Acta Physiologiae Plantarum (2024) 46:60
https://doi.org/10.1007/s11738-024-03684-2

ORIGINAL ARTICLE

Antioxidant metabolism in forage cactus genotypes intercropped with *Gliricidia sepium* in a semi-arid environment

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Received: 22 July 2022 / Revised: 29 April 2023 / Accepted: 14 April 2024 / Published online: 7 June 2024

Abstract

Although forage cactus is a CAM plant capable of adjusting to adverse conditions, little is known about the regulation of the antioxidant defense system under typical growth conditions and even less under competitive environment such as intercropping system. Therefore, this study aimed to investigate the regulation of the antioxidant defense system in three forage cactus genotypes grown in intercropping. The experimental design was in randomized blocks set up in a 3×2 factorial with three replications, consisting of three forage cactus genotypes ('Orelha de Elefante Mexicana'—*Opuntia stricta* (Haw.), 'Miúda'—*Nopalea cochenillifera* (L.) Salm. Dyck, and 'Baiana' – *N. cochenillifera* (L.) Salm. Dyck) and two cropping systems (monocropping and intercropping with *Gliricidia sepium*). Intercropping significantly affected the antioxidant metabolism of forage cactus genotypes. 'Orelha de Elefante Mexicana' in intercropping showed higher contents of photosynthetic pigments and H_2O_2, and activities of SOD (EC 1.15.1.1) and CAT (EC 1.11.1.6), and lower contents fresh mass, dry mass, and MDA, and activity of APX (EC 1.11.1.11), than under monocropping. 'Miúda' showed similar results except for dry mass, which was the same in both cropping systems. In turn, 'Baiana' in intercropping showed higher contents of fresh and dry mass, water, photosynthetic pigments, and H_2O_2, activities of SOD, CAT, and APX, and lower lipid peroxidation than under monocropping. Thus, intercropping favored the 'Baiana' as a function of the effective role of the antioxidant enzyme system, reducing lipid peroxidation, maintaining the stability of the photosynthetic process, and increasing growth.

Keywords Forage cactus · Lipid peroxidation · Antioxidant system · Cropping system

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Communicated by S. Srivastava.

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Introduction

Limited agricultural production in arid and semi-arid regions around the world is mainly due to low water availability (Ashoori et al. 2021). Furthermore, climate change has intensified this scenario, causing greater vulnerability in these regions by limiting activities such as livestock farming, e.g., cattle, which depend on forage (Marengo et al. 2016; Jardim et al. 2020). Given these conditions, it is increasingly necessary to search for crops adapted to the climatic conditions of semi-arid regions.

Among such adapted crops, forage cactus (*Opunti*a spp. and *Nopalea* spp.), members of the Cactaceae family that originated in Mexico, shows minimum water demand and tolerates high temperatures. Furthermore, it is widely used as animal feed, especially for ruminants, and can be provided throughout the year, including during drought (Jardim et al. 2020, 2021). Therefore, forage cactus is the most important cultivated cactus in the world, with a planted area of 1,000,000 ha (Cardador-Martínez et al. 2011). In turn, Brazil has a representative planted area of 600,000 ha, located mainly in the Northeast Region and used for animal feed (Dubeux Júnior et al. 2013).

Forage cactus is a crop of high fresh biomass yield, water content, abundant energy source, good palatability, high acceptability, and low production costs (Marques et al. 2017; Moura et al. 2020). However, it has a low nutrient value to be used as an exclusive animal feed. From this perspective, intercropping systems could be a viable alternative, especially if intercropped with a highly nutritive crop in terms of protein and fiber, increasing the yield of the production area (Bakhtiyari et al. 2020; Lira et al. 2022). Moreover, the plant used for intercropping should be carefully chosen since this practice can result in competition for nutrients, water, light, and space, characterizing an unfavorable growth condition, which potentially leads to production losses and induces oxidative stress in plants (Gecaite et al. 2021; Alves et al. 2021). *Gliricidia sepium* is a fast-growing leguminous tree species that is commonly used in agroforestry systems due to its ability to fix nitrogen and improve soil fertility. Thus, intercropping *G. sepium* with forage cactus can improve forage quality and production, leading to improved animal performance in terms of weight gain and milk production (Camelo et al. 2021; Lira et al. 2022; Singh and Sahoo 2015).

Although forage cactus can be classified as a CAM plant with an adapted metabolism to unfavorable conditions, this does not prevent severe damage with the overproduction of reactive oxygen species (ROS). ROS can be formed under stress through two main processes: the first is metabolic, in which ROS are generated due to interruptions in the metabolic activity, and the second consists of ROS signaling, generated to signal the transduction pathway in response to stress (Miller et al. 2010; Mittler et al. 2012; Berwal et al. 2020). Under normal conditions, ROS are merely the by-products of metabolic pathways, e.g., photosynthetic processes, respiration, and photorespiration. However, the overproduction of ROS such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide ($O^-{}_2$), and hydroxyl radicals indicates that plants face unfavorable conditions (Czarnocka and Karpiński 2018; Kaur et al. 2019; Sharma et al. 2019).

The overproduction of ROS within plant cells immediately triggers defense mechanisms in order to reduce or eliminate excess ROS. The antioxidant defense system, composed of enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GSH-Px), peroxidases (POX), and glutathione reductase (GR), stands out among such defense mechanisms (Gratão et al. 2015; Alves et al. 2018, 2021). This system is also composed of non-enzymatic compounds such as ascorbate (AsA) and glutathione (GSH), which are present in virtually all subcellular compartments (Schmutz et al. 2010; Upadhyaya et al. 2011; Muhammad et al. 2021).

In light of the aforementioned, we address how an intercropped system can affect stress modulation responses in three forage cactus genotypes. We hypothesized that through the modulation of the antioxidant defense system, plants can to maintain their growth in an intercropped system.

