Paixão-Daruichi *et al.*
Journal of Cotton Research (2025) 8:39
https://doi.org/10.1186/s42397-025-00234-0

Journal of Cotton Research

RESEARCH Open Access

Impact of nitrogen sources and rates under irrigated and rainfed systems on photosynthetic pigments, nitrogen and antioxidant metabolism and cotton yield

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Abstract

Background Irrigation has been a strategy used to reduce losses due to drought, which combined with a good supply of nitrogen (N), can improve the protective system of cotton plants. The objective of this study was to investigate the effects of irrigated and rainfed cotton cultivation using different rates and sources of N. Cotton cultivation was carried out in Selvíria-MS field in the 2017/2018 harvest. The experiment was conducted in randomized blocks, which were designed in a 4 × 2 × 2 factorial scheme. The factors were composed of 0, 40, 80, and 150 kg·hm^{-2} level of N, using two sources of N under rainfed and irrigated systems.

Results The provision of irrigation provided an increase in the levels of chlorophylls (Chl) a, Chl b, total Chl, carotenoids, pheophytin, leaf chlorophyll index (LCI), N content, nitrate (NO_3^-), sucrose (SUC), the number of vegetative and reproductive branches, boll mass, and seed cotton productivity. There was no effect of N sources on any of the characteristics evaluated. Application of 150 kg·hm^{-2} level of N increased in 11%, 59%, 22%, 15%, 15% and 17% in LCI, NO_3^-, N, total amino acids (TA), SUC, and proline concentration in leaves, compared with 0 kg·hm^{-2} of N, respectively. Application of 150 kg·hm^{-2} level of N improved the leaf catalase activity (CAT) under the irrigation system; however, in a rainfed system, the highest CAT was observed at rates of 0 and 150 kg·hm^{-2} level of N. Irrigation increased in 55%, 117%, 68%, 46%, 8%, 36%, 24%, 118%, 48%, 10%, 11% and 72% in Chl a, Chl b, total Chl, CAR, LCI, pheophytins (Pheo), SUC, NO_3^-, the number of vegetative branches, the number of reproductive branches, mass of 20 bolls and seed cotton yield compared with rainfed system, respectively, however, the antioxidant system and the ammonium content of plants was stimulated by rainfed cultivation.

Conclusions Antioxidant responses increased during droughts in cotton farming, which may be connected to oxidative stress-related losses. Better N metabolism, photosynthetic pigments, and manufacturing components were all

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made possible by irrigated cultivation. The delivery of 150 kg·hm^{-2} of N in topdressing in cotton agriculture promoted the N metabolism, sucrose, total amino acids, and the plant's defense mechanism against oxidative stress.

Keywords Drought stress, Catalase, Photosynthetic pigments, Proline, Foliar amino acids

Introduction

The herbaceous cotton is a tropical Malvaceae plant with tolerance to the effects of drought compared with crops such as soybeans, rice, and corn (Almeida et al. 2017). The tolerance occurs due to the ability of the root system to grow at greater depths; in conditions of water deficiency, the root can absorb water in deeper layers of soils (Rosolem 2007). For its growth and development, the cotton plant frequently needs an adequate amount of water defined according to the soil and climate. The lack of water in critical periods of the cycle compromises the growth, development, and productivity of cotton (Luo et al. 2014; Lima et al. 2024). Most of the Brazilian cotton production is in the Cerrado region, as it presents favorable climatic conditions for the development of the crop, but with a high chance of drought periods occurring during its cultivation (Daniel et al. 2021).

The effect of water stress on cotton depends on the severity and duration of the stress, and another determining factor is the growth stage at which the stress occurs. Water is the limiting factor for cotton growth and yield; the greatest losses occur when water is lacking during the stages of flowering and boll development, causing flower and boll abortion (Zonta et al. 2017). Several studies have demonstrated that persistent water stress during the cotton growing cycle affects its growth, yield, and fiber quality (Snowden et al. 2013; Witt et al. 2020). The use of strategies to mitigate the effects of prolonged periods of drought during cultivation is crucial for the expansion of cotton cultivation in the Brazilian Cerrado.

Nitrogen (N) fertilization is essential for the growth, yield, and quality of cotton. As a result, farmers often apply large amounts of N to achieve these outcomes (Khan et al. 2021). Adequate nitrogen supply promotes the accumulation of dry matter, increases chlorophyll content, enhances the net photosynthetic rate, and improves carboxylation in cotton plants (Niu et al. 2021). However, excess nitrogen disrupts carbon (C) and nitrogen metabolism, which can impair plant growth. Furthermore, it promotes vegetative growth and may delay maturity, leading to decreased fiber quality and yield, while also contributing to environmental pollution (Lehrsch et al. 2015; Chen et al. 2021). Using nitrogen sources with lower volatilization losses can be an effective strategy to improve nitrogen availability for cotton plants during their growth and reproductive stages (Watts et al. 2017). Using N sources with lower volatility in cotton topdressing, associated with the rainfed and irrigated cultivation system, can reduce N consumption by the crop.

The prolonged drought period in rainfed farming systems has become a global problem. Studies have indicated that oxidative stress induced by drought stress results in the production of reactive oxygen species (ROS), such as H_2O_2. These ROS are highly cytotoxic due to the damage caused to lipids, proteins, nucleic acids, cell membranes, and enzymatic inactivation by the effect of lipid peroxidation (Kohli et al. 2019; Hasanuzzaman et al. 2020; Sachdev et al. 2021). The primary organelles responsible for ROS production in cells include chloroplasts, mitochondria, peroxisomes, apoplast, and the endoplasmic reticulum. To mitigate and prevent the harmful effects of these ROS, plants have developed highly regulated enzymatic and non-enzymatic mechanisms to maintain a balance between ROS production and degradation, ensuring cellular redox homeostasis (Nadarajah 2020). The main enzymes involved in scavenging ROS include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and glutathione reductase (GR) (Sachdev et al. 2021).

N fertilization has a positive effect on stress tolerance. In recent years, the impact of N supply on plant stress tolerance and the antioxidant system has garnered significant attention (Abid et al. 2016), in addition to enhancing activity of the antioxidant system, osmotic adjustment, and alterations in N metabolism (Iqbal et al. 2020). Poor N metabolism reduces the activity of enzymes such as glutamate synthase, glutamine synthetase, and nitrate and nitrite reductase, which are involved in the synthesis of amino acids derived from plant N absorption. These enzymes are essential in cellular osmotic regulation responsible for increasing cellular turgor, which helps to mitigate water stress in cotton plants (Zahoor et al. 2017; Iqbal et al. 2020).

