



Article Impact of Drought and Flooding on Alkaloid Production in Annona crassiflora Mart

Ana Beatriz Marques Honório ¹, Iván De-la-Cruz-Chacón ², Mariano Martínez-Vázquez ³, Magali Ribeiro da Silva ⁴, Felipe Girotto Campos ¹, Bruna Cavinatti Martin ¹, Gustavo Cabral da Silva ¹, Carmen Sílvia Fernandes Boaro ¹, ^{*}, and Gisela Ferreira ¹

- ¹ Plant Biology Sector, Biostatistics, Plant Biology, Parasitology and Zoology Department, Institute of Biosciences, São Paulo State University, UNESP, Street Prof. Dr. Antônio Celso Wagner Zanin 250, Botucatu 18618-689, SP, Brazil; beatriz.honorio@unesp.br (A.B.M.H.); felipe.girotto@unesp.br (F.G.C.); bruna.cavinatti@unesp.br (B.C.M.); gustavo.cabral-silva@unesp.br (G.C.d.S.); gisela.ferreira@unesp.br (G.F.)
- ² Laboratorio de Fisiología y Química Vegetal, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, UNICACH, Libramiento Norte Poniente 1150, Tuxtla Gutiérrez 29039, CHIS, Mexico; ivan.cruz@unicach.mx
- ³ Chemistry Institute, Universidad Nacional Autónoma de México, UNAM, Circuito Exterior S/N, C.U., Coyoacán 04510, CDMX, Mexico; marvaz@unam.mx
- ⁴ Forest, Soil and Environmental Sciences Department, Faculty of Agronomic Science, São Paulo State University, UNESP, Avenida Universitária 3780, Botucatu 18610-034, SP, Brazil; magali.ribeiro@unesp.br
- * Correspondence: carmen.boaro@unesp.br; Tel.: +55-(14)-3880-0124

Abstract: The Brazilian Cerrado is the second largest Brazilian biome. In recent decades, a reduction in rainfall has indicated an extension of the dry season. Among the many native species of the Cerrado of the Annonaceae family and used in folk medicine, *Annona crassiflora* Mart. has fruits of high nutritional value and its by-products are sources of bioactive compounds, such as alkaloids. The aim of the study was to investigate how water stress impacts the production of alkaloids. The study was carried out in a nursery, and the knowledge was flood, field capacity and drought. Gas exchange, chlorophyll *a* fluorescence, antioxidant enzymes, total soluble sugars, starch, reducing sugars, sucrose, total alkaloids and liriodenine were analyzed. We observed that plants subjected to drought had an increase in the production of total alkaloids and liriodenine, without a reduction in photosynthetic metabolism. Plants kept under drought and flood conditions dissipated higher peroxidase activity, while catalase was higher in flooded plants. Starch showed the highest concentration in flooding plants without differing from drought plants; the lowest trehalose concentrations were found in both drought and flooding plants. The drought stimulated the synthesis of total alkaloids and liriodenine without reducing the primary metabolism, which suggests adaptation to Cerrado conditions.

Keywords: Annonaceae; antioxidant enzymes; carbohydrates; liriodenine; photosynthesis

1. Introduction

The Brazilian Cerrado is the second largest Brazilian biome, considered one of the 25 global biodiversity hotspots, present in more than twelve states and occupying approximately 25% of the national territory, with native flora characterized by small and twisted trees [1].

This biome covers important aquifers and rivers [2] and is located in the central area of the country, being the origin of large hydrographic regions in Brazil and in the South American continent [3,4]. However, Lee et al. [5], Debortoli et al. [6] and Penereiro et al. [7] reported reduction of approximately 70 mm in annual precipitation in the Cerrado region between 1979 and 2006, indicating an extension of the dry season. Furthermore, although with great biological diversity, the biome is under continuous threat of extinction due to the expansion of agriculture and pastures, as occurs with other biomes [2,8].



Citation: Honório, A.B.M.; De-la-Cruz-Chacón, I.; Martínez-Vázquez, M.; da Silva, M.R.; Campos, F.G.; Martin, B.C.; da Silva, G.C.; Fernandes Boaro, C.S.; Ferreira, G. Impact of Drought and Flooding on Alkaloid Production in *Annona crassiflora* Mart. *Horticulturae* 2021, *7*, 414. https://doi.org/10.3390/ horticulturae7100414

Academic Editor: Yanyou Wu

Received: 26 September 2021 Accepted: 14 October 2021 Published: 19 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The Cerrado vegetation is exposed to high irradiances (1500 to 2500 μ mol. m⁻² s⁻¹), high temperatures (25–40 °C at midday) and in the dry season, low relative humidity (10 to 20%) [9]. Although there is abundance of light, the seasonality of the rainfall regime is one of the factors that limits vegetation growth, leading to greater investment in root formation to explore deeper soil layers [3]. As a consequence, species present in the Cerrado biome tend to have smaller specific leaf area; on the other hand, they invest more in the bark in order to guarantee their survival in situations of water and temperature stress [10], and present lower growth rates and greater hydraulic conductance per leaf area unit when compared to species from other biomes [11,12].

Many native Cerrado species belong to the Annonaceae family and are widely used in folk medicine for the treatment of different diseases [13,14]. Native *Annona crassiflora* Mart., widely spread throughout the Cerrado biome, popularly known as araticum do cerrado, marolo, araticum cortiça or bruto, is among the species with the most consumed fruits in this biome, with pleasant sensory characteristics and high nutritional value, rich in phenolic and oligosaccharide compounds [15], carotenoids and vitamins [16], in addition to alkaloids found in by-products such as leaves and stem bark [17], representing a natural source of bioactive compounds due to their antioxidant properties [14] and seeds with high lipid yield [18].