Materials and methods

Experimental area

The field study was conducted at Experimental Unit of Semi Arid National Institute (Instituto Nacional do Semiárido – INSA)/Brazilian Ministry of Science, Technology, and Innovation, in Frei Martinho, Paraíba State, Brazil (6°24′15’’ S, 36°26′94’’ W, at an elevation of 369 m above sea level). According to the Köppen classification, the climate is classified as BSh (summer rainfall), with a mean annual rainfall of 461 mm and a mean annual temperature of 25.6 °C.

Experimental conduction

The experimental design adopted was set up in randomized block in a 3 × 2 factorial with three replications (blocks), consisting of three forage cactus genotypes: *O. stricta* var. ‘OE Mexicana’; *N. cochenillifera* var. ‘Miúda’; *N. cochenillifera* var. ‘Baiana’ (Fig. 1) in monocropping and intercropping with cropping with *Gliricidia sepium* (Fig. 2), totaling 18 experimental units, each represented by three plants. Cactus seeds (leaves) were purchased in local commercial

Fig. 1 Cladodes of the three forage cactus genotypes used in the experiment

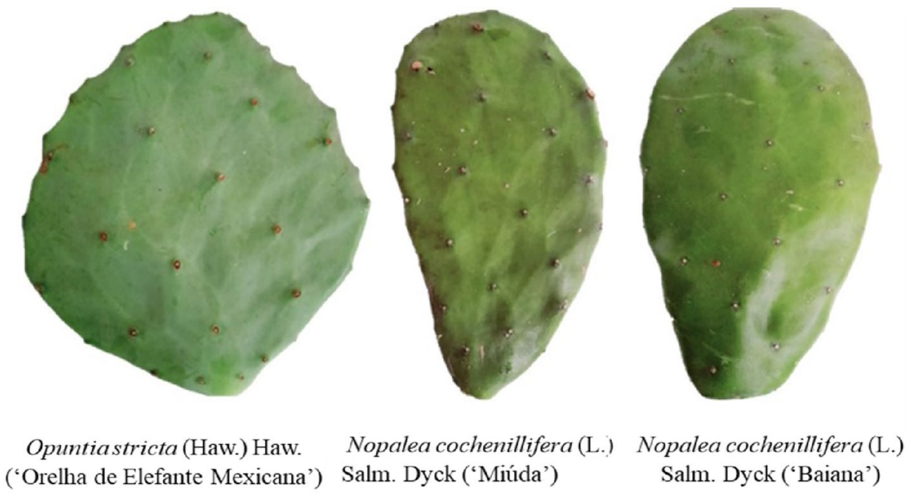

Fig. 2 *O. stricta* var. ‘OE Mexicana’ intercropping with *Gliricidia sepium* (**A**), *N. cochenillifera* var. ‘Miúda’ intercropping with *Gliricidia sepium* (**B**), *N. cochenillifera* var. ‘Baiana’ intercropping with *Gliricidia sepium* (**C**), *N. cochenillifera* var. ‘Baiana’ representing the monocropping (**D**)

planting with no pests and diseases. The G. sepium plants used in the intercropping system were planted through seedlings, produced at the Instituto Nacional do Semiárido Experimental Station. The seeds were sown in 1.4-L plastic bags containing 1.0 kg of substrate composed of soil and manure tanned in the proportion of 2:1 (v:v) and taken to the field 30 d after sowing. Forage cactus and G. sepium were planted at the same time.

The plants were irrigated with treated domestic wastewater. The effluent was treated in three stages: facultative lagoon (primary), stabilization pond, and equalization reservoir (Table 1). Irrigation with domestic wastewater was adopted, because it was an experimental area inserted in a place where this water was available, being a way to reuse a source of water with adequate standards for use in agriculture, allowing for the saving of drinking water. A drip irrigation system was adopted in the experiment, with emitters operating at a flow rate of 1.6 L/h. Irrigation was performed every 7 d throughout the experiment, with the exception of the week in which there was precipitation with accumulation ≥ 6 mm, according to Lira et al. (2022). The irrigation regime was the same for both the intercropping and monocropping systems. Forage cactus genotypes were planted in double rows, spaced 1.50 by 0.50 by 0.50 m, totaling 20,000 plants ha^{-1}. The plant spacing was the same in the intercropping system, and the second crop was inserted between each forage cactus row, spaced by 1 m.

Table 1 Analysis of wastewater used in irrigation

Parameters	
pH	7.7 ± 0.8
Color (uC)	882 ± 140
Turbidity (NTU)	124 ± 74
Salinity (g/L)	1.25 ± 0.13
Total dissolved solids (TDS) (mg/L)	1615 ± 213
Electric conductivity (mS/cm)	2.57 ± 0.24
Chemical oxygen demand (COD) (mg/L)	412 ± 291
Ammonia (mg/L)	74.3 ± 25.6
Phosphor (mg/L)	13.5 ± 5.7
Sodium (mg/L)	201 ± 117
Potassium (mg/L)	63 ± 36
E coli (NMP/100 mL)	4.96E + 05
Helminth eggs (eggs/L)	0

The forage cactus samples were harvested in the second year of cropping, close to the harvest day of *G. sepium*, which occurs every 4 months. The harvest was performed by removing a cladode from three plants of each experimental unit, after which the plant material was immediately taken to the Plant Production Laboratory (from INSA) and washed under running water, followed by cladode fractioning and immediate freezing in liquid nitrogen (aiming at stopping any physiological or biochemical plant reaction). After freezing, the samples were stored in a freezer at – 80 °C. Then, the samples were ground with mortar and pestle in liquid nitrogen to provide a homogeneous tissue and the following analyses were performed: concentration of chlorophylls and carotenoids, lipid peroxidation, hydrogen peroxide concentration, enzymatic extraction, determination of total proteins, and analysis of antioxidant systems. Plant growth was determined by collecting a whole plant from each experimental unit and quantifying the dry and fresh mass and the water content.

Analyses

Growth and water content

Plant growth was determined by measuring the fresh and dry plant weight. The fresh weight was determined by cutting the whole plant, which was later put in a plastic bag and weighed on an analytical balance accurate to 0.01 g. Dry weight was determined by cutting the plants, oven-drying them on trays for 72 fours at 65 °C, and weighing the material on an analytical balance accurate to 0.0001 g. Both the dry and fresh weight results were expressed as g plant^{-1}. The water content was determined by the following equation: (FPW – DPW)/FPW*100; where FPW = fresh plant weight and DPW = dry plant weight, with results expressed as %.