According to Ahmad et al. (2022), increasing N application was found to enhance the activities of SOD, CAT, and POD in two sorghum varieties, thus improving their morphological and physiological functions. Similarly, Liao et al. (2023) reported that higher N fertilizer application regulated the activities of SOD, POD, and CAT in rice cultivars, thereby helping plants respond to oxidative stress. Additionally, cotton plants subjected to water deficit exhibited increased SOD, POD, and CAT activity

when provided with a higher nitrogen supply, compared with a lower nitrogen supply. This highlights the crucial role of adequate nitrogen in enhancing cotton's ability to cope with both water and oxidative stress caused by drought stress (Iqbal et al. 2020). These findings indicate that antioxidant responses to N supply vary significantly among different crops and even among different varieties of the same crop. Adequate N nutrition can be effective in mitigating the effects of drought periods in field cultivation, resulting in enhanced enzyme activity, osmotic adjustment, and changes in N metabolism (Zahoor et al. 2017; Iqbal et al. 2020; Liao et al. 2023).

N fertilization was previously reported to be effective in alleviating the effects of drought periods in field cultivation, favoring increased crop yields (Abid et al. 2016). Adequate N supply could increase leaf photosynthetic pigments and accumulation of dry mass, thereby improving carbon metabolism, and reduce flower abortion (Xue et al. 2006). Furthermore, an adequate N supply improves plant growth, cotton yield, and cotton fiber quality (Dong et al. 2012).

Therefore, given the importance of an adequate supply of N in cotton cultivation, particularly in Cerrado regions, Brazil, which experience prolonged drought periods during cultivation, this study aimed to explore the responses of morphophysiological and biochemical characteristics of cotton to water supplementation, levels, and sources of N. Specifically, it investigated photosynthetic changes, N metabolism, the antioxidant system, and possible changes in cotton productivity depending on the cultivation system, rates, and sources of N.

Material and methods

Location and experimental design

The study was conducted in the experimental area (farm) FEPE - UNESP of Ilha Solteira, located in Selvíria, Mato Grosso do Sul, Brazil. The geographical coordinates of the study area are 20°20′ of South Latitude and 51°24′ of West Longitude at an average altitude of 344 m. The climate of the region is classified according to Köppen as Aw type, defined as humid tropical with a rainy season in summer and drought season in winter. It has an average annual temperature of 24.5 °C, average annual precipitation of 1 232 mm, and average annual relative humidity of 64.8%.

The data of minimum temperature, average temperature, maximum temperature, and daily rainfall were collected at an automatic climate station installed in the experimental area; the water and irrigation deficiency calculations were carried out based on the water requirement in each cotton growth stage. The minimum temperature, average temperature, maximum temperature, daily rainfall, and water deficiency are presented in Fig. 1 in rainfed cultivation and irrigated cultivation during the field experiment in Selvíria-MS.

The soil in the cultivation area is classified as a typical clayey Latossolo Vermelho distrófico, according to the Brazilian soil classification (Santos et al. 2018). Before starting the experiment, soil samples were collected from the area and chemical analyses were carried out according to the methodology proposed by Raij et al. (1983), obtaining the following chemical characteristics: P (resin): 11 mg·dm^{-3}; organic matter: 26 g·dm^{-3}; pH ($CaCl_2$): 4.7; K: 2.5 mmol·dm^{-3}; Ca: 13 mmol·dm^{-3}; Mg: 11 mmol·dm^{-3}; aluminum (Al): 2 mmol·dm^{-3}; hydrogen (H) + Al: 42 mmol·dm^{-3}; cation exchange capacity (CTC): 68.5 mmol·dm^{-3}, and base saturation (V): 39%.

The predecessor crop in the same experimental area was cotton, too. Based on the results of the soil analysis, liming was performed according to the recommendation for cotton cultivation, raising the V to 70%. Limestone was incorporated to a depth of 30 cm using a moldboard plough and a harrow. According to the soil analysis, sowing fertilization was carried out with 350 kg·hm^{-2} of the formulated N–P–K (4–30–10). The herbaceous cotton cultivar used was TMG 81 WS, a late cycle and

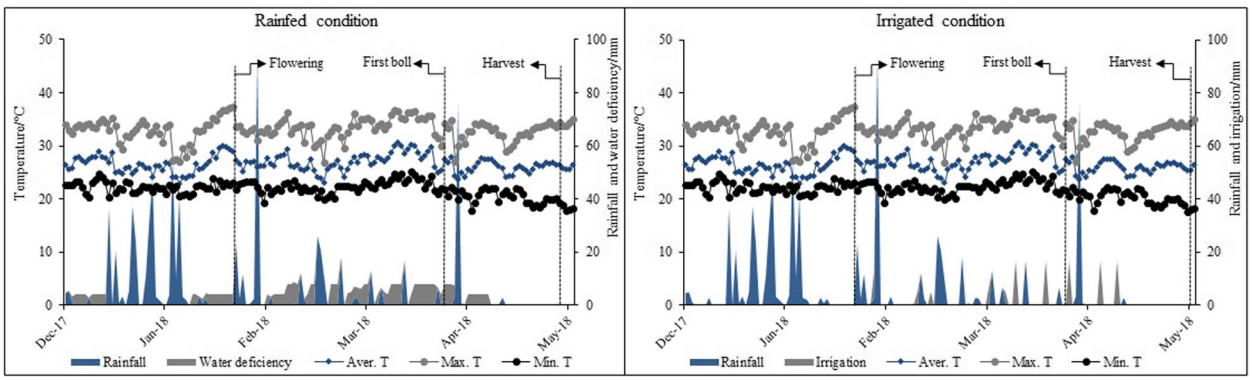


Fig. 1 Minimum, average, and maximum daily temperatures, and precipitation during the experiment with herbaceous cotton, water deficiency in rainfed cultivation and supplementary irrigation in the irrigated experiment, from December 2017 to May 2018, Selvíria-MS

tall variety, planted at a spacing of 0.9 m between rows with 8 seeds per linear meter. The experimental plot consisted of 9 lines of 4 m in length with a spacing of 1 m between the plots. The 4 central lines were designated as the usable areas, with 0.5 m discarded at each end of the plot.

