminates hydrogen peroxide, such as APX, is significant for the conservation of membrane stability (Naudts et al., 2014). According to Choudhury (2017), reactive oxygen

species can favor the adequacy of metabolism and acclimatization of plants in the face of abiotic stress.

Associated with this, the synchrony between the behavior patterns of the enzymes that act in the antioxidant defense plays an important role in regulating the level of ROS produced in the plant cell (Guimarães et al., 2018). The increase of 6.3 °C in air temperature promoted an increase in APX and CAT activity (Figs. 4 and 5) that favored the production of stem dry mass (Table 3). This can be explained by the tolerance of the cultivar to superoxide (O2-), and by the activity of H<sub>2</sub>O<sub>2</sub>-scavenging enzymes (CAT and APX) capable of reducing oxidative stress, reflecting the maintenance of production even at high temperatures. The antioxidant enzymes CAT and APX are two fundamental tools in the regulation of oxidative stress caused by H<sub>2</sub>O<sub>2</sub> (Das and Roychoudhury 2014).

Thus, Guimarães et al. (2020) indicate that when using enzymatic variables to determine plant tolerance to abiotic stresses, it is necessary to associate changes in enzymatic activities with the production parameters of the cultures evaluated. They claim that plants that present enzymatic alterations without synchrony between the lines of defense (SOD – first line and CAT, APX – second line of defense) and reduced production can be considered sensitive to the applied stress. On the other hand, plants that do not show changes or exhibit changes with synchrony between defense lines associated with maintenance or an increase in production can be considered tolerant.

Different types of abiotic stresses, such as temperature extremes, can intensify the generation of ROS in plant cells (Gill and Tuteja, 2010). In a study carried out by Djanaguiraman et al. (2014), sorghum plants showed an increase in the superoxide radical

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(O2-) in response to the increase of 6 °C and 9 °C in the average air temperature. Mohammed and Tarpley (2010) also found a 43 % increase in  $O_2^-$  and a 124 % increase in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content in sorghum plants exposed to heat stress (40/30 °C, day/night). Balancing the level of ROS within cells is critical for the acclimatization of plants to stress situations (Cheah, 2017). Therefore, despite the negative impacts on plant metabolism, the overproduction of ROS triggered by heat stress can benefit them with signals for the activation of stress response pathways (Baxter et al., 2014; Choudhury, 2017).

The activities of SOD, CAT, and APX enzymes can be explained by the acclimatization process of genetic materials in face of the established thermal increase (Almeselmani et al., 2009; Sarkar et al., 2016). Since high temperatures stimulate the antioxidant defense system, with response variation between genotypes (Hasanuzzaman et al., 2012; Ding, 2016; Awasthi et al., 2017).

#### CONCLUSIONS

In this study, sorghum cultivars responded differently to the increase in temperature, reinforcing the importance of selecting cultivars tolerant to high temperatures as a measure of adaptation for farmers in the face of global warming. Associating biometric, enzymatic, and productive response the cultivars AGRI-002E, BRS 506, BRS Ponta Negra, and Volumax showed better defense of the antioxidant system with increasing air temperature, with less accumulation of reactive oxygen species and greater biomass production. These cultivars can be classified as tolerant to an increase of up to 6.3 °C in air temperature, with emphasis on the cultivar BRS 506, which showed higher production of stem dry mass. Cultivars BRS Ponta Negra and BRS 716 tolerate the increase of up to 6.3 °C.

# **AUTHOR'S PARTICIPATION**

Conceptualization: Francislene Angelotti, Anderson Ramos de Oliveira.

Investigation: Glaucia Suêrda Gomes do Nascimento, Elioenai Gomes Freire Silva.

Formal analysis: Glaucia Suêrda Gomes do Nascimento, Miguel Júlio Machado Guimarães, Hiram Marinho Falcão, Juliane Rafaele Alves Barros.

Writing: Glaucia Suêrda Gomes do Nascimento, Francislene Angelotti.

# ACKNOWLEDGMENT

To the Foundation for Support to Science and Technology of the State of Pernambuco (N° IBPG-0640-5.01/20) and the Coordination for the Improvement of Higher Education Personnel (CAPES) Brazil–Finance code 001.

# **CONFLICT OF INTEREST**

The authors manifest not having any type of conflict of interest.

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# TABLES AND FIGURES.

**Table 1.** Temperature regimes, with minimum, average and maximum temperature variation

 throughout the day.

		Time/Temperature (°C)					
Temperature regime	Increase	6 h (am)	10 h (am)	3 h (pm)	8 h (pm)		
		to 10 h (am)	to 3h (pm)	to 8 h (pm)	to 6 h (am)		
T1: 20.0 – 26.0 – 33.0 °C		26.0	33.0	26.0	20.0		
T2: 24.8 – 30.8 – 37.8 °C	4.8 °C	30.8	37.8	30.8	24.8		
T3: 26.3 – 32.3 – 39.3 °C	6.3 °C	32.3	39.3	32.3	26.3		
T4: 27.8 – 33.8 – 40.8 °C	7.8 °C	33.8	40.8	33.8	27.8		

**Table 2.** Plant height, stem diameter and leaf length +3 of seven sorghum cultivars submittedto different temperature regimes (°C).