Chlorophyll and carotenoids content

Chlorophyll and carotenoids were quantified by weighing 250 mg of each sample. Under lowlight conditions, the samples were placed in Eppendorf tubes containing 2 mL of acetone (80%) and then maintained under 48 h under refrigeration. After this period, the readings were performed in triplicate with a spectrophotometer at the following wavelengths: Chlorophyll α at 663 nm; Chlorophyll β at 647 nm, and Carotenoids (carotene [c] + xenophiles [x]) at 470 nm (Lichtenthaler 1987).

Lipid peroxidation

The characterization of oxidative stress in forage cactus plants was performed by quantifying the content of substances with reactivity to thiobarbituric acid (TBARS) as the final product of lipid peroxidation. This procedure was performed using 0.5 g cladode samples macerated in liquid nitrogen with the following reagents in the reactive medium: trichloroacetic acid (TCA), thiobarbituric acid (TBARS), and polyvinylpolypyrrolidone (PVPP). The reaction medium was put in a dry bath at a temperature of 96 °C for 30 min, an essential step of the reaction. The readings were performed in a spectrophotometer at 535 and 600 nm, and the content of TBARs was calculated according to the specific equation for the reaction (Shimizu et al. 2006).

Hydrogen peroxide

The hydrogen peroxide concentration (H_2O_2) in the plant samples was determined in a reaction medium composed of 0.1% TCA, 100 mM potassium phosphate buffer (pH 7.5), 1 M potassium iodide, and 0.5 g of plant sample. The reading was performed in a spectrophotometer at 390 nm (Alexieva et al. 2001).

Extraction and quantification of total protein

The plant samples previously collected and stored in the freezer at – 80 °C were weighted for 0.250 g, macerated in liquid N_2, and homogenized in 1 M potassium phosphate buffer (pH 7.5) containing 1 mM of ethylenediaminetetraacetic acid (EDTA), 50 mM sodium chloride (NaCl), 1 mM ascorbic acid, and 2% PVPP. The mixture was centrifuged at 10.000 g for 30 min at 4 °C. The supernatant was divided into aliquots kept at – 80 °C to be used in the enzymatic assays (Souza et al. 2013; Alves et al. 2018). The quantification of total soluble proteins was performed according to Bradford (1976).

Superoxide dismutase—SOD (EC 1.15.1.1)

SOD activity was determined in a spectrophotometer. The reaction was conducted in a reaction chamber (box) illuminated by a 15 W fluorescent lamp at 25 °C. The mixture consisted of a solution containing 150 µl of the sample, 50 mM potassium phosphate (pH 7.8), 50 mM methionine, 10 mM EDTA, 1 mM blue neotetrazolium chloride (NBT), and 0.1 mM riboflavin. The reaction was placed inside the box, avoiding any exterior light, after which the lamp was lit in the interior of the box for 15 min, during which the blue formazan compound was formed by NBT photoreaction. One unit of enzyme activity was defined as the amount of enzyme required to inhibit the nitrotetrazolium blue chloride reduction by 50%. The readings were performed at 560 nm (Giannopolitis and Ries 1977), with modifications according to the reaction time. SOD activity was expressed as U SOD mg^{-1} protein.

Catalase—CAT (EC 1.11.1.6)

Catalase activity was determined with a spectrophotometer based on the consumption of H_2O_2 by monitoring the decrease in absorbance for 1 min at 240 nm. The reaction medium was composed of 100 µl of vegetal extract, 100 mM potassium phosphate (pH 7.5), and 10 mM H_2O_2 (Luck et al. 1974), with modifications (Alves et al. 2018). The activity was calculated with an extinction coefficient 39.4 mM^{-1} cm^{-1}, and was expressed as µmol H_2O_2 min^{-1} mg^{-1} protein.

Ascorbate peroxidase—APX (EC 1.11.1.11)

APX activity was determined in a spectrophotometer based on the oxidation of ascorbate peroxidase by monitoring the reduction in absorbance at 290 nm for 1 min. The reaction medium was composed of 150 µl of plant extract, 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbic acid, and 1 mM H_2O_2 (Nakano and Asada 1981) with modifications (Alves et al. 2018). The activity was calculated with an extinction coefficient of 2.8 mM^{-1} cm^{-1}, and was expressed as µmol ascorbate min^{-1} mg^{-1} protein.

Peroxidases—POX (EC 1.11.1.7)

POX activity was determined by the method of Kar and Mishra (1976), according to which the enzymes contained in the extract oxidize guaiacol to produce purpurogallin and reduce the H_2O_2 provided to the medium. The reactive medium consisted of the plant extract, 25 mM potassium buffer extract (pH 6.8), 20 mM pyrogallic acid, 20 mM H_2O_2, and 0.5% H_2SO_4. The mixture was incubated at 25 °C for 5 min. Subsequently, the reaction was neutralized by adding H_2SO_4 at 0.5%, and the absorbance was monitored at 420 nm for 3 min. POX activity was calculated based on the molar extinction coefficient of 2.47 mM^{-1} cm^{-1} (pyrogallic acid) (Chance and Maehley 1955) and considering the increase of one unit (UA) of absorbance, with data expressed as UA guaiacol g^{-1} min^{-1} protein.

Statistical analysis

Analysis of variance was performed and the variables that showed a significant response by the F-test had treatments means compared by the Tukey test at 5% probability. To determine the potential relationships between variables, the Pearson correlation coefficient was estimated. Associations were considered significant if the p-value was less than 0.05. The statistical analyses were performed with the R v4.0.0 software (R Core Team 2018).