The experimental design involved randomized blocks, in a 4×2×2 factorial scheme, totalizing 16 treatments with 3 replications, reresulting in 48 plots. The treatments consisted of 4 rates of N in coverage, 0 kg·hm^{-2} (the control) and rates of 40, 80, and 150 kg·hm^{-2}, 2 sources of N (urea "46% of N, equivalent to the application of 86.9, 173.9, and 326.0 kg·hm^{-2} of urea" and ammonium nitrate "34% of N, equivalent to the application of 117.6, 235.2, and 441.2 kg·hm^{-2} of ammonium nitrate") and 2 cultivation systems (rainfed "cultivation system without interference with water supply" and irrigated "water supply to cotton plants based on crop need and evapotranspiration rate"). Applications of N rates and sources were performed manually, distributing the fertilizers over the soil surface in top dressing, alongside the plant line and without incorporation into soil, at 45 days after emergence (DAE). After top dressing, the entire experimental area was irrigated by sprinkling to minimize N losses from ammonia volatilization (only in the irrigated cultivation system).

Irrigation management

The area was irrigated with a fixed sprinkler irrigation system with a flow rate of 3.3 mm·h^{-1} in the sprinklers, operating pressure of 0.25 MPa, and spacing between the sprinklers of 6×6 m. Water evaporation (ECA) was obtained daily from the Class A tank, installed at the agrometeorological station located approximately 500 m from the experimental area. The class A tank coefficient (Kp) was proposed by Doorenbos et al. (1976), which is a function of the surrounding area, wind speed, and relative humidity.

The reference evapotranspiration was determined by the expression:

ETo = Kp × ECA, where:

ETo: Reference evapotranspiration in mm·d^{-1};

Kp: Class A tank coefficient;

ECA: Class A tank evaporation in mm·d^{-1};

Crop evapotranspiration (ETc) was determined by the following expression:

ETc = Kc × ETo, where:

ETc: Crop evapotranspiration in mm·d^{-1};

Kc: Crop coefficient;

In water management, four crop coefficients (Kc) were used for the four stages of plant development, as proposed in FAO Bulletin 56 (Allen et al. 1998), specific for cotton (Table 1).

Table 1 Crop coefficients (Kc), reference evapotranspiration (ETo) and crop evapotranspiration (ETc) used during the stages of development of the herbaceous cotton crop in Selvíria - MS, 2017/2018

Phenological stage	Phase	Kc	ETo / (mm·d^{-1})	ETc
I	Initial: 0–25 days after emergence (DAE)	0.45	4.0	1.8
II	Development: 26–70 DAE	0.75	4.0	3.0
III	Fruit development: 71–120 DAE	1.15	3.7	4.3
IV	Maturity: 121 DAE – harvest	0.70	3.1	2.2

ETc reached the pre-established values close to the available soil water (ASW). Irrigations were ended at 125 DAE when 70% of bolls opened. Weed control was carried out with herbicides recommended for cultivation and manual weeding. Growth regulator was used at 55 and 78 DAE, and pest control was carried out with insecticides recommended for cotton at the beginning of infestations.

Evaluations

Biometrics traits

At 120 DAE, the number of vegetative and reproductive branches was manual counted. At harvest time (140 DAE), seed cotton productivity (yield that manual harvesting from the 4 central lines, weighing and estimation for kg·hm^{-2}), the mass of 20 bolls (random harvest of 20 bolls per plot in the middle third of the plants, weighing with the aid of a digital scale and expressed in g), the number of bolls per plant (5 random plants at the time of harvest) were manual counted.

Nitrogen concentration in leaf

Leaf analysis of the cotton plant was carried out by collecting 20 leaves from each experimental plot (blade of the 5th leaf of the main stem, from the apex to the base) at 80 DAE, in accordance with the recommendations of Silva (1999), in order to verify the effect of the treatments on the concentration of nutrients. After drying, weighing, and grinding leaf materials in a Wiley mill, the concentrations of N in the leaves of cotton were determined according to the method of Malavolta et al. (1997).

Biochemistry analysis

For all physiological and biochemical analyses, only the 2nd to the 4th fully expanded leaves (from the apex, without signs of senescence) were used, from five cotton

plants per plot as described by Santos et al. (2021). The leaves were collected at 95 DAE, macerated in liquid N, separated, identified, and stored in a freezer at −80 °C until processed for the quantification of nitrogenous compounds, soluble sugar, sucrose, amino acids, proline, chlorophylls, protein, and antioxidant enzymes as described in detail below.

Extraction of nitrogenous compounds and sugars

N compounds were extracted according to the method described by Bieleski (1966). For 1 g of the frozen plant material, 10 mL of 60% methanol, 25% chloroform, and 15% H_2O (MCW) solution was added. Then, it was left in a refrigerator in a sealed 15 mL Falcon tube for 48 h. Once this was done, the 4 mL supernatant was removed, and 1 mL of chloroform and 1.5 mL of deionized water was added. The tubes were returned to the refrigerator and sealed for 24 h so that the alcoholic and aqueous phases were separated. Only the water-soluble (aqueous) phase was used for the analysis of nitrogenous compounds (nitrate, ammonia, amino acids, sucrose, and total soluble sugars).

Ammonium and nitrate analysis

The method of Cataldo et al. (1975) was used to quantify variations in the concentration of nitrate present in cotton leaves. From the supernatant (aqueous phase), a 0.1 mL-aliquot was removed, and 0.4 mL of 5% (w/v) salicylic acid in H_2SO_4 was added. After 20 min at room temperature, 9.5 mL of 2 mol·L^{-1} NaOH was added slowly. After cooling to room temperature, a spectrophotometer reading was taken at 410 nm. Nitrate concentration was evaluated using a standard curve for nitrate solution. It was carried out according to the method proposed by McCullough (1967). From the supernatant, a 0.1 mL-aliquot was removed, and 0.5 mL of phenol solution (2.5 g phenol + 12.5 mg sodium nitroprusside was added in a final volume of 250 mL) + 0.5 mL of phosphate solution (1.25 g NaOH + 13.4 g $Na_2HPO_4.7H_2O$ + 2.5 mL 5% NaOCl in a final volume of 250 mL). The assay was incubated in a water bath for 1 h at 37 °C and then read on a spectrophotometer at 630 nm. Ammonia concentration was evaluated using a standard curve of ammonium sulfate solution.