Plant height (cm)								
Temperature Regime	AGRI 002E	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax	
20.0-26.0-33.0 °C	21.20 Bb	31.37 Bc	26.55 Ba	24.92 Ba	28.50 Ba	27.82 Bb	48.32 Aa	
24.8-30.8-37.8 °C	36.97 Ca	72.05 Aa	43.80 Ca	40.57 Ca	33.30 Ca	53.00 Ba	35.12 Ca	
26.3-32.3-39.3 °C	31.12 Ba	53.57 Ab	33.05 Ba	31.20 Ba	22.87 Ba	35.30 Bb	34.10 Ba	
27.8-33.8-40.8 °C	35.90 Aa	44.37 Ab	35.12 Aa	27.25 Aa	34.55 Aa	31.00 Ab	37.32 Aa	
CV % 24.83								
Stem diameter (mm)								
Temperature Regime	AGRI 002E	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax	
20.0-26.0-33.0 °C	15.81 Ac	17.98 Ab	14.74 Ab	14.33 Ac	14.53 Ab	15.65 Ac	13.02 Ac	

24.8-30.8-37.8 °C	21.45 Ab	17.74 Bb	22.19 Aa	22.36 Aa	20.59 Ba	20.04 Bb	22.75 Aa		
26.3-32.3-39.3 °C	24.56 Aa	23.55 Aa	23.10 Aa	23.33 Aa	18.48 Ba	23.63 Aa	23.85 Aa		
27.8-33.8-40.8 °C	20.16 Ab	19.10 Ab	17.23 Ab	18.34 Ab	16.87 Ab	18.17 Ac	18.06 Ab		
CV %		11.63							
Leave length +3 (cm)									
					IAC Santa	BRS Ponta			
<b>— — — — — — — — — —</b>									
Temperature Regime	AGRI 002E	BRS 506	BRS 716	SF 15	Elisa	Negra	Volumax		
20.0-26.0-33.0 °C	AGRI 002E 67.50 Bc	BRS 506 79.82 Ab	BRS 716 69.37 Bb	SF 15 79.87 Ab	Elisa 72.62 Bb	Negra 79.92 Ab	Volumax 62.25 Bb		
20.0-26.0-33.0 °C           24.8-30.8-37.8 °C	AGRI 002E 67.50 Bc 98.00 Aa	BRS 506 79.82 Ab 89.50 Aa	BRS 716 69.37 Bb 94.00 Aa	SF 15 79.87 Ab 94.50 Aa	Elisa 72.62 Bb 94.75 Aa	Negra 79.92 Ab 92.50 Aa	Volumax 62.25 Bb 84.75 Aa		
20.0-26.0-33.0 °C           24.8-30.8-37.8 °C           26.3-32.3-39.3 °C	AGRI 002E 67.50 Bc 98.00 Aa 84.00 Bb	BRS 506 79.82 Ab 89.50 Aa 77.50 Bb	BRS 716 69.37 Bb 94.00 Aa 92.00 Aa	SF 15 79.87 Ab 94.50 Aa 94.00 Aa	Elisa 72.62 Bb 94.75 Aa 84.25 Bb	Negra 79.92 Ab 92.50 Aa 87.25 Aa	Volumax 62.25 Bb 84.75 Aa 77.00 Ba		
20.0-26.0-33.0 °C         24.8-30.8-37.8 °C         26.3-32.3-39.3 °C         27.8-33.8-40.8 °C	AGRI 002E 67.50 Bc 98.00 Aa 84.00 Bb 95.67 Aa	BRS 506 79.82 Ab 89.50 Aa 77.50 Bb 75.25 Bb	BRS 716 69.37 Bb 94.00 Aa 92.00 Aa 75.10 Bb	SF 15 79.87 Ab 94.50 Aa 94.00 Aa 80.12 Bb	Elisa 72.62 Bb 94.75 Aa 84.25 Bb 80.27 Bb	Negra 79.92 Ab 92.50 Aa 87.25 Aa 76.22 Bb	Volumax 62.25 Bb 84.75 Aa 77.00 Ba 75.75 Ba		

Averages followed by the same uppercase letter in the row and lowercase in the column do not differ from each other by the Scott-Knott test at 5 % probability.