Results

Statistical data analysis showed a significant effect of the interaction between forage cactus genotypes and cropping systems on the total chlorophylls ($F=3.47$, $P=0.0390$), MDA ($F=16.72$, $P=0.000$), H_2O_2 ($F=3.91$, $P=0.0266$), SOD ($F=5.94$, $P=0.005$), CAT ($F=0.86$ $P=0.042$), APX ($F=80.63$ $P=0.000$), FPW ($F=49.22$, $P=0.000$), and DPW ($F=11.48$, $P=0.002$). The interaction had no significant effect on the total carotenoids ($F=0.29$, $P=0.75$), which were only influenced by the cropping systems ($F=229.82$, $P=0.00$). The POX ($F=0.57$, $P=0.566$) was not influenced by the interaction but showed a significant effect of the forage cactus genotypes, similar to the water content ($F=1.81$, $P=0.205$), which was only significantly influenced by the cropping system (Tables 2 and 3).

The concentration of total chlorophylls was significantly affected by the interaction between genotypes and cropping systems. The intercropping system increased the concentration of total chlorophylls in the forage cactus *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Miúda', with no statistical difference for the *N. cochenillifera* var. 'Baiana'. The forage cactus genotypes only differed when were grown in monocropping, with the *N. cochenillifera* var. 'Baiana' showing the highest concentration of total chlorophylls (Fig. 3A). For total carotenoids, there was a reduction in concentration when the genotypes were cultivated in monocropping (Fig. 3B).

The H_2O_2 concentration was affected by the interaction between forage cactus genotypes and cropping system. Three forage cactus genotypes grown in intercropping showed increased H_2O_2 concentration when compared with monocropping system. It was also possible to observe

Table 2 Summary of analysis of variance

Average squares						
V.S	GL	TCh	CT	FPW	DPW	WC
Genotypes	2	4.085*	0.0156 ns	2,168,887.50**	7967.80*	0.219 ^{ns}
Intercroping	1	30.448**	12.313**	7,334,450.00**	19.996 ns	10.25 **
GxI	2	3.952*	0.016 ns	164,533.33**	15,606.77*	1.817 ns
Residuo	48	1.137	0.054	8,098,287.50	1358.96	1.001
Tukey's CV		10.56	7.44	7.39	10.88	1.07

TCh Total Chlorophyll, *CT* carotenoids, *FPW* fresh plant weight, *DPW* dry plant weight, *WC* water content. *, ** significant at 5 and 1% probability, respectively, by the *F* test. Data

Table 3 Summary of analysis of variance

Average squares							
V.S	GL	TBARs	H_2O_2	SOD	CAT	APX	POX
Genotypes	2	0.031**	0.04**	1114.7**	10.01**	14.73**	374.30**
Intercroping	1	0.225**	0.24**	2088.0**	58.21**	5.34*	0.419 ns
GxI	2	0.067**	0.008*	138.56**	0.246*	65.109**	1.0406 ns
Residuo	48	0.0040	0.0020	23.3230	0.2781	0.8074	1.8090
Tukey's CV		17.91	10.75	12.33	15.37	13.60	16.17

TBARs Thiobarbituric acid reactive substances, *H_2O_2* hydrogen peroxide, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *POX* peroxidases. *, ** significant at 5 and 1% probability, respectively, by the *F* test. Data

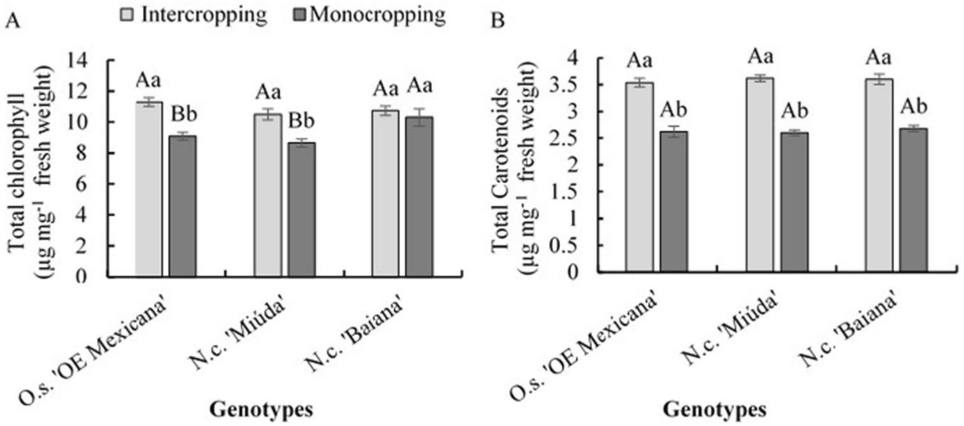

Fig. 3 Total chlorophylls (**A**) and total carotenoids (**B**) in three forage cactus genotypes in different cropping systems (with and without intercropping). Uppercase letters represent a statistical difference between forage cactus genotypes within each cropping systems. Lowercase letters represent a statistical difference of the cropping system within each genotype according to the Tukey test (α = 0.05)

that *O. stricta* var. 'OE Mexicana' showed the highest concentration of H_2O_2 within the intercropping system, while *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Baiana' showed the highest concentrations within the monocropping system (Fig. 4A). On the other hand, the concentration of the TBARs showed effect of the interaction between forage cactus genotypes and cropping system. Plants of *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Baiana' showed increased TBARs concentration when were grown in monocropping system. The TBARs concentration was similar between the genotypes in intercropped system (Fig. 4B).

The enzymatic antioxidant system is essential to eliminate or reduce the oxidative damage generated by ROS. As a first defense, the SOD (Fig. 5A) breaks the superoxide, forming H_2O_2, whereas enzymes such as CAT, APX, and POX (Fig. 5B, C, and D) play an important role by eliminating this peroxide generated by the SOD and other metabolic pathways.