Amino acids analysis

The method of Yemm et al. (1955) was used to quantify variations in the concentration of total soluble amino acids in cotton leaves. From the supernatant (aqueous phase), a 0.3 mL-aliquot was removed, and 500 µL of 0.2 mol·L^{-1} sodium citrate, 200 µL of ninhydrin solution (ninhydrin diluted in ethylene glycol monomethyl ether with a concentration of 5%) and 1 mL of 0.2 mmol·L^{-1} KCN solution were added. The test was heated to 100 °C for 20 min and then cooled for 10 min in running water. One mL of 60% ethyl alcohol was added, followed by a spectrophotometer reading at 570 nm. The concentration of total soluble amino acids was evaluated using a standard curve of methionine solution.

Sucrose and total soluble sugars analysis

To quantify sucrose, the Van Handel (1968) method was used. Fifty µL of supernatant + 500 µL of 30% KOH and 2 mL of concentrated sulfuric acid (H_2SO_4) were used. The mixture was homogenized by a vortex and heated in a water bath at 100 °C for 10 min. After cooling to room temperature, the samples were read in a spectrophotometer at a wavelength of 490 nm. The concentration was determined using a standard curve for sucrose solution.

The method used to measure total soluble sugars was according to Dubois et al. (1956), with following modification. The phenol-sulfuric method is based on the determination of simple sugars, polysaccharides, and their derivatives after their dehydration by sulfuric acid and subsequent complexation of the products formed with phenol. Fifty µL of supernatant sample + 500 µL of 5% phenol in H_2O + 2 mL of concentrated sulfuric acid (H_2SO_4) were used, placed directly on them, followed by homogenization. As the reaction is exothermic, it cooled at room temperature for 20 min. Next, readings were taken at a wavelength of 490 nm. The concentration of sugars was determined using a standard curve for sucrose solution.

Proline extraction and determination

The method proposed by Bates et al. (1973) was used, with some modifications. A half gram of frozen plant material was macerated in 10 mL of 3% sulfosalicylic acid. Then, the samples were transferred to Falcon tubes and centrifuged at 10 000 r·min^{-1} for 10 min. After centrifugation, 1 mL of the supernatant was collected and placed in test tubes, and 1 mL of acid ninhydrin and 1 mL of glacial acetic acid were added. The tubes were left in a water bath at 100 °C for 1 h. After that, the tubes were cooled in an ice bath. The samples were read in a spectrophotometer at a wavelength of 520 nm. The absorbances obtained were compared with the proline standard curve.

Photosynthetic pigments

Extraction for direct reading of photosynthetic pigments was carried out using the method proposed by Arnon (1949). A sample of 500 mg of frozen macerated plant material was used with the addition of 5 mL of 80% (v/v) acetone, stored for 24 h in the refrigerator. The contents of total chlorophyll, carotenoids, and pheophytins were determined.

Protein extraction

A total of 300 mg of frozen plant material was transferred to 15 mL falcon tubes, and 5 mL of 0.1 mol·L^{-1} potassium phosphate buffer, pH 6.8, containing 1 mmol·L^{-1} ethylene diaminetetraacetic acid (EDTA), 3 mmol·L^{-1} dithiothreitol (DTT), and polyvinylpolypyrrolidone (PVPP) 4% (w/v), using methodology adapted from Gomes Junior et al. (2007). The material was homogenized in a mixer and then centrifuged at 9 000 r·min^{-1} for 20 min at 4 °C.

Protein determination

The concentration of total soluble proteins was determined according to the method of Bradford (1976), using bovine serum albumin (BSA) as standard. A sample of 500 µL of the aliquot and 5 mL of the Bradford reagent, which had been previously prepared, were pipetted into test tubes. The absorbance reading was performed on a spectrophotometer at 595nm and the results were expressed in mg·g^{-1} (protein) in fresh weight (FW).

Statistical analysis

Data of all variables presented normal distribution and homogeneous variances (Shapiro-Wilk test), so they were submitted to variance analysis. The significance of the mean squares obtained in the variance analysis was tested by the *F*-test at the 5% probability level. The means for treatments differ by Tukey's test at the 5% probability level.

Results

Photosynthetic pigments

There was no significant effect ($P > 0.05$) of sources and rates of N on the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t), carotenoids (CAR), and pheophytin (Pheo) in cotton leaves, and there was no interaction between cultivation systems, sources, and rates of N for all these characteristics (Table 2, Suppl Table 1). It was observed that the irrigated cultivation system provided an increase of 55%, 117%, 68%, 46%, and 36% in the leaf content of Chl a, Chl b, Chl t, CAR, and Pheo compared with the rainfed cultivation system, respectively (Table 2).

Nitrogenous compounds, amino acids, sugars, and antioxidant activity

The use of different N sources did not influence ($P > 0.05$) the leaf chlorophyll index (LCI), N, nitrate (NO_3^-) and leaf ammonium (NH_4^+) content. There was an extremely significant effect ($P < 0.01$) of the cultivation system on the LCI and the content of nitrate and ammonium in the leaves but no significant effect on N content as well as the N rates on the foliar N content. However, there was a significant effect of the interaction of the cultivation systems and the N rates inleaf (Table 3, Suppl Table 2).

Cotton cultivation in the irrigated system favored an increase by 8% and 118% in LCI and foliar NO_3^- content

Table 2 Contents of photosynthetic pigments: chlorophyll a (Chl a) (mg·g^{-1} FW), chlorophyll b (Chl b) (mg·g^{-1} FW), total chlorophyll (Chl t) (mg·g^{-1} FW), carotenoids (CAR) (mg·g^{-1} FW), and pheophytins (Pheo) (µg·g^{-1} FW) extracted from fresh leaf mass of plants of herbaceous cotton depending on the cultivation systems, rates and sources of N, at 95 DAE

Treatments	Chl a /(mg·g^{-1} FW)	Chl b	Chl t	CAR	Pheo /(µg·g^{-1} FW)
Cultivation systems					
Rainfed	4.03 b	1.72 b	5.75 b	4.25 b	12.24 b
Irrigated	6.23 a	3.73 a	9.66 a	6.19 a	16.61 a
N sources					
Urea	5.07 a	2.55 a	7.62 a	5.25 a	15.32 a
Ammonium nitrate	5.18 a	2.60 a	7.79 a	5.21 a	13.52 a
N rates/(kg·hm^{-2})					
0	3.73	1.92	5.65	3.73	10.30
40	5.35	2.65	8.01	5.28	15.71
80	5.87	2.84	8.71	5.87	17.90
150	5.56	2.90	8.44	5.99	13.76
Average	5.13	2.58	7.71	5.22	14.42
CV/%	24.47	23.63	24.77	24.00	24.55

