



Article

Pollen Viability, and the Photosynthetic and Enzymatic Responses of Cowpea (*Vigna unguiculata* (L.) Walp., Fabaceae) in the Face of Rising Air Temperature: A Problem for Food Safety

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Abstract: Rising temperature affects agricultural production, causing food insecurity. Thus, the objective of this study was to evaluate how increased temperature influences pollen viability, photosynthetic and enzymatic responses, and their consequences on the final yield of cowpea cultivars. The cultivars BRS Acauã, BRS Guariba, BRS Gurguéia, and BRS Pajeú were used, kept in growth chambers under two temperature regimes: 24.8–30.8–37.8 °C and 20–26–33 °C. The cultivars BRS Acauã, BRS Guariba, and BRS Pajeú showed prolonged flowering and greater flower abortion, at 23.58%, 34.71%, and 25.55%, respectively, under an increase of 4.8 °C in temperature. This increase also reduced the viability of BRS Acauã and BRS Pajeú pollen by 34 and 7%, respectively. Heating increased stomatal opening and transpiration but reduced chlorophyll content. The enzymatic response varied according to cultivars and temperature. Changes in photosynthetic and enzymatic activities contribute to reducing pollen viability and productivity. BRS Acauã was the most affected, with an 82% reduction in the number of seeds and a 70% reduction in production. BRS Gurguéia maintained its production, even with an increase of 4.8 °C, and can be selected as a cultivar with the potential to tolerate high temperatures as it maintained pollen viability, with less flower abortion, with the synchrony of physiological and biochemical responses and, consequently, greater production.

Keywords: heat stress; oxidative stress; physiological activity; pollen grain; *Vigna unguiculata*



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1. Introduction

Climate change poses a threat to food security, compromising the final yield of crops [1], as it negatively affects plant growth, damaging their morphological, physiological and biochemical characteristics [2,3]. Among these changes in the climate, we highlight the increase in temperature, causing significant losses in the production of several species [4], including cowpea (*Vigna unguiculata* (L.) Walp.) [5]. In this sense, the recent estimate of the Intergovernmental Panel on Climatic Changes (IPCC) of a 4 °C increase in the global average temperature by the end of the century [6] predicts significant impacts for cowpea production. This is because temperatures above 33 °C alter the physiological and biochemical parameters of plants, reducing photosynthetic rates, and increasing reactive oxygen species [5] and the unviability of pollen grains, which can cause flowers to abort, reducing the final grain yield [7]. Thus, studies that report how high temperatures will affect cowpea will have great relevance since this crop has socioeconomic importance, being one of the sources of protein and carbohydrate in the diet, especially in semi-arid regions [8,9].

Thus, considering current climate change scenarios and forecasts for the end of the century, the impact on food security will be even greater. Therefore, scientific research to elucidate the impacts of climate change on cowpea production will be imperative for regions with adverse climates, such as the Brazilian semi-arid region, ensuring food security. The authors in [10] observed that to tolerate increased temperature, plants alter their metabolism through the production of compatible solutes that are capable of organizing proteins and cellular structures, providing osmotic adjustment and modifying the antioxidant system to re-establish the cellular redox balance and homeostasis. Furthermore, plants modify the expression of genes involved in the direct protection against high temperatures, such as osmoprotectants, antioxidant enzymes, and regulatory proteins [11]. These changes in gene expression cause changes in physiological and biochemical processes, gradually contributing to the development of heat tolerance, which is ideal for plant adaptation [12].

Assessing the impact of temperature stress on pollen is also of great importance, as pollen grain viability is one of the main parameters for determining plant tolerance to temperature stress [13]. For cowpea cultivars in the Brazilian semi-arid region, there are still no studies in the literature that address the effect of the high temperatures predicted by the authors in [6] on the viability of pollen grain and its consequences for grain yield. This type of study will allow the selection of cowpea cultivars tolerant to temperature stress and may contribute to the maintenance/increase in the yield of this crop. Therefore, the objective was to evaluate how increased temperature influences the pollen viability, photosynthetic and enzymatic responses, and final yield of cowpea cultivars.

2. Materials and Methods

This study was carried out in growth chambers of the Phytotron type, where temperature, humidity, and the photoperiod were carefully controlled. The design was in a 4×2 factorial scheme (cultivars \times temperature), with four replications. Seeds of Brazil seeds (BRS) of the BRS Acauã, BRS Guariba, BRS Gurguéia, and BRS Pajeú cowpea cultivars were used. The temperature regimes (Table 1) were determined from the averages (\pm range) of the minimum, average, and maximum temperatures, with values of 20, 26, and 33 °C, respectively, in the sub-middle area of the São Francisco Valley, of the last 30 years (T1). In this study, an increase of 4.8 °C (T2) over the current temperature was used, based on the scenario of temperature increase by the IPCC [6].

Table 1. Temperature regimes used in the experiment.

Temperature Regimes	Time/Temperature (°C)			
	20 h to 6 h	6 h to 10 h	10 h to 15 h	15 h to 20 h
T1 (20–26–33 °C)	20	26	33	26
T2 (24.8–30.8–37.8 °C)	24.8	30.8	37.8	30.8

The seeds were planted in a seven-liter-capacity pot, and fertilization was conducted two days prior to planting. This fertilization was based on the findings of the soil chemical analysis and followed the recommended guidelines for the specific crop [14]. In the foundation fertilization, superphosphate was used in the amount of one gram per pot. Fifteen days after plant emergence, cover fertilization was performed using ammonium sulfate (0.6 g/pot), applied via fertigation. These amounts were based on the vessel diameter, which measured as 24 cm, and volume.

2.1. Phenological Cycle

The plants were assessed daily post-sowing to ascertain the phenological cycle, employing the scale outlined by the authors in [15]. The vegetative phase included the following stages: V0—germination; V1—emergence; V2—primary leaves; V3—first open composite leaf; and V4—third open trifoliolate leaf. The reproductive phase was divided into: R5—pre-flowering; R6—flowering; R7—formation of pods; R8—filling of pods;

and R9—maturation. During flowering, the count of flower buds aborted per plant was performed.