The SOD activity was significantly influenced by the interaction between genotypes and cropping systems. In all genotypes, the highest activity was observed in the intercropping system. When comparing system cropping, the highest SOD activity occurred in the *O. stricta* var. 'OE

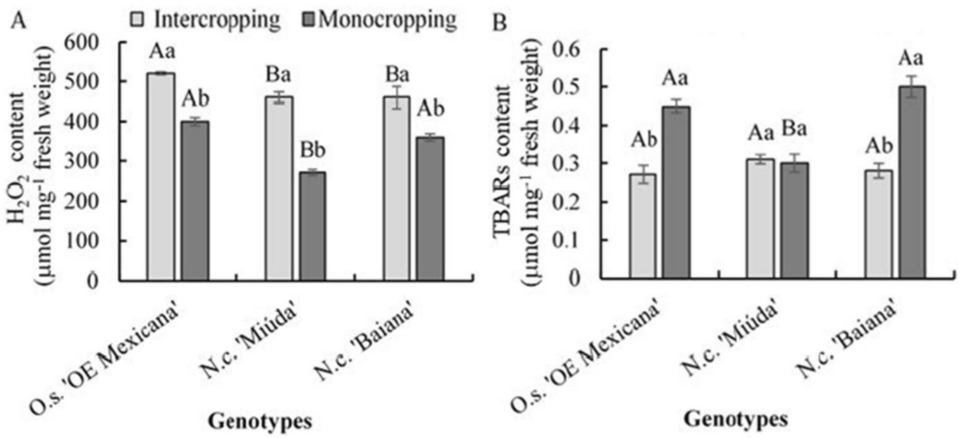

Fig. 4 Concentration of H_2O_2 (**A**) and TBARs (**B**) in three forage cactus genotypes in different cropping systems (with and without intercropping). Uppercase letters represent a statistical difference between forage cactus genotypes within each cropping systems. Lowercase letters represent a statistical difference of the cropping system within each genotype according to the Tukey test (α = 0.05)

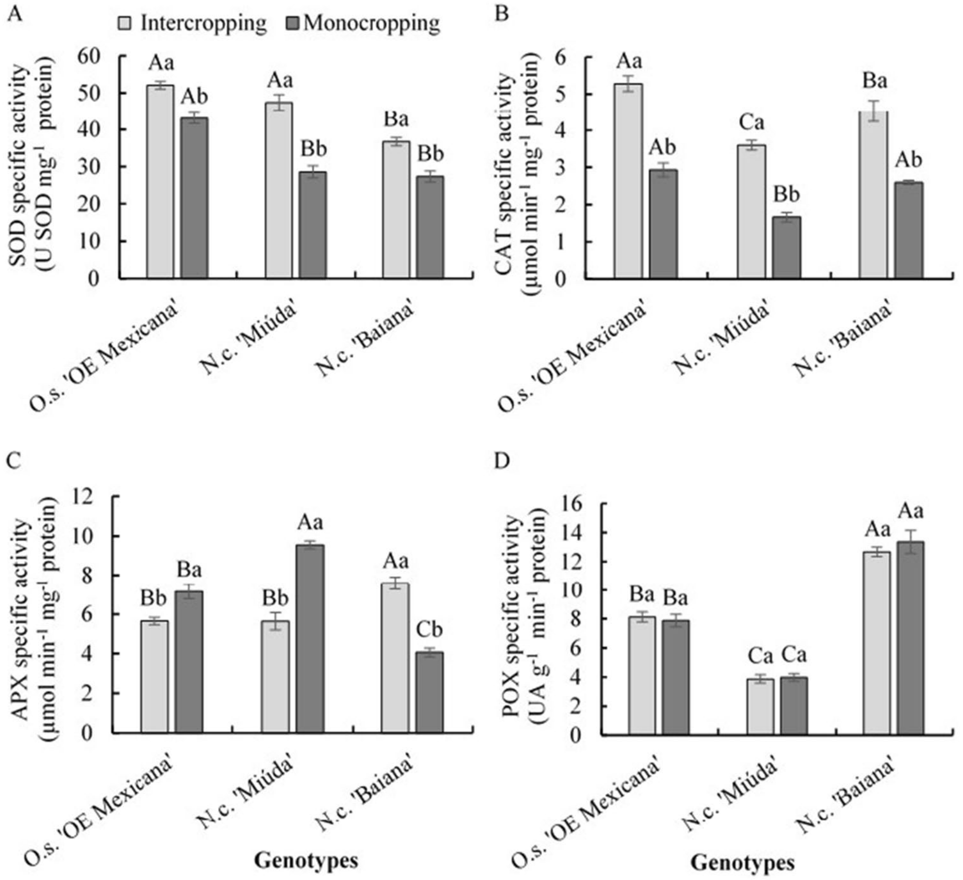

Fig. 5 SOD specific activity (**A**), CAT specific activity (**B**), APX specific activity (**C**), and POX specific activity (**D**) in three forage cactus genotypes in different cropping systems (with and without intercropping). Uppercase letters represent a statistical difference between forage cactus genotypes within each cropping systems. Lowercase letters represent a statistical difference of the cropping system within each genotype according to the Tukey test (α = 0.05)

Mexicana' and *N. cochenillifera* var. 'Miúda' in the intercropping system, no differing statistically from each other. However, in the monocropping system the highest SOD activity was observed in the *O. stricta* var. 'OE Mexicana' (Fig. 5A).

Similar to SOD, the CAT activity increased in all genotypes grown in intercropping. However, when the cropping systems were analyzed between genotypes, the highest CAT activity in the intercropping system was observed for the *O. stricta* var. 'OE Mexicana', in contrast, the lowest activity was observed for the *N. cochenillifera* var. 'Miúda'. In the monocropping, the highest activity occurred in the *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Baiana', no differing between each other (Fig. 5B).

The APX also showed a significant effect of the interaction between genotypes and cropping system. For the *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Miúda', the highest APX activity occurred in the monocropping, with the *N. cochenillifera* var. 'Baiana' showing the highest activity in the intercropping. When the cropping systems were compared between genotypes, the highest activity in the monocropping occurred in the *N. cochenillifera* var. 'Miúda', whereas the lowest activity occurred in the *N. cochenillifera* var. 'Baiana'. In the intercropping, the highest activity occurred in the genotype 'Baiana', with no difference between *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Miúda' (Fig. 5C).

The cropping systems significantly influenced the POX activity, and its highest value was observed in the genotype *N. cochenillifera* var. 'Baiana' in both cropping systems, whereas the lowest activity was observed in the genotype *N. cochenillifera* var. 'Miúda' (Fig. 5D).