Averages followed by different letters in the columns differ significantly from each other by the Tukey test at 5% probability

Table 3 Leaf chlorophyll index (LCI), leaf nitrogen content (g·kg^{-1}), and nitrate (NO_3^-) (µmol·g^{-1} FW) and ammonia (NH_4^+) (µmol·g^{-1} FW) contents at 95 DAE in leaf tissues of herbaceous cotton plants as a function of N rates and sources in cultivation systems (rainfed and irrigated)

Treatments	LCI –	N /(g·kg^{-1})	Nitrate /(µmol·g^{-1} FW)	Ammonium
Cultivation systems				
Rainfed	52.77 b	49.46 a	18.42 b	0.392 a
Irrigated	56.94 a	50.91 a	40.24 a	0.315 b
N Sources				
Urea	55.62 a	50.64 a	28.14 a	0.339 a
Ammonium nitrate	54.09 a	49.73 a	30.52 a	0.368 a
N rates/(kg·hm^{-2})				
0	51.82	47.12	23.36	0.393
40	54.31	49.32	27.67	0.362
80	55.52	51.08	29.10	0.352
150	57.77	53.22	37.18	0.307
Average	54.85	50.69	29.33	0.354
CV/%	7.07	7.15	32.98	26.48

Averages followed by different letters in the columns differ significantly from each other by the Tukey test, at 5% probability

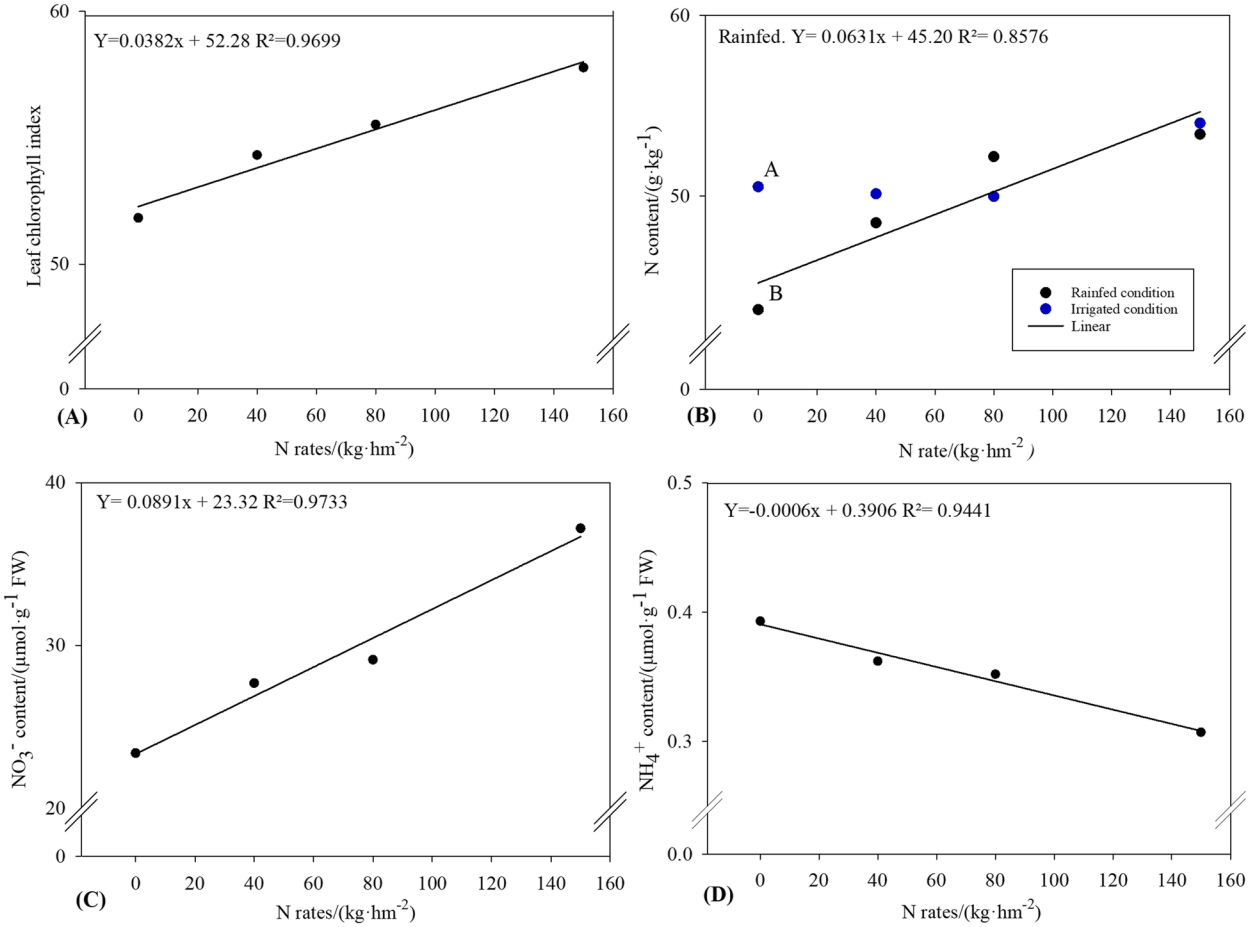


Fig. 2 Leaf chlorophyll index (**A**), leaf nitrate (**C** - NO_3^-), and leaf ammonia (**D** - NH_4^+) contents as a function of N rates, and interaction between N rates and cultivation systems on leaf N content (**B**), as a function of N rates in cotton cultivation at 95 DAE

compared with the rainfed cultivation system, respectively. However, the leaf NH_4^+ content in rainfed cultivation system was 24% higher than that of irrigated cultivation system (Table 3). Providing 150 kg·hm^{-2} of N in top dressing favored an 11% increase in LCI and 59% in leaf NO_3^- content in cotton compared with not supplying N in top dressing (Fig. 2A, C). Under the application of 0 kg·hm^{-2} of N in top dressing, a 15% increase in leaf N content was observed in the irrigated cropping system compared with the rainfed cropping system. Furthermore, a 22% increase in leaf N content was observed under the dose of 150 kg·hm^{-2} of N compared with the dose of 0 kg·hm^{-2} of N in the rainfed cropping system (Fig. 2B). In contrast to this, a 22% reduction in leaf NH_4^+ content was observed under the dose of 150 kg·hm^{-2} of N compared with the dose of 0 kg·hm^{-2} of N in top dressing in cotton (Fig. 2D).