2.2. Pollen Viability

To evaluate pollen viability, after the beginning of flowering, in the pre-anthesis, the flower buds were collected and transferred immediately to a 50 mL plastic collector with a lid containing a solution of alcohol 70% and stored in a refrigerator (4 °C) until the preparation of the slides.

The pollen grains stained with acetic carmine were analyzed and classified as normal/viable (stained grains with intact exine and larger size) and abnormal/unviable (visually small size and weak color). While acetic carmine is not clearly discriminant of viable pollen grains, it has been employed previously to differentiate between empty, deceased pollen grains and those that might be potentially viable [16]. For the preparation of the slides, the corolla of the floral bud was removed with the aid of a scalpel; then, the removal of the banner, wings, and keel was performed for the exposure of the stamens; then, the fillet was removed, leaving only the anthers containing the pollen grains (Figure 1a–f) [17]. The anthers were macerated for the release of the grains using needles from light beats. After the removal of impurities, 15 μ L of the dye was applied to the pollen grains, and the material was covered with 22 \times 22 mm laminae and observed under an optical microscope with an objective lens of 10 \times increase for the count of viable and unviable pollen grains.

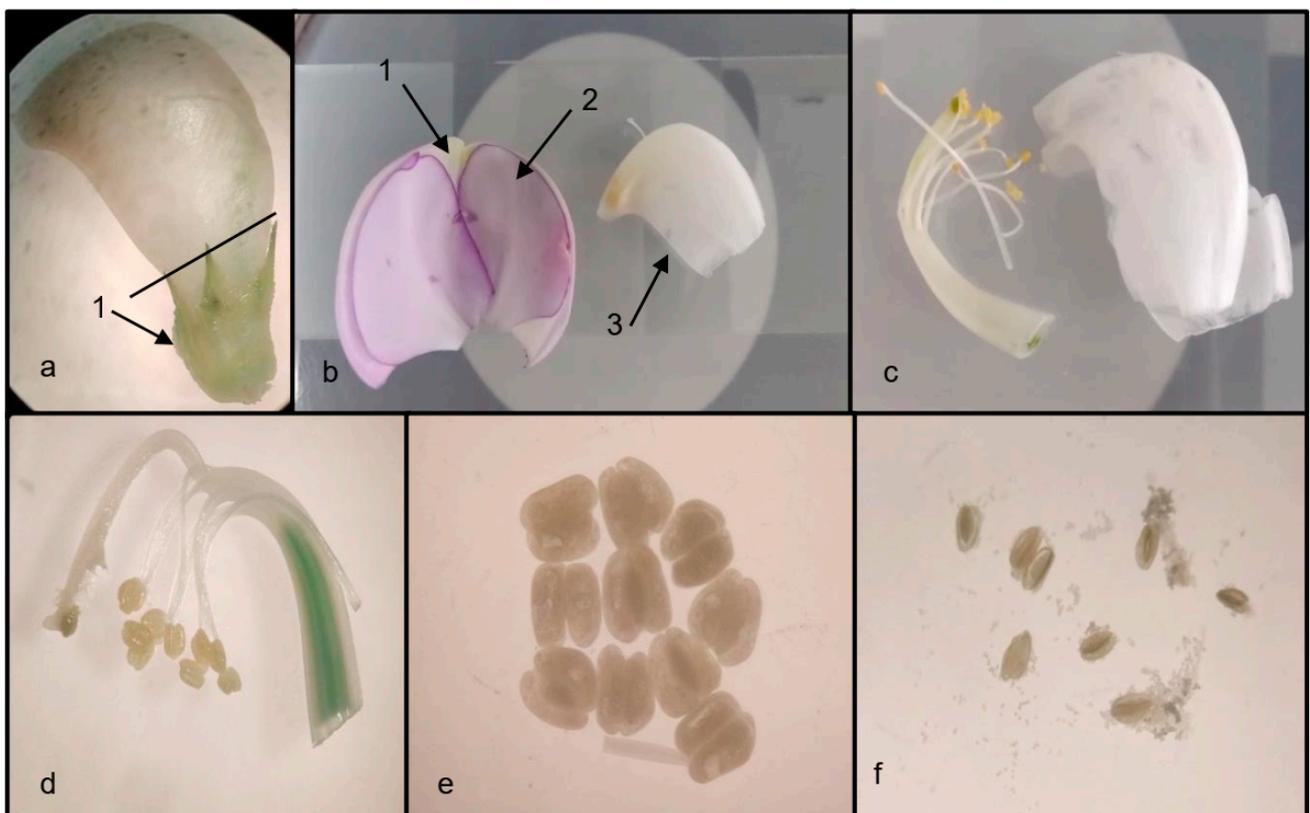


Figure 1. Removal of the corolla (1) (a); removal of the banner (1), removal of the wings (2), and removal of the keel (3) (b); separation of the stamen keel (c); stamen (d); separation of fillet from anthers (e); and removal of pollen grain from inside the anthers (f).

Stained grains with an intact exine were classified as viable, and those with an unstained interior, visually small size, and weak color were classified as unviable [18] (Figure 2).

For each cowpea cultivar, four slides were made, accounting for 100 pollen grains/blade, totaling 400 pollen grains/cultivar.