**Table 3.** Fresh and dry mass of leaf and stem of sorghum cultivars subjected to different temperature regimes.

Leaf Fresh Mass (g)								
Temperature Regime	AGRI 002e	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax	
20.0-26.0-33.0 °C	33.72 Bb	50.17 Ab	35.00 Bb	31.12 Bb	30.77 Bb	37.57 Bb	21.60 Bc	
24.8-30.8-37.8 °C	73.82 Aa	66.67 Aa	52.58 Ba	48.47 Ba	28.62 Cb	62.16 Aa	60.91 Aa	
26.3-32.3-39.3 °C	62.66 Aa	67.34 Aa	64.48 Aa	58.19 Aa	52.56 Aa	65.46 Aa	63.47 Aa	
27.8-33.8-40.8 °C	67.81 Aa	65.22 Aa	45.37 Bb	41.32 Bb	42.35 Ba	46.52 Bb	46.05 Bb	
CV %				20.89				
Stem Fresh Mass (g)								

Temperature Regime	AGRI 002e	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax
20 0-26 0-33 0 °C	29.00 Ab	55 10 Ac	34 35 Ab	30 37 Ab	30.62 Aa	34 95 Ab	41.80 Ab
20.0 20.0 55.0 C	29.00 110	55.10 MC	34.33710	30.37 110	50.02 / M	54.55710	41.00 / 10
24.8-30.8-37.8 °C	99.12 Ba	166.78 Aa	81.81 Ca	81.55 Ca	33.85 Da	112.99 Ba	112.05 Ba
26.3-32.3-39.3 °C	84.32 Ca	157.59 Aa	97.83 Ba	104.88 Ba	59.48 Ca	111.21 Ba	108.77 Ba
27.8-33.8-40.8 °C	82.02 Aa	105.52 Ab	58.32 Bb	49.05 Bb	50.77 Ba	53.92 Bb	66.20 Bb
CV %				24.51			
		L	eaf Dry Mass	(g)			
Temperature Regime	AGRI 002e	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax
20.0-26.0-33.0 °C	5.62 Ac	8.22 Ab	7.02 Ac	4.97 Ac	5.45 Ac	5.97 Ab	4.03 Ac
24.8-30.8-37.8 °C	18.37 Aa	19.39 Aa	19.37 Aa	15.99 Ba	13.94 Ba	17.12 Aa	15.59 Ba
26.3-32.3-39.3 °C	16.99 Aa	16.54 Aa	15.64 Ab	11.84 Bb	8.34 Cb	14.24 Aa	16.67 Aa
27.8-33.8-40.8 °C	13.57 Ab	10.57 Bb	9.32 Bc	7.15 Bc	8.45 Bb	8.25 Bb	9.37 Bb
CV %				17.54			
Stem Dry Mass (g)							
Temperature Regime	AGRI 002e	BRS 506	BRS 716	SF 15	IAC Santa Elisa	BRS Ponta Negra	Volumax
20.0-26.0-33.0 °C	2.82 Ac	5.20 Ad	4.32 Ab	2.70 Ac	3.45 Ab	4.82 Ab	4.42 Ab
24.8-30.8-37.8 °C	15.89 Ba	24.09 Ab	18.19 Ba	16.12 Ba	10.32 Ca	19.04 Ba	17.59 Ba
26.3-32.3-39.3 °C	14.87 Ca	28.19 Aa	15.54 Ca	12.09 Db	6.69 Eb	19.46 Ba	18.69 Ba
27.8-33.8-40.8 °C	8.57 Ab	9.35 Ac	6.07 Bb	4.15 Bc	5.87 Bb	5.57 Bb	6.57 Bb
CV %				19.71			

Averages followed by the same uppercase letter in the row and lowercase in the column do not differ from each other by the Scott-Knott test at 5 % probability.



**Figure 1.** Sorghum plants at different stages of growth are grown under controlled conditions in a growth chamber.





**Figure 2.** Fresh mass (a) and dry mass (b) of sorghum plants as a function of different temperature regimes: T1: 20.0-26.0-33.0 °C; T2: 24.8-30.8-37.8°C; T3: 26.3-32.3-39.3°C; T4: 27.8-33.8-40.8°C. Averages followed by the same letter did not differ from each other by the Scott Knott test (p<0.05).



**Figure 3.** Superoxide dismutase (SOD) in sorghum cultivars subjected to different temperature regimes. \*Averages followed by the same letter do not differ statistically from each other, capital letters for cultivars and lowercase for temperature regime followed by the Scott-Knott test (p<0.05).



**Figure 4.** Ascorbate Peroxidase (APX) in sorghum cultivars subjected to different temperature regimes. \*Averages followed by the same letter do not differ statistically from each other, capital letters for cultivars and lowercase for temperature regime followed by the Scott-Knott test (p<0.05).



**Figure 5.** Catalase specific activity (CAT) in sorghum cultivars subjected to different temperature regimes. \*Averages followed by the same letter do not differ statistically from each other, capital letters for cultivars and lowercase for temperature regime followed by the Scott-Knott test (p<0.05).



**Figure 6.** Principal component analysis (PCA) of data collected from sorghum. (a) PCA for temperature regime 20.0-26.0-33.0°C; (b) PCA for temperature regime 24.8-30.8-37.8°C; (c) PCA for temperature regime 26.3-32.3-39.3°C; (d) PCA for temperature regime 27.8-33.8-40.8°C.