There were no significant effects of sources and rates of N under sucrose (SUC), total soluble sugars (TSS), total free amino acids (TA), and total soluble proteins (TSP). And no significant effect of the cultivation system was selected on TSS, TA or TSP. There was no significant interaction between the factors for TSS and TSP characters (Table 4, Suppl Table 3). There was an extremely significant effect ($P < 0.01$) of the cultivation system on the SUC; an increase of 24% in SUC was observed under the irrigated cropping system compared with the rainfed cropping system (Table 4).

An increase of 15% in leaf SUC was observed under fertilization with 150 kg·hm^{-2} of N compared with the fertilization at 0 kg·hm^{-2} of N in top dressing in cotton cultivation (Fig. 3A). Providing 150 kg·hm^{-2} of N in top dressing favored an increase in 15% in leaf TA in cotton compared with no fertilization with N in top dressing (Fig. 3B).

There were no significant effects of N rates and sources ($P > 0.05$) on the activity of SOD and CAT, but there was an extremely significant effect ($P < 0.01$) of N rates on leaf proline content (Suppl Table 4). There was also an extremely significant effect ($P < 0.01$) of cultivation

Table 4 Contents of sucrose (SUC) (mg·g^{-1} FW), total soluble sugars (TSS) (mg·g^{-1} FW), total amino acids (TA) (mg·g^{-1} FW), total soluble proteins (TSP) (mg·g^{-1} FW) in leaf tissues of herbaceous cotton depending on cultivation systems, sources, and rates of N at 95 DAE

Treatments	SUC /(mg·g^{-1} FW)	TSS	TA	TSP
Cultivation systems				
Rainfed	64.51 b	100.24 a	19.09 a	4.63 a
Irrigated	79.73 a	107.17 a	20.14 a	6.07 a
N Sources				
Urea	72.28 a	105.68 a	19.62 a	5.70 a
Ammonium nitrate	71.87 a	101.73 a	19.61 a	5.00 a
N rates/(kg·hm^{-2})				
0	67.44	99.77	18.51	5.99
40	69.72	100.48	19.53	4.95
80	73.60	107.66	19.44	5.08
150	77.53	114.90	20.98	5.37
Average	11.39	23.89	9.29	17.93
CV (%)	72.07	103.70	19.61	5.35

Averages followed by different letters in the columns differ significantly from each other by the Tukey test, at 5% probability

systems on the activities of SOD and CAT, and an increase of 26% in SOD and an increase of 61% in CAT were observed in cotton leaves under the rainfed cultivation system compared with the irrigated cultivation system (Table 5, Suppl Table 4). An extremely significant interaction ($P<0.01$) of cultivation systems and N rates was observed on CAT activity (Table 5, Suppl Table 4). There was no significant effect of the other interactions for SOD, CAT, or proline (Table 5, Suppl Table 4).

The CAT activity was 200% higher at the rate of 0 kg·hm^{-2} in the rainfed cultivation compared with the irrigated cultivation, and the behavior of the activity of the CAT in the rainfed system was quadratic with reduction of the enzyme activity with the supply of N and increasing again CAT activity at the rate of 150 kg·hm^{-2}. However, a 136% increase in CAT activity was observed under the irrigated cultivation system at the rate of 150 kg·hm^{-2} of N in relation to the rate of 0 kg·hm^{-2} of N in top dressing in cotton cultivation (Fig. 4A). The highest leaf proline content was observed at the rate of 150 kg·hm^{-2} of N, being 17% higher than 0 kg·hm^{-2} of N (Fig. 4B).

Cotton productive components

There were no significant effects ($P>0.05$) of N sources and rates on the number of vegetative and reproductive branches, mass of 20 bolls (M20), and seed cotton yield, and there was no interaction between the factors on these characteristics either (Table 6, Suppl Table 5). There was a extremely significant effect ($P<0.01$) of the cultivation systems on the number of vegetative and reproductive branches, M20, and yield (Suppl Table 5). Cotton grown in the irrigated cultivation system showed an increase of 48% in the number of vegetative branches, 10% in the number of reproductive branches, 11% in M20, and 72% in yield compared with those in the rainfed cultivation system (Table 6).

Discussion

Prolonged periods of drought in the Brazilian Cerrado favor the degradation of the contents of Chl a, Chl b, Chl T, CAR, Pheo, and LCI in leaves, in response to which higher contents of these parameters were observed in the irrigated system (Tables 2 and 3). Chl b plays an essential role in the absorption of blue and violet light, and Chl a is the reaction center pigment that can convert light energy into electrical energy, important for improving the light-trapping ability of the leaf (Wang et al. 2011).

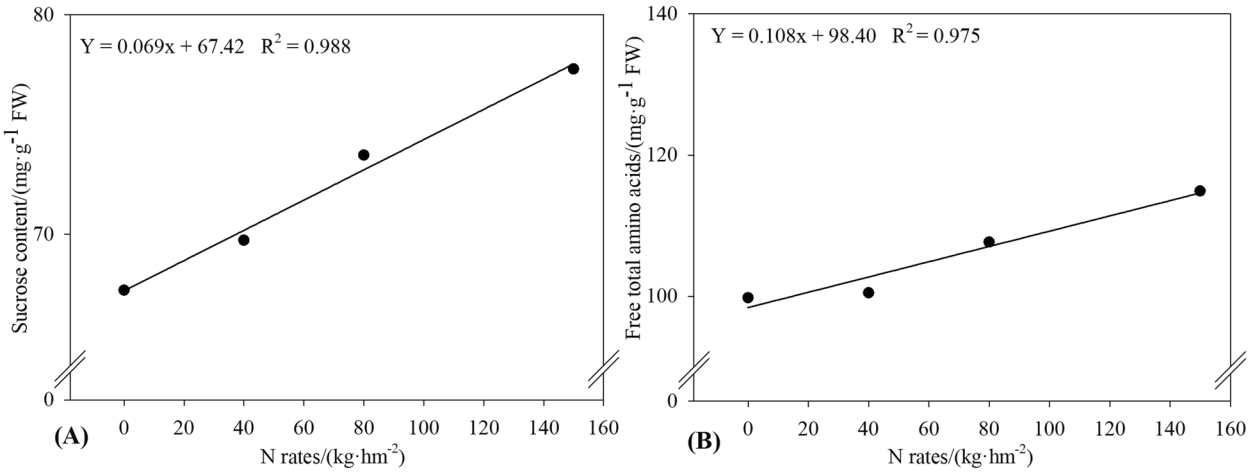


Fig. 3 Sucrose (**A**) and total free amino acids (**B**) contents as a function of N rates in cotton cultivation at 95 DAE