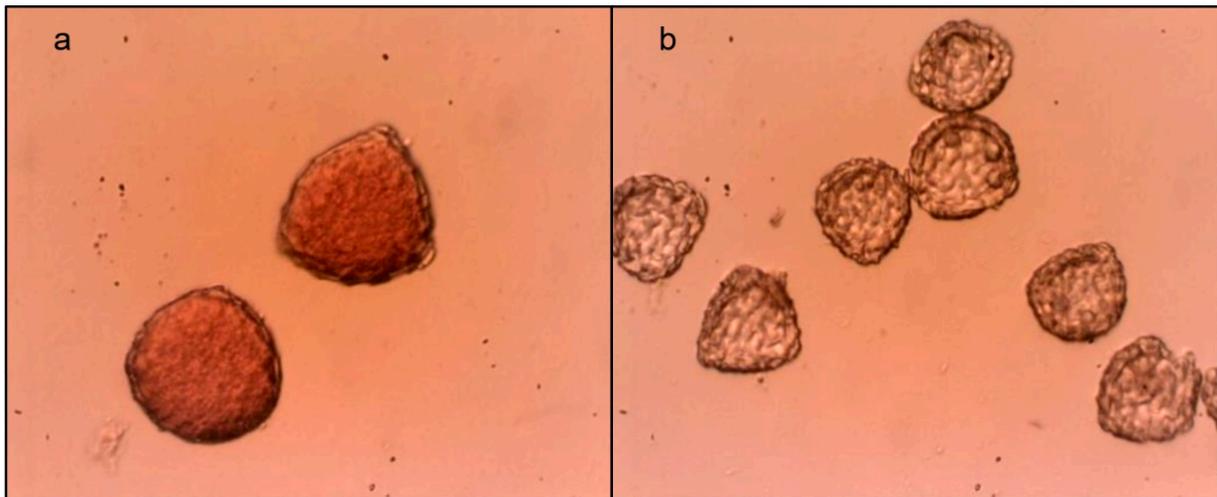


Figure 2. Viable pollen grain (stained grains with intact exine and larger size) (a) and unviable (visually small size and weak color) (b) in cowpea, colored with acetic carmine 2%.

2.3. Physiological Evaluation and Enzymatic Activity

For physiological and enzymatic activity evaluation, reading and collection were performed on fully expanded leaves, without injuries and with green coloration in the medium third of the plant, respectively. The evaluations were carried out 30 days after planting, in the vegetative stage V4 [15], at 09:00 in the morning.

The gas exchange was analyzed via the portable Infrared Gas Analyzer (IRGA), model Li-6400, manufacturer LI-COR, Lincoln, Nebraska, using artificial light fixed at $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The variables analyzed were photosynthesis rate (A), stomatic conductance (gs), transpiration (E), and leaf temperature (Tf). To evaluate the chlorophyll content of the leaves, the relative chlorophyll index was determined with the aid of the portable chlorophyll meter, model CFL 1030 FALKER, manufacturer FALKER agricultural automation Ltda., Porto Alegre, Brazil. To analyze the activities of antioxidant enzymes, samples were promptly sealed in aluminum foil envelopes and submerged in liquid nitrogen (N_2). Plant extracts were prepared by macerating 1 g of plant material in liquid nitrogen, incorporating 3 mL of extraction buffer (pH 7.5) with a concentration of 100 mM potassium phosphate.

Subsequently, the extract was centrifuged at $15,000 \times g$ for 15 min at 4°C , and the resulting supernatant served as the crude enzymatic extract. The determination of the total soluble protein content was conducted following the Bradford method [19] at 595 nm. The activity of superoxide dismutase (SOD) was assessed in accordance with the methodology of the authors in [20], with spectrophotometer readings at a wavelength at 560 nm, and defining the SOD unit as the amount of enzyme necessary to inhibit Nitroblue Tetrazolium Chloride (NBT) photoreduction by 50%.

For ascorbate peroxidase (APX) activity, it was determined as described by the authors in [21], by monitoring the oxidation rate of ascorbate using a spectrophotometer with a wavelength at 290 nm at 25°C for 60 s. The catalase activity (CAT) was determined following the decomposition of H_2O_2 for 60 s, through spectrophotometric readings at 240 nm and a temperature of 25°C , according to the method described by the authors in [22].

2.4. Productive Parameters

To evaluate the reproductive parameters of cowpea, the pods were harvested, then the seeds were removed and quantified to obtain the number of seeds per plant; later, these seeds were weighed using an analytical balance to determine the production. These evaluations were performed when the plants reached the maturation stage of the pods (R9) [15], varying according to the cycle of each cultivar.

2.5. Statistical Analysis

The obtained results underwent an analysis of variance (ANOVA), wherein isolated significant effects and interactions between sources of variation were tested. A p -value < 0.05 was deemed indicative of statistical significance. Mean comparisons were conducted using the ScottKnott test, facilitated by the SISVAR Version 5.6 program.

3. Results

3.1. Phenological Cycle and Pollen Viability

The results of the phenological cycle of cowpea are represented in Table 2. It is observed that the increase of 4.8 °C in air temperature did not significantly affect the average number of days of germination (V0), emergence (V1), pre-flowering (R5), and maturation (R9) phases for the evaluated cowpea cultivars. However, the increase in temperature resulted in the extension of flowering (R6) for the cultivars BRS Acauã, BRS Guariba, and BRS Pajeú (Table 2).

Table 2. Mean number of days for each phenological stage and cycle duration of four cowpea cultivars, maintained in two temperature regimes (20–26–33 °C and 24.8–30.8–37.8 °C).

Cultivar	Temperature	Phenological Cycle (Average of Days)										Ciclo
		V0	V1	V2	V3	V4	R5	R6	R7	R8	R9	
BRS Acauã	20–26–33 °C	1.0 a	1.0 a	8.0 a	10.3 a	26.5 b	2.0 a	1.0 b	9.0 a	7.3 a	3.0 a	69.0
	24.8–30.8–37.8 °C	1.5 a	1.3 a	4.5 b	9.8 a	32.0 a	2.3 a	23.0 a	7.0 a	6.3 a	3.0 a	90.5
BRS Guariba	20–26–33 °C	1.0 a	1.5 a	8.0 a	7.5 b	26.3 a	2.0 a	1.3 b	10.0 a	7.0 a	3.0 a	67.5
	24.8–30.8–37.8 °C	1.0 a	1.0 a	5.3 b	11.0 a	25.8 a	2.0 a	7.3 a	8.8 b	5.5 a	3.0 a	70.5
BRS Gurguéia	20–26–33 °C	1.0 a	1.5 a	7.5 a	8.5 a	43.8 a	2.0 a	1.3 a	8.8 a	4.3 a	3.0 a	79.5
	24.8–30.8–37.8 °C	1.0 a	1.0 a	6.8 a	9.0 a	44.8 a	2.0 a	2.0 a	10.5 a	5.0 a	3.0 a	81.7
BRS Pajeú	20–26–33 °C	1.0 a	1.8 a	8.5 b	12.5 a	34.0 a	2.0 a	2.5 b	10.3 a	6.0 a	3.0 a	81.5
	24.8–30.8–37.8 °C	1.0 a	1.0 a	6.0 a	9.5 b	34.8 a	2.3 a	19.5 a	8.5 a	5.3 a	3.0 a	90.8

Averages followed by the same letter do not differ from each other by the ScottKnott test at 5% probability.