The interaction between genotypes and cropping systems affected the plant growth variables (fresh weight, dry weight, and water content). The highest fresh weight values were observed in the *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Miúda' monocropping (6365.00 and 5215.00 g, respectively), which increased by 12.02 and 15.15% compared to the intercropping system. For the *N. cochenillifera* var. 'Baiana' the highest fresh weight was observed with intercropping (7570.00 g), increasing by 50.26% compared monocropping. When comparing the cropping systems between genotypes, the highest fresh weight value was observed in the *N. cochenillifera* var. 'Baiana' with intercropping, increasing by 26.02% compared to the genotype *O. stricta* var. 'OE Mexicana' and by 41.54% compared to the *N. cochenillifera* var. 'Miúda'. For the system monocropping, the highest fresh weight was observed in the *O. stricta* var. 'OE Mexicana', whereas the lowest was observed in the *N. cochenillifera* var. 'Baiana' (Fig. 6A).

The genotype *O. stricta* var. 'OE Mexicana' showed the highest dry weight when grown monocropping (425.12 g), increasing by 24.66% compared to the intercropping system. There was no significant difference for the genotype *N. cochenillifera* var. 'Miúda', whereas the genotype *N. cochenillifera* var. 'Baiana' showed the highest dry weight with intercropping (392.59 g), increasing by 25.04% compared with monocropping. The results were similar when the cropping systems were compared between genotypes, with the genotype *O. stricta* var. 'OE Mexicana' showing the highest dry weight with monocropping and the genotype *N. cochenillifera* var. 'Baiana' the highest dry weight with intercropping (Fig. 6B).

For the water content, only the genotype *N. cochenillifera* var. 'Baiana' showed a significant difference between cropping systems, and the plants grown in the intercropping system showed the highest water content (Fig. 6C).

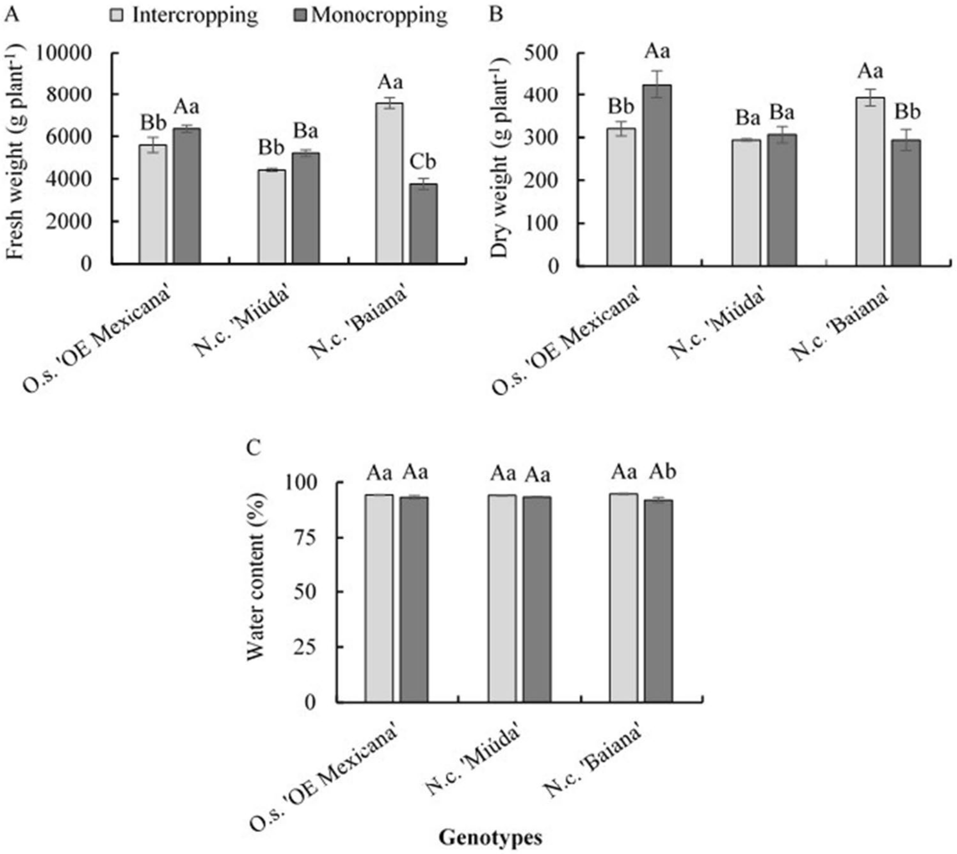

Fig. 6 Fresh weight (**A**), dry weight (**B**), and water content (**C**) of three forage cactus genotypes grown in different cropping systems (with and without intercropping). Uppercase letters represent a statistical difference between forage cactus genotypes within each cropping systems. Lowercase letters represent a statistical difference of the cropping system within each genotype according to the Tukey test ($\alpha = 0.05$)

Discussion

Unfavorable conditions considered stressful for plants can directly affect their photosynthetic rate. Therefore, a low concentration of photosynthetic pigments could indicate a stress condition that could reduce growth. Pigments play a key role in the reaction centers of photosystems I and II by performing the first stage of photosynthesis, one of the most important plant processes, converting solar energy into chemical energy (Sudhakar et al. 2016; Alves et al. 2021), in addition, they are compounds with antioxidant capacity and contribute to greater efficiency of the enzymatic defense system (Kharrat et al. 2018).

The results showed that when forage cactus was intercropped with *G. sepium*, the concentration of total chlorophyll was similar for all genotypes, which may be related to the ability of plants to regulate their metabolism even in a competitive environment. Interestingly, the total chlorophyll in 'OE Mexicana' and 'Miúda' genotypes were significantly higher when intercropped as compared to monocropping, showing a genotype-dependent response to intercropping that may be associated with competition for light, water, space or nutrients (Verdaguer et al. 2017). Furthermore, the concentration of total carotenoids was higher in all genotypes when intercropped with *G. sepium*, which could be related to the antioxidant role of these components, thus acting as a secondary defense metabolite (Falk and Munné-Bosch 2010; Alves et al. 2021).