Table 5 Activity of the antioxidant enzymes superoxide dismutase (SOD: Unit per mg of protein^{-1}), catalase (CAT: µmol H_2O_2 decomposed min^{-1} mg of protein^{-1}) and peroxidase (POD: µmol of H_2O_2 decomposed min^{-1} mg of protein^{-1}), Proline amino acid content (mg·g^{-1} FW) in leaf tissues of herbaceous cotton as a function of N rates and N sources in cultivation systems (rainfed and irrigated), at 90 DAE

Treatments	SOD /(U·mg^{-1} (protein))	CAT /(µmol·(H_2O_2 decomposed)·min^{-1}·mg^{-1} (protein))	POD	Proline /(mg·g^{-1} (FW))
Cultivation systems				
Rainfed	17.19 a	0.098 a	0.313 a	0.140 a
Irrigated	13.61 b	0.061 b	0.234 a	0.132 a
N sources				
Urea	14.11 a	0.080 a	0.272 a	0.133 a
Ammonium nitrate	16.68 a	0.081 a	0.275 a	0.141 a
N rates/(kg·hm^{-2})				
0	15.48	0.093	0.242	0.133
40	15.46	0.071	0.350	0.136
80	15.68	0.057	0.214	0.120
150	14.98	0.097	0.288	0.155
Average	15.40	0.080	0.274	0.136
CV/%	30.32	29.19	34.64	18.11

Averages followed by different letters in the columns differ significantly from each other by the Tukey test, at 5% probability

Small periods of drought during cultivation benefit the opening of the photosystem II (PSII) reaction center; thus, a greater amount of light is used to promote the transport of photosynthetic electrons (Zhao et al. 2007). Irrigation was provided to the cotton plants only when the KC of the crop for each developmental stage possibly improved the fluorescent activity of the plants. However, in the rainfed system, there were periods of drought of up to 15 days after cotton flowering and maturation, which damaged the photosynthetic system of cotton plants (Lima et al. 2024). The electron transport rate in cotton plants was strongly affected by prolonged periods of drought, reaching 80% in severe drought conditions (Deeba et al. 2012). The degradation of the photosynthetic system of cotton plants caused by oxidative stress in the rainfed cultivation system impaired the chlorophyll contents and the quantum yield of PSII (Zhang et al. 2021).

Adequate water supply favors better regulation of carbon (C) metabolism, increasing mass accumulation in cotton plants. As C metabolism is regulated to efficiently utilize limited photosystem I (PSI) resources, C is partitioned into free amino acids for carbon skeletons (Paul et al. 2003), which also affects crop quality (Halford 2010). During the flowering period, the LCI and SUC content in leaves increased under the irrigated system cultivation in relation to the rainfed system cultivation (Tables 3 and 4), as it obtained higher SUC and LCI under fertilization with 150 kg·hm^{-2} of N compared with the application of 0 kg·hm^{-2} of N in top dressing in cotton cultivation (Figs. 2 and 3). Physiologically, as the number of fruiting branches and bolls increases, cotton growth shifts from the vegetative growth to the reproductive growth, and C from older leaves that previously had stored sugars becomes efficiently exported to bolls (Liu et al. 2014). This increases the efficiency of mass accumulation in the vegetative and productive branches, and favors an increase in seed productivity (Liu et al. 2015).

N metabolism is related to C metabolism in plants, as sucrose is used to indicate the N nutrition status of plant

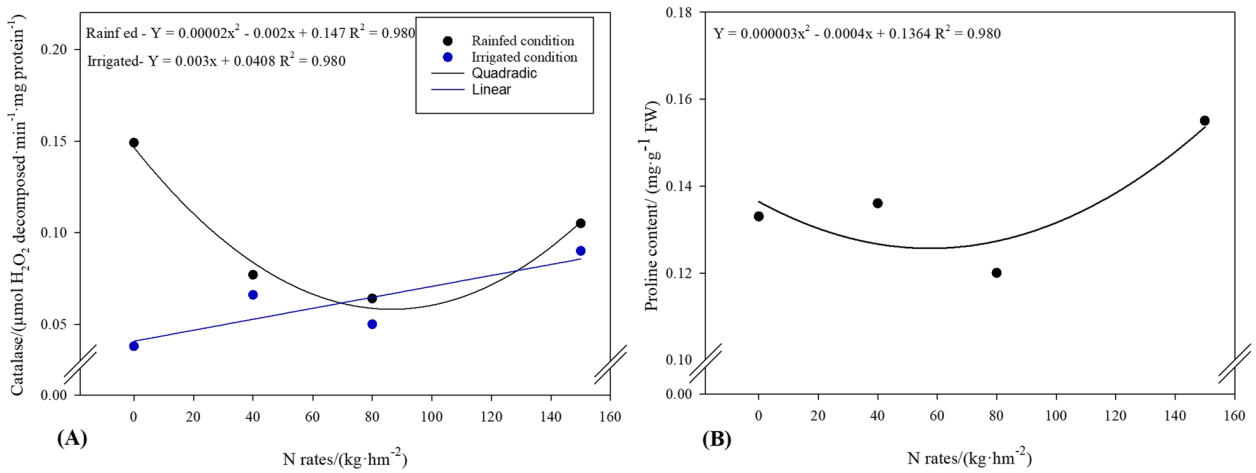


Fig. 4 Activity of the catalase enzyme (**A**), proline content (**B**) in leaves of herbaceous cotton as a function of N rates and in different cultivation systems

Table 6 Number of vegetative and reproductive branches (No·plant^{-1}), mass of 20 bolls (M20) (g), and seed cotton productivity (Yield) (kg·hm^{-2}) of herbaceous cotton plants as a function of cultivation systems, rates, and sources of N

Treatments	No. of branches		M20	Yield
	Vegetatives	Reproductives	/g	/(kg·hm^{-2})
Cultivation systems				
Rainfed	1.97 b	22.60 b	98.85 b	2.003 b
Irrigated	2.90 a	24.80 a	109.66 a	3.437 a
N Sources				
Urea	2.45	23.66	103.23	2.843
Ammonium nitrate	2.42	23.74	105.29	2.596
N rates/(kg·hm^{-2})				
0	2.37	24.41	103.04	2.719
40	2.39	25.40	105.10	2.721
80	2.30	26.00	105.77	2.737
150	2.69	27.82	103.13	2.702
Average	2.44	23.70	8.68	17.44
CV/(%	19.86	2.11	104.26	2 720