The prolongation in flowering was associated with increased flower abortion (Figure 3a), causing the plants to take longer to enter the pod-formation phase (R7).

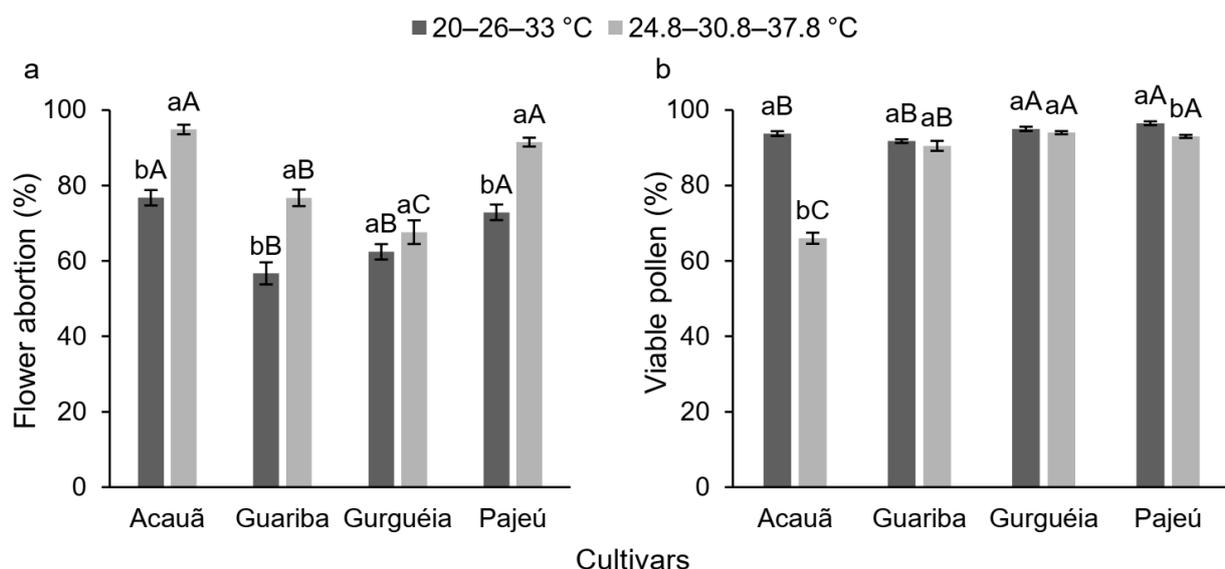


Figure 3. Number of aborted flowers (%) (a) and percentage of viable pollen grains (b) per plant of four cowpea cultivars subjected to different temperature regimes. Lowercase letters for temperature and uppercase for cultivars. Values represent averages of four biological replicates. Different letters indicate significant differences ($p < 0.05$, by ScottKnott test). Error bars (T) show SE.

This prolongation in flowering (R6) due to the 4.8 °C increase in air temperature contributed to a longer cycle of the BRS Acauã, BRS Guariba, and BRS Pajeú cultivars, with an average increase of 21.5, 3, and 9.3 days, respectively, in relation to plants maintained in the current temperature regime (20–26–33 °C) (Table 2).

In the temperature regime of 24.8–30.8–37.8 °C, flower abortion was 23.58%, 34.71%, and 25.55% for cultivars BRS Acauã, BRS Guariba, and BRS Pajeú, respectively, when compared with the regime of 20–26–33 °C (Figure 3a).

Among the cultivars, the response of the cultivar BRS Gurguéia, which did not present an alteration in the number of aborted flowers with the increase of 4.8 °C (Figure 3a), the lack of prolongation in the flowering phase (Table 2) also stands out.

The increase of 4.8 °C in the air temperature reduced the percentage of viable pollen grains of the cultivars BRS Acauã and BRS Pajeú by 34% and 7%, respectively (Figure 3b). Although temperature stress did not reduce the formation of viable pollen grains in the flowers of the cultivar BRS Guariba, there was an increase of 34.71% in aborted flowers (Figure 3a). It can be observed that the increase of 4.8 °C in the average air temperature did not reduce the percentage of viable pollen grains of the BRS Gurguéia cultivar and did not increase the percentage of aborted flowers.

3.2. Physiological Evaluation and Enzymatic Activity

Regarding gas exchange, the cowpea cultivars responded differently to each other in relation to the environment in which they were maintained. The cultivar BRS Acauã did not reduce photosynthetic activity with increasing temperature (24.8–30.8–37.8 °C) (Figure 4a). However, this increase of 4.8 °C promoted the increase in stomatic opening (Figure 4b) and, consequently, higher transpiration (Figure 4c). A similar result was observed for the cultivar BRS Guariba, although the photosynthetic rate was 93.32% higher when compared to the plants maintained in the temperature regime 20–26–33 °C (Figure 4b), and stomatic conductance and transpiration were higher in the environment with temperature stress (Figure 4a,c).

For the cultivar BRS Pajeú, a high temperature did not cause an increase in stomatic opening and transpiration; however, it reduced photosynthetic activity by 17.21% (Figure 4a–c). For the cultivars BRS Acauã, BRS Guariba, and BRS Pajeú, changes in physiological responses caused by an increased temperature contributed to the increase in floral abortion and unviability of pollen grains (Figure 3a,b).