Griffiths (1989) proposed that CAM plants could prevent ROS formation since the daytime CO_2 concentrations prevented the over energization of photosynthetic mechanisms. However, CAM plants can increase their oxidative load since they transport electrons even with closed stomata, increasing the internal O_2 concentrations (Spalding et al. 1979). Therefore, with oxygen release and consumption, the formation of ROS generates oxidative stress in CAM plants (Niewiadomska and Borland 2008).

The results demonstrate that ROS can be formed in CAM plants since the H_2O_2 content increased in the three forage cactus genotypes with intercropping (Fig. 4A). At the same time, a positive and significant correlation was recorded for the H_2O_2 content with the total chlorophyll and total carotenoids contents (Fig. 7). These results can be attributed to the competition for nutrients and/or space, which is established between the forage cactus and the consortium species (Camelo et al. 2021; Lira et al. 2022), resulting in a decrease in the antioxidant defense system of plants, making them more susceptible to oxidative stress (Alves et al. 2021; Anjum et al. 2022). In turn, under moderate low light conditions, plants can experience oxidative stress due to the accumulation of excess energy in the chloroplasts, which can lead to the production of reactive oxygen species, including H_2O_2 (Fahnenstich et al. 2008). Therefore, the high H_2O_2 content in forage cactus under consortium conditions indicates that H_2O_2 can act as a signaling molecule that triggers the up-regulation of secondary metabolite production as a defense response to oxidative stress (Ibrahim and Jaafar 2012).

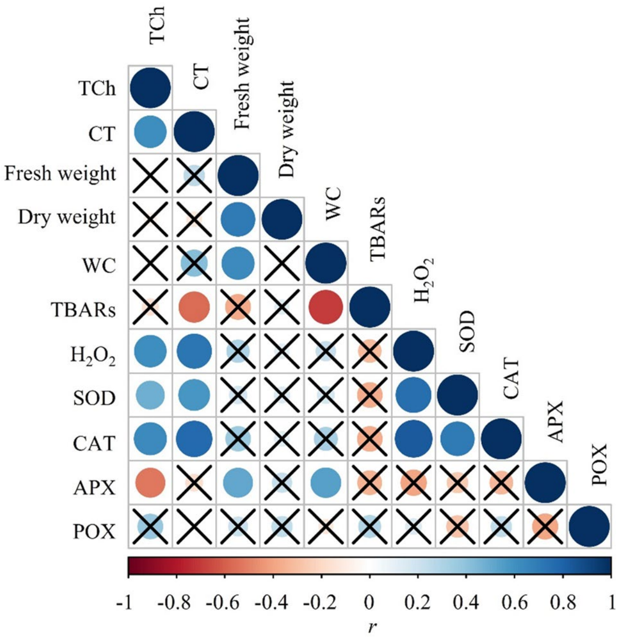

Fig. 7 Pearson's *r* correlation. *TCh* Total Chlorophyll, *CT* carotenoids, *WC* water content. *TBARs* Thiobarbituric acid reactive substances; *H_2O_2* hydrogen peroxide, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *POX* peroxidases. × significance was not obtained at 5% level ($p < 0.05$)

Hydrogen peroxide is a potent signaling molecule that is produced under stress or non-stressed conditions, triggering the regulation of antioxidant defense components, and may even be involved in the regulation of plant growth (Niu and Liao 2016; Alves et al. 2021; Anjum et al. 2022). In turn, this signaling allowed the antioxidant defense system to work effectively (Fig. 4), reducing oxidative damage in plants through the lower generation of TBARs (Fig. 4B) since H_2O_2 can be synthesized through enzymatic and non-enzymatic pathways (Niu and Liao 2016). When the genotypes were grown in monocropping, there was an increase in TBARs concentration, generating oxidative stress, since the defense system was not effective in reducing TBARs content. Although the levels of H_2O_2 were lower, this could be related to the oxidative stress generated, and part of the H_2O_2 may have been eliminated by the antioxidant defense system.

Formation of TBARSs demonstrates the importance of lipid peroxidation, so when oxidative stress is generated, it can cause inhibition of antioxidant defense mechanisms and even damage to biological systems (Hatamleh et al. 2022). According to the results of the TBARs concentration, the forage cactus genotypes in intercropping did not undergo oxidative damage since the TBARs concentration values were lower than in the plants grown with monocropping. This reduced level of oxidative damage may be the result of balanced and efficient antioxidant metabolism in scavenging ROS, indicated by positive and significant correlation values for H_2O_2 content with CAT and SOD activities and total carotenoids content (Fig. 7). It is also interesting to mention the positive and significant correlation values for APX activity with fresh weight and water content in forage cactus, indicating the role of this important antioxidant enzyme in maintaining cactus production and quality.

Intercropping *G. sepium* with forage cactus has been shown to improve soil fertility by increasing the levels of nitrogen, phosphorus, and organic matter in the soil. This is because *G. sepium* is a nitrogen-fixing plant that can add nitrogen to the soil, while forage cactus can help to conserve soil moisture and reduce soil erosion (Camelo et al. 2021; Mekuria et al. 2011). According to Gomathinayagam et al. (2013), the consortium system can increase the forage yield compared to monoculture forage cactus because *G. sepium* can provide shade and reduce water stress for the forage cactus, leading to increased growth and yield. However, the mechanisms of the underlying antioxidant system and their relationships with the performance of the consortium system of these species are still poorly understood.

The plants under unfavorable conditions can adjust their metabolism through the antioxidant defense system by interrupting uncontrolled oxidation chain reactions and maintaining their normal growth. The enzymatic activity of SOD, CAT, and APX clearly shows this ability to prevent uncontrolled oxidative damage in forage cactus genotypes when the intercropping cropping system was used. However, the *O. stricta* var. 'OE Mexicana' showed reduced growth when intercropped (Fig. 6), confirming the genotype-dependent response to intercropping, with variations in their antioxidant system.