Averages followed by different letters in the columns differ significantly from each other by the Tukey test at 5% probability

tissues (Liu et al. 2015). In the present study, the contents of N, NO_3^-, SUC, and AT in the leaves increased with increasing N rates in top dressing, and the reduction in NH_4^+ levels with increasing N supply (Figs. 2 and 3). These results indicate that good nutrition with N favors both N metabolism and C metabolism, which promotes increased transport of carbohydrates from branches and leaves to bolls (Liu et al. 2014). The amount of carbohydrates, proteins, amino acids increase with the increases of N supply (Poorter et al. 2009). The increase in amino acids with the greater supply of N contributes to the regulation of nitrate in the acquisition, transport, and assimilation of N by the plant (Barbosa et al. 2010), and also contributes to the osmotic regulation that plays a fundamental role in the cell expansion of the plant leaf (Jing et al. 2009).

In irrigated cultivation, there were a greater number of vegetative and reproductive branches, greater boll mass, and higher seed cotton yield. The losses observed in rainfed cultivation are mainly related to the dry periods that occurred during the cotton cultivation period. Drought periods during the rainfed cultivation have been a reality in recent years, and their effects on the productive components of cotton have been reported in several regions of the Brazilian Cerrado (Echer et al. 2024; Lorençone et al. 2025). Losses due to prolonged drought exceed 50% of seed cotton yield in relation to water supply with irrigation as indicated for the crop (Witt et al. 2020; Echer et al. 2024). There was greater activity of CAT and SOD enzymes in the rainfed system compared with the irrigated one, possibly due to the activation of the plant's defense system to combat oxidative stress caused by prolonged water stress (Table 5). The results suggested that part of the oxidative stress caused by drought was mitigated by the activity of CAT and SOD. SOD activity acts primarily to convert superoxide radicals into hydrogen peroxide (H_2O_2) and cellular oxygen (O_2) (Weydert et al. 2010), and CAT activity is often used by cells to rapidly catalyze the decomposition of reactive oxygen species (ROS), mainly H_2O_2 into oxygen and water, preventing ROS formation, and maintaining cellular redox homeostasis (Gratão et al. 2005; Glorieux et al. 2017).

There was an increase in CAT activity with increasing N rates in the irrigated cultivation system. However, in the rainfed cultivation system at all N rates it was mainly because of the plant's defense system (Fig. 4A). These results demonstrate that adequate N supply favors the protection of cotton plants from oxidative stress caused by the increase in ROS under drought conditions (Iqbal et al. 2020). Lack of nitrogen supply, combined with stress conditions in plants, exacerbates oxidative stress. Consequently, providing nitrogen to plants may be linked to enhanced protection of leaf tissues due to increased metabolic and physiological activity within the tissues (Yue et al. 2021). At the rate of 150 kg·hm^{-2} of N, it was possible to see an increase of 17% in the concentration of proline in the leaves compared with not applying N as a top dressing on cotton (Fig. 4B). Proline is an osmolyte that accumulates in plants in response to abiotic stress conditions (Grigore et al. 2011). It plays an important role in the process of cellular osmotic adjustment of plants under water stress conditions, directly stabilizing subcellular structures (membranes and proteins), scavenging free radicals that buffering redox potential, alleviating cellular acidosis, and acts as a signaling molecule in stress responses (Rana et al. 2017). In this form, the increase in leaf proline content is most often associated with increased tolerance to water stress (Al Hassan et al. 2016).

In this study, it was possible to highlight that the irrigated cultivation system provides higher contents of Chl a, Chl b, Chl T, CAR, Pheo, LCI, NO_3^-, and N in cotton leaves compared with the rainfed cultivation system. In addition, a greater number of vegetative and reproductive branches, M20, and yield were verified under the irrigated system in relation to the rainfed system. When exploring the different cultivation systems, it was found that under conditions of drought stress (in the rainfed cultivation system), the activities of CAT and SOD enzymes in cotton leaves were higher than in the rainfed system, demonstrating the functioning of the plant's defense system to combat the period of water deficit.

The N sources used did not influence the characteristics evaluated. However, the supply of 150 $kg \cdot hm^{-2}$ of N in top dressing provided an improvement in the contents of N, NO_3^-, SUC, AT, CAT, proline, and LCI in cotton leaves when compared with applying 0 $kg \cdot hm^{-2}$ of N in top dressing. These findings demonstrate the behavior of cotton plants in relation to their response to nitrogen fertilization, mainly in relation to the absorption and use of N and its conversion into amino acids and sucrose, as in the plant's defense system. A new perspective regarding N supply behavior during cotton cultivation will assist in responding to excessive ROS production under stress conditions.

Conclusions

Drought periods in cotton cultivation in rainfed systems stimulated an increase in antioxidant responses, which may be related to losses caused by oxidative stress. Adequate water supply in irrigated systems provided better functioning of N metabolism, photosynthetic pigments, and production components. The contents of N, NO_3^-, sucrose, total amino acids, leaf chlorophyll index, proline, and CAT activity in leaves were favored by the supply of 150 $kg \cdot hm^{-2}$ of N in topdressing in cotton cultivation, improving the use and conversion of N and the plant's defense system. The N sources used in the study were not able to influence any of the physiological, metabolic, nutritional, and production characteristics of cotton.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-025-00234-0.

Supplementary Material 1.

Acknowledgements
This study was partly funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES) – Finance Code 001.

Authors' contributions
Paixão-Daruichi AP, Martins AR, de Camargo LS, and Oliveira CES wrote the first draft of the manuscript. Paixão-Daruichi AP and Furlani Junior E designed the experiments. Paixão-Daruichi AP, Rodrigues RAF, da Silva DB, Pereira TC, De Lima MMF, Vieira NCS, and De Faria-Nocchi RC collected and analyzed the data. Paixão-Daruichi AP, Furlani Junior E, de Camargo LS, Oliveira CES, and Martins AR interpreted the results and revised the manuscript. All authors read and approved the final revision of the manuscript.

Funding
This study was partly funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES) – Finance Code 001.

Data availability
Data will be made available on request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 4 November 2024 Accepted: 15 July 2025
Published online: 03 November 2025

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