The results of the cultivar BRS Gurguéia indicate an increase of 37.31% in photosynthetic activity in the temperature regime 24.8–30.8–37.8 °C (Figure 4a) resulting in a lower percentage of aborted flowers and grains of unviable pollens (Figure 3a,b).

For the total chlorophyll content, there was no interaction between temperature and cultivars. The 4.8 °C increase in air temperature reduced chlorophyll content by 6.82% (Figure 4d).

Leaf temperature showed no statistical difference in the evaluated treatments, with an average of 31.53 in the 20–26–33 °C regime and 31.58 in the 24.8–30.8–37.8 °C regime.

The increase in temperature also causes metabolic changes, leading to the accumulation of reactive oxygen species (ROS). For the activity of antioxidant enzymes, there was significant interaction of temperature × cultivars for SOD and APX. For CAT, only the effect that was isolated from the cultivars was significant (Figure 5a–c).

The cultivars BRS Acauã, BRS Guariba, BRS Gurguéia, and BRS Pajeú presented significant reductions of 62.78%, 78.07%, 72.83%, and 77.60% in SOD activity, respectively, in plants maintained in the regime of 24.8–30.8–37.8 °C (Figure 5a).

In the environment with heat stress, it is observed that when the cultivars are compared to each other, the BRS Pajeú cultivar showed greater SOD activity in relation to the other cultivars (Figure 5a). This result points to the sensitivity of this cultivar to temperature stress (Figure 3a,b). In addition, the cultivar BRS Pajeú also showed an increase of 73.45% in the specific activity of the APX enzyme with the increase in air temperature (Figure 5b).

The increase in temperature provided a synchrony response of these enzymes, with stability in the activity of APX in the cultivars BRS Guariba and BRS Gurguéia, and a reduction of 26.94% in the cultivar BRS Acauã (Figure 5b). The specific activity of CAT was higher for cultivar BRS Gurguéia in relation to the other cultivars (Figure 5c).

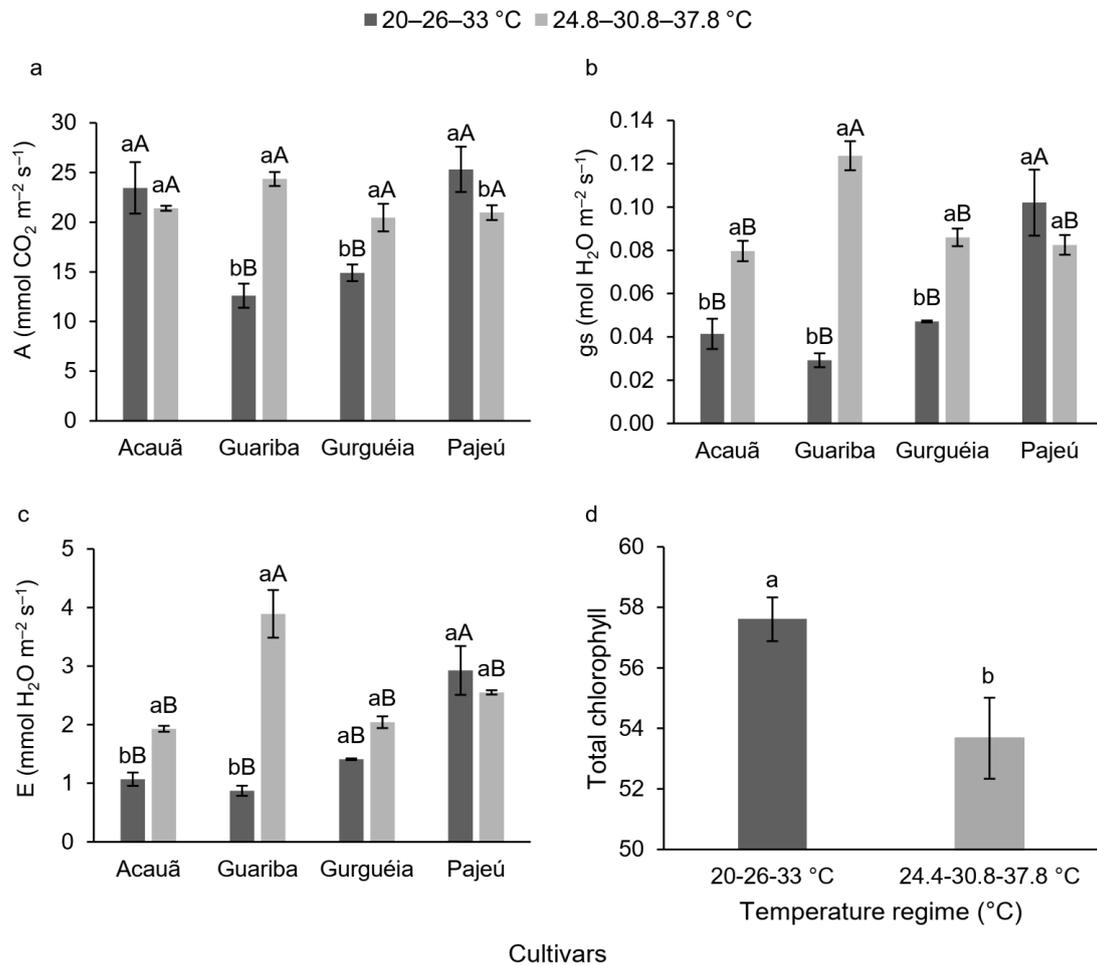


Figure 4. Photosynthetic parameters of cowpea cultivars subjected to two temperature regimes. (a) Photosynthetic rate; (b) stomatic conductance; (c) transpiration rate; (d) total chlorophyll. Lower-case letters for temperature and uppercase for cultivars. Values represent averages of four biological replicates. Different letters indicate significant differences ($p < 0.05$, by ScottKnott test). Error bars (T) show SE.