Alkaloids make up the most diverse group among nitrogenous compounds. Multiple biological activities have alkaloid origins, and there are several drugs available on the market produced from natural plant alkaloids [19]. Several alkaloids are found in *Annona crassiflora* Mart. leaves, peels and stems. Gonçalves et al. [20] isolated two alkaloids, namely atherospermidine and liriodenine, from the stem; Pereira et al. [21] isolated and characterized alkaloid stephelagin from the fruit peel and Egydio et al. [22] and Ferraz et al. [23] identified dimethoxy-dihydroxy-tetrahydroprotoberberine, isolaurelin, xylopine, anonaine, anoretin and romucosine in leaves.

Liriodenine, an alkaloid found in abundance in the Annonaceae family [20,24–27], has several potent biological activities [28,29], including potential antibacterial [24], antiprotozoal [30,31], cytotoxic [32,33] and antifungal activities [34]. In particular, it has activity against more than 20 phytopathogens, including *Rhizopus stolonifer* and *Aspergillus glaucus*, fungi that impair seed germination [25].

Although there are several reports of alkaloids in Annonaceae, so far, there are no reports on how drought conditions, similar to those periodically found in the Cerrado, impact alkaloid production in the species, or how the species tolerate flooding conditions and how these conditions would reflect on the synthesis of specialized metabolites such as alkaloids. Thus, the aim of this study was to investigate how water stress impacts the production of total alkaloids and liriodenine in *Annona crassiflora* Mart.

2. Materials and Methods

2.1. Plant Material

Annona crassiflora Mart. seedlings were obtained in the municipality of Paraguaçu— Minas Gerais (April 2019) and transported to the nursery of the Department of Forest Science of the Faculty of Agronomic Sciences, Unesp—Botucatu (coordinates 22°51′ latitude S and 48°26′ longitude W), where they were submitted to a 6-month acclimation period. Transplantation to 5-L polyethylene pots was carried out in November 2019. To fill the pots, medium-texture Dystrophic Red Latosol was used [35,36], collected from the surface layer (0–20 cm in depth).

During the period in which the experiment was conducted, plants were submitted to humidity and temperature conditions shown in Figure 1.



Figure 1. Average humidity and temperature between the 13th and 31st of May 2020 in the seedling nursery of the Department of Forestry Science of the Faculty of Agronomic Sciences (FCA) (DAT: days after transplanting).

2.2. Experimental Design

The experimental design was completely randomized, with three water stress levels (Flooding (>0.01 MPa); Field Capacity (-0.01 MPa) (Control) and Drought (-1.5 MPa)), with six replicates of two plants per plot. Plants remained in treatment (13–31 May 2020) until the permanent wilting point was reached. Two days after this stress condition was reached, the entire experiment was collected.

From the water retention curve, the percentage of water needed for the soil to reach -0.01 MPa (Field capacity) (Control) and -1.5 MPa (Drought) was calculated, which corresponded to 16% and 8% of water, respectively. After placing pots under the established conditions, they were weighed daily and the evapotranspiration difference was replaced to maintain previous conditions (-0.01 MPa and -1.5 MPa). To maintain plants under flooding, pots were kept in flooded trays throughout the experiment.

The moisture values corresponding to the water retention tension obtained through the soil water retention curve are shown below (Table 1).

Tension	Humidity (%)		
Flooding (>0.01 MPa)	37		
Field capacity (-0.01 MPa)	16		
Drought (-1.5 MPa)	8		

2.3. Gas Exchanges

Gas exchanges were monitored weekly in all treatments from 9:00 a.m. to 11:00 a.m., with the aid of a CO₂ gas and infrared gas analyzer ("InfraRed Gas Analyzer—IRGA", model GSF 3000 Fl WALZ, Germany) with saturating light of 450 m⁻² s⁻¹ determined by means of a light curve. For monitoring, six replicates (1 plant per replicate) of each treatment were evaluated, taking measurements on the 2nd and 3rd fully expanded leaves.

CO₂ assimilation rate (*Anet*, μ mol CO₂ m⁻² s⁻¹), transpiration rate (*E*, mmol water vapor m⁻² s⁻¹) and stomatal conductance (*gs*, mol m⁻² s⁻¹) were determined. Water use efficiency (WUE, μ mol CO₂ (mmol H₂O⁻¹)) was calculated using the relationship between assimilated CO₂ and the transpiration rate (*Anet/E*). The apparent carboxylation efficiency was calculated according to the relationship between CO₂ assimilation rate and leaf intercellular CO₂ concentration (*Anet/Ci*, mol m⁻² s⁻¹ Pa⁻¹).

2.4. Chlorophyll a Fluorescence

Chlorophyll *a* fluorescence was performed from 9:00 a.m. to 11:00 a.m. using a fluorometer (LED-Array/PAM-Module3055-FL) on 18 plants (six replicates of 1 plant each) and leaves were acclimated to a period of 30 min in the dark by covering them with aluminum foil; then, an actinic light pulse of 4500 μ mol m⁻² s⁻¹ was applied to obtain Fm (maximum dark-adapted fluorescence) and Fm' (maximum light-adapted fluorescence). In addition to the maximum leaf light-adapted and dark-adapted fluorescence, Fo (minimum dark-adapted fluorescence) and Fo' values (minimum light-adapted fluorescence) were also obtained.

The maximum quantum yield (Fv/Fm) [37], effective quantum yield (ϕ PSII) [38], photochemical quenching (qP) [39], non-photochemical quenching (NPQ) [40] and electron transport rate (ETR) were calculated through Fm, Fo, Fm' and Fo', considering that 84% of light is absorbed by chlorophyll, with 50% of photons activating photosystem II