SOD is a key enzyme that plays a role in ROS formation, breaking superoxide into oxygen and peroxide, the latter of which is then converted into water and oxygen by CAT and APX inside plant cells and by POX in the extracellular space (Rajput et al. 2021). Indeed, the enzymatic complex was essential to prevent lipid peroxidation in the forage cactus genotypes grown with intercropping, especially in the genotype *N. cochenillifera* var. 'Baiana', which showed the highest system regulation effectiveness, thus favoring plant growth even with intercropping (Figs 5 and 6).

The increase in CAT and APX activity observed in the genotype *N. cochenillifera* var. 'Baiana' with intercropping could be related to the increase in H_2O_2 (Fig. 7), as compared to monocropping, which is its substrate, whereas the regulation in the H_2O_2 content within plant cells under stress conditions is due to the action of SOD (Gill and Tuteja 2011). Our results confirm this hypothesis since the increase in CAT and APX activity in the *N. cochenillifera* var. 'Baiana' with intercropping could be related to the increase in SOD activity. Based on the results obtained, the antioxidant defense system protected the membrane lipids and other cell structures (Dumont and Rivoal 2019; Alves et al. 2020), preventing ROS from causing negative damage to the *N. cochenillifera* var. 'Baiana' in the intercropping system. The *O. stricta* var. 'OE Mexicana' and *N. cochenillifera* var. 'Miúda', although showing increased SOD and CAT activity in the intercropping system, were not as effective as the *N. cochenillifera* var. 'Baiana' (Fig. 5). Furthermore, these enzymes are also essential in plant growth and development since they are involved in cell modulation and subcellular processes (Bela et al. 2015; Sofy et al. 2020; Rajput et al. 2021).

Plant growth analysis is a simple tool used to understand plant behavior under different situations since environmental and biological factors change the characteristics of plants, e.g., the distribution of photoassimilates and cell expansion (Alves et al. 2021). With intercropping, species usually differ with regard to morphological features, potentially inducing the competition for light, nutrients, water, and space. Under these conditions, the larger crop prevents a higher light incidence on the lower crop, reducing the leaf area and plant growth.

A graphic summary of the influence of intercropping on the growth and antioxidant metabolism of forage cactus is shown in Fig. 8. The results of the growth variables (fresh weight, dry weight, and water content) observed in the present study highlight the influence of the intercropping system since each genotype showed a different behavior, decreasing in the *O. stricta* var. 'OE Mexicana' and increasing in the *N. cochenillifera* var. 'Baiana', whereas the *N. cochenillifera* var. 'Miúda' was indifferent to both systems. Therefore, these results are directly related to the observations of the antioxidant defense system, also highlighting that, in addition to acting against ROS, enzymes are also essential components in plant growth regulation.

What possibly unleashed this response on plant growth was that oxidative damage was generated in the *O. stricta* var. 'OE Mexicana', preventing the regulation of the enzymatic defense system, whereas the genotype *N. cochenillifera* var. 'Baiana' showed greater efficiency, preventing the uncontrolled production of ROS. Thus, choosing the best arrangement and time of harvest for *Gliricídia* is crucial to the performance of the intercropping system in order to maximize the production of forage cactus genotypes and, consequently, reduce oxidative damage.

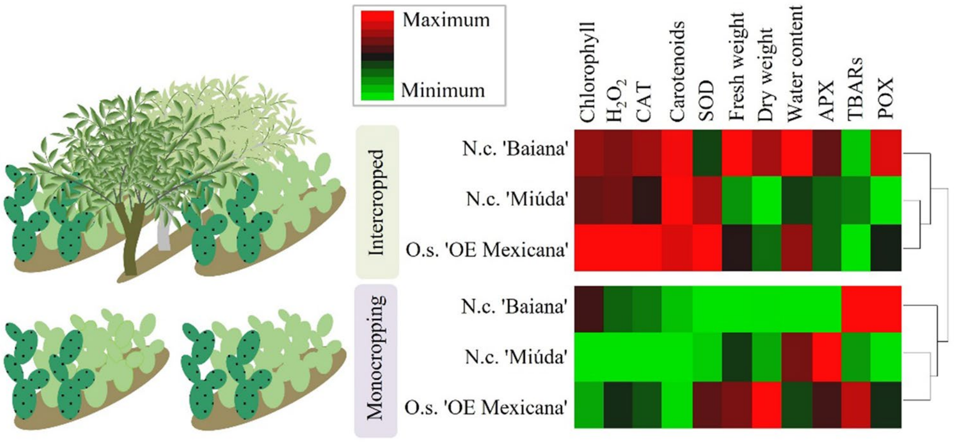

Fig. 8 Graphical abstract of results

Conclusion

Changes in antioxidant metabolism in forage cactus intercropped with *Gliricidia sepium* is genotype-dependent. Intercropping forage cactus *N. cochenillifera* var. 'Baiana' resulted in better regulation of the antioxidant enzymatic defense system, reducing lipid peroxidation, maintaining the stability of photosynthetic processes, and favoring plant growth as compared to monocropping. Although the highest accumulation of H_2O_2 was registered in the intercropped forage cactus, these plants showed lower TBRAS concentration, probably due to the crucial role of the antioxidant system in maintaining the oxidative balance as indicated by the high CAT and SOD activity and high carotenoid content.

Acknowledgements The authors would like to thank the Brazilian Northeast Bank (BNB—Sei Process 01201.000138/2017-61) and Semi-Arid National Institute (INSA) for their financial support for design of the study and all the analyzes carried out.

Author contributions Rita de Cássia Alves: conceptualization, methodology, investigation, field work, laboratory analyses, data processing, writing—original draft. Evaldo dos Santos Felix: methodology and field work. Tarcísio José de Oliveira Filho: field work and laboratory analyses. Elder Cunha lira: field work and laboratory analyses. Renato Pereira Lima: writing—review and editing. Maria do Perpétuo Socorro Damasceno Costa: laboratory analyses. Jaqueline de Araújo Oliveira: conceptualization and laboratory analyses. José Thyago Ayres Souza: methodology, data processing. Emmanuel Moreira Pereira: conceptualization and review. Priscila Lupino Gratão: writing—review and editing. Jucilene Silva Araújo: conceptualization, methodology, writing—review and editing and funding acquisition. All authors have read and approved the final.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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