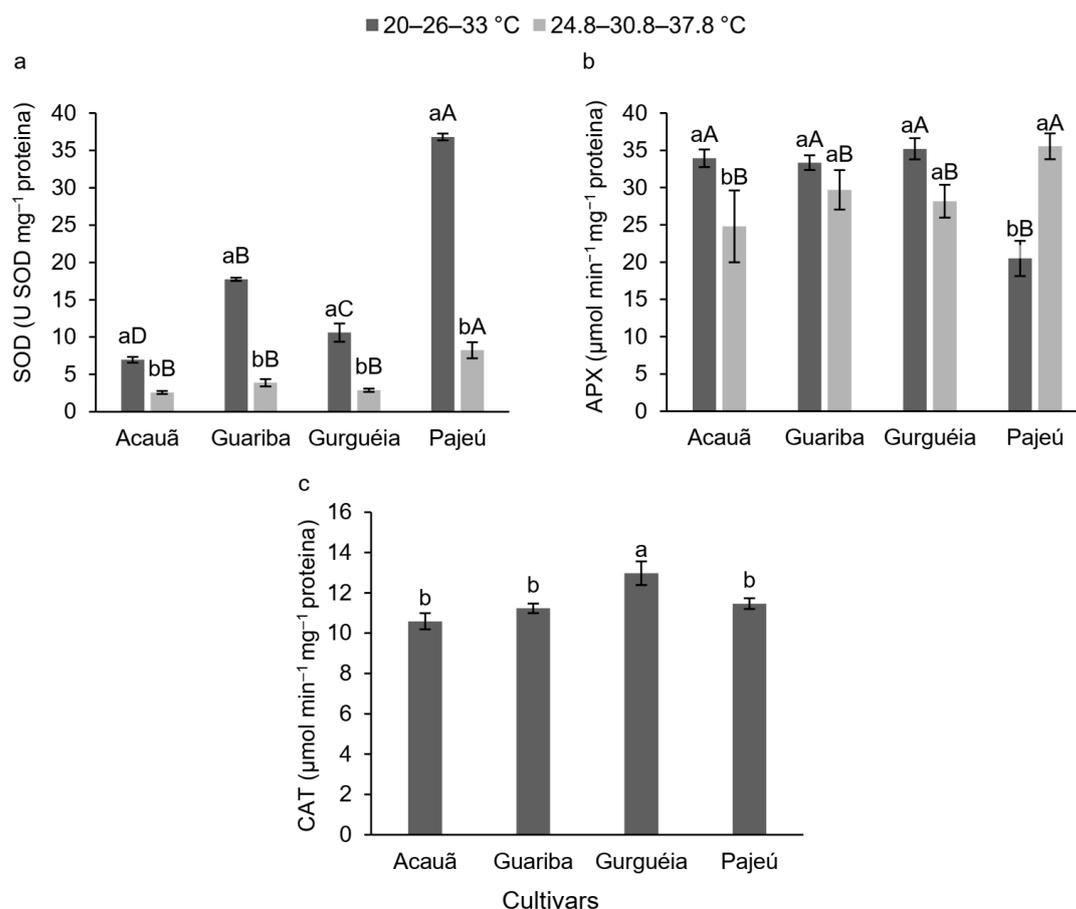


Figure 5. Enzyme activity: (a) superoxide dismutase (SOD) (U SOD mg⁻¹ protein); (b) ascorbate peroxidase (APX) (μmol min⁻¹ mg⁻¹ protein); and (c) catalase (CAT) (μmol min⁻¹ mg⁻¹ protein) in cowpea cultivars submitted to two temperature regimes. Lowercase letters for temperature and uppercase for cultivars. Values represent averages of four biological replicates. Different letters indicate significant differences ($p < 0.05$, by ScottKnott test). Error bars (T) show SE.

3.3. Productive Parameters

Regarding yield, the cowpea cultivars evaluated presented different performances in response to the environment (Figure 6a,b). The increase in the percentage of aborted flowers (Figure 3a), the unviability of pollen grains (Figure 3b), and changes in photosynthetic and enzymatic activities (Figures 4 and 5), due to the increase of 4.8 °C in air temperature, negatively influenced the production of cowpea cultivars (Figure 6).

There was a significant reduction in grain yield for the cultivars BRS Acauã, BRS Guariba, and BRS Pajeú (Figure 6a,b). The cultivar BRS Acauã was the most affected by the increase in temperature, with a reduction of 82% in the number of seeds and 70% in production (Figure 6a,b). The cultivars BRS Guariba and BRS Pajeú showed a reduction of 34 and 46% in the number of seeds, respectively (Figure 6a). Consequently, there was a decrease in production of 32% for the BRS Guariba cultivar and 52% for the BRS Pajeú cultivar (Figure 6b) due to the increase in temperature.

We highlight the seed production of the cultivar BRS Gurguéia, which maintained the number of seeds and the production with the increase in temperature (Figure 6a,b).

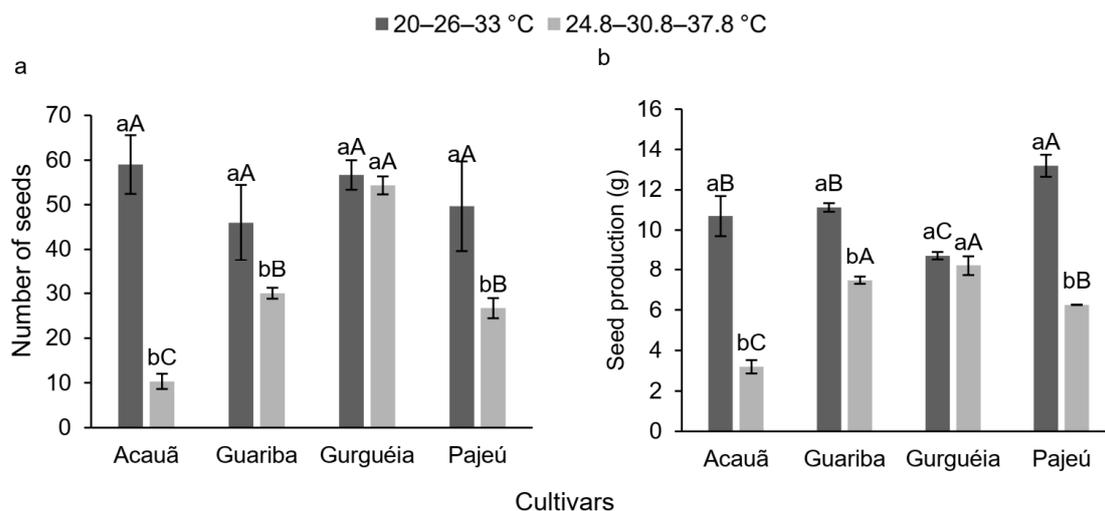


Figure 6. Number of seeds (a) and seed production (g) (b) per plant of four cowpea cultivars subjected to different temperature regimes. Lowercase letters for temperature and uppercase for cultivars. Values represent averages of four biological replicates. Different letters indicate significant differences ($p < 0.05$, by Scott-Knott test). Error bars (T) show SE.

4. Discussion

4.1. Phenological Cycle

Temperature is a fundamental element for the regulation of the phenological development of plants [23] and may shorten the phenological cycle due to the accumulation of degree days [24], or cause the prolongation of a given phase, as observed in this study (Table 2). Thus, delays will occur in the harvest, causing economic losses and, in addition, these plants will be exposed for a longer period of time to environmental weather, affecting the final yield [25].

Studies with other cowpea cultivars in the Brazilian semi-arid region confirm the sensitivity of this crop in the face of climatic scenarios. This is especially true when these climate changes coincide with the reproductive phase of the crop, negatively affecting the flowering of cowpea with the greater abortion of flowers [5,26], resulting in the loss of pods [27].

The cultivar BRS Gurguéia did not show any change in the number of aborted flowers with an increase of 4.8 °C (Figure 3a) and showed no extension in the flowering phase (Table 2), differing from the other cultivars studied. Phenological stages differ in their sensitivity to heat stress and vary between the species and genotypes of the same species [28,29].

4.2. Pollen Viability

The results show that an increase of 4.8 °C in air temperature reduces the percentage of viable pollen grains of cowpea cultivars (Figure 3b). According to the authors of [30], the increase in temperature has an impact on the size, number, and viability of pollen grain, leading to male sterility. Temperature stress increases the formation of unviable pollens during the development of the floral bud due to the low carbohydrate content in flowers, causing floral abortion [31].

It was observed that heat stress did not reduce the formation of viable pollen grains in the flowers of the BRS Guariba cultivar; however, there was an increase in aborted flowers (Figure 3a). Reactive oxygen species (ROS) suggest a negative effect of temperature not only on pollen viability, but also in the stages after pollination, such as the receptivity and retention of pollen grains on the stigma surface, pollen hydration, pollen tube germination, and egg formation [32,33]. So, even if the pollen grain is viable, temperature stress affects the viability of the egg [34], contributing to the abortion of flowers.

The authors of Ref. [35] observed that tomato plants with a higher number of viable pollen grains present a lower rate of flower abortion and higher number of fruits, even at high temperatures, and that, therefore, pollen viability can be used as a screening approach to identify heat-stress-tolerant cultivars. Furthermore, high temperatures during anthesis cause poor anther dehiscence, impair pollen tube growth, and hinder fertilization, resulting in lower seed production [36].

During the reproductive phase, gametogenesis and fertilization are highly sensitive to increased temperature. This negatively affects meiosis in male and female organs, impairing pollen germination and pollen tube growth, in addition to reducing the viability and size of the ovule. Heat stress also alters stigmatic and style positions, reduces stigma receptivity, alters embryo fertilization processes, and impedes endosperm growth [37,38]. This will cause an increase in aborted flowers, as it reduces pollen viability, since pollen grain development is a stage that is sensitive to temperature stress [39], as can be observed in this research.

4.3. Physiological Evaluation and Enzymatic Activity

At a subcellular level, increased temperature affects crucial processes for plants, such as photosynthesis, respiration, membrane functioning, and water relations, in addition to causing negative impacts on the activity of enzymes [29,40]. Heat stress can also increase the accumulation of reactive oxygen species (ROS), generate organelle malfunction, and alter phytohormone production and signaling [41].

The cultivars BRS Acauã, BRS Guariba, and BRS Gurguéia did not reduce the photosynthetic rate, even with an increase of 4.8 °C in the average air temperature (Figure 4a). Studies indicate that the high availability of carbohydrates, such as sucrose, during heat stress represents an important physiological characteristic associated with tolerance to heat stress [42]. This is because sucrose is the main product of photosynthesis, regulating plant development and stress responses through carbon allocation and sugar signaling [43], as has been observed in tolerant tomato genotypes [44].

Likewise, the carbohydrate content of developing and mature pollen grains could be a crucial factor in determining pollen quality. This is particularly evident in heat-tolerant tomato cultivars, which seem to possess a mechanism to sustain sufficient carbohydrate levels even under temperature stress [45].

The increase in stomatic opening in an environment with high temperatures may result in greater water losses to the atmosphere [46], and a reduction in the accumulation of photosynthates, proline contents, and total soluble sugars [47], which are necessary for the formation of viable pollens. The negative impact of high temperatures on plant physiological parameters [48] has a consequence on the formation of floral components and the development of new flowers [49]. This is because pollen grain is an important photosynthetic drain, requiring the high accumulation of photo assimilates for its development [48]. Thus, the impact of the increase in temperature on the physiological activities of plants may result in the formation of unviable pollen grains, as observed in this study (Figure 3a,b).

It was observed that the temperature reduced the chlorophyll content (Figure 4d). According to the authors in [46], chlorophyll reduction is one of the first physiological responses of plants to temperature stress, due to leaf sensitivity to high temperatures. For the specific activities of reactive oxygen enzymes, it was observed that increasing the temperature reduced the activity of SOD, which was already expected, as heat stress can reduce the activity of this enzyme [50]. The increase in temperature can cause a greater accumulation of reactive oxygen species (ROS) in plant tissues due to oxidative damage and lipid peroxidation [51], causing pollen unviability and floral abortion [50].

However, plants have developed several adaptation mechanisms under elevated temperatures, such as changing leaf orientation, transpirational cooling or changes in membrane lipid composition, or short-term stress avoidance and acclimation mechanisms [41].

The reduction in the specific activity of SOD directly reflects the activity of the APX enzyme, since this enzyme acts on the detoxification of H_2O_2 produced by the action of SOD [52]. With this, it is observed that the increase in temperature provided a synchrony response of these enzymes, with stability in the activity of APX in the cultivars BRS Guariba and BRS Gurguéia, and a reduction in the cultivar BRS Acauã (Figure 5b).

The specific activity of CAT was higher for cultivar BRS Gurguéia, in relation to the other cultivars (Figure 5c). This contributed to the formation of viable pollen grains (Figure 3b), since this enzyme acts directly on the detoxification of H_2O_2 in the cells of the flower buds, contributing to pollen viability [50]. Tolerant plants synthesize several antioxidant components such as ascorbic acid or glutathione, and ROS-scavenging enzymes (SOD, APX, CAT, or GPX). These components were found in several cellular compartments, indicating the importance of ROS detoxification for cell survival [53].

Studies have shown that the activity of antioxidant enzymes is directly associated with the tolerance of several plant species to temperature increase [12,54], including cowpea [5,55]. However, plant responses to high temperatures clearly depend on genotypic parameters, as certain genotypes are more tolerant [56].

Following exposure to elevated temperatures and the perception of signals, changes occur at the molecular level, altering gene expression and the accumulation of transcripts. This leads to the synthesis of stress-related proteins as a tolerance strategy [53]. Notably, the expression of heat shock proteins is recognized as a crucial adaptive mechanism in this context.

In a study carried out in rice, it was observed that the accumulation of heat shock proteins responsive to stress in the anthers contributed to greater plant tolerance and a high percentage of pollen germination at high temperatures. Thus, the increased accumulation of heat shock proteins could play an important role in protecting the cell's metabolic activities and is a key factor in the adaptation of organisms to high temperatures [36]. The tolerance conferred by heat shock proteins results in improved physiological phenomena such as photosynthesis, assimilated partitioning, water and nutrient use efficiency, and membrane stability [57]. These improvements make it possible for plants to grow and develop under heat stress.

Regarding plant tolerance responses at the reproductive stages, strategies used to discover the molecular mechanisms that confer heat tolerance during pollen development are crucial to develop heat-tolerant germplasms [40]. The results found in this study corroborate this, contributing to showing how climate changes will affect pollen viability in cowpea crops in association with physiological and biochemical responses.

4.4. Productive Parameters

During the reproductive stages, heat stress negatively affects the seed production and yield of food legumes [58,59]. Other cowpea cultivars produced in the Brazilian semi-arid region also showed a reduction in their production due to the increase in temperature, as observed by the authors in [5] when evaluating the cultivars Carijó, Pujante, Rouxinol, and Tapahium. The authors observed that the 4.8 °C increase in air temperature reduced cowpea seed production by up to 96%.

The two primary yield components in grain-producing crops are grain number and grain weight, both of which are sensitive to high temperatures [60]. Grain number is contingent upon successful fertilization, which depends mainly on viable pollen and the functioning of the ovule. Adverse environmental conditions during floral development and anthesis can negatively influence gamete viability and functionality, leading to decreases in flower fertility and, consequently, seed production [60]. Similarly, elevated temperatures during the grain filling period decrease the individual grain sizes due to a shorter filling duration [56], thereby leading to reduced yields.

High temperatures reduce pollen viability with a direct effect on production [13,35]. In addition to the negative effect during the flowering phase of the crop, physiological changes such as the reduction in carbon fixation and assimilation [61] hinder the formation

of floral components and the development of new flowers, reducing the number of pods and seeds [49].

We highlight the seed production of the cultivar BRS Gurguéia, which maintained the number of seeds and the production with the increase in temperature (Figure 6a,b). The positive response of this cultivar is explained by the high percentage of viable pollen grains, by the maintenance in the percentage of aborted flowers, and in the photosynthetic and enzymatic activities under the increase of 4.8 °C in air temperature. Thus, the success of the crop yield is determined by the combination of the number and weight of the seeds, resulting from the viability of pollen [62].

Cowpea is a crop of great socioeconomic importance. In addition, this legume is one of the main components of the diet, and contributes to the generation of employment and income. The production of this grain occurs mainly through family farming, notably being the main source of accessible protein and subsistence for the populations of the semi-arid regions [25] and can be considered a key crop in the issue of food security [8].

In the environment, increasing temperature is often associated with reduced water availability. Therefore, crops grown in tropical and subtropical environments must be evaluated for their response to elevated temperatures [63].

Therefore, studies that verify the vulnerability or risk of cowpea cultivation are strategic because they represent the first step towards the adoption of adaptation measures in the face of future climate change. Thus, the selection of thermotolerant cultivars, through the understanding of the reproductive, physiological, and biochemical responses of plants will contribute to face the challenge of reducing losses and even maintaining cowpea productivity in high-temperature areas.

5. Conclusions

In conclusion, the study revealed that the 4.8 °C increase in average air temperature increased the number of aborted flowers and reduced the formation of unviable pollen grains in some cultivars. Changes in photosynthetic activities and the accumulation of reactive oxygen species, caused by increased temperature, contribute to reduce pollen grain viability. The cultivar BRS Gurguéia maintained pollen viability with a lower flower abortion rate and, consequently, a higher production, in addition to the synchrony of physiological and biochemical responses, being tolerant to the 4.8 °C increase. Future research will be of the utmost importance to ensure the sustainability of cowpea cultivation and ensure the food security of a rapidly growing world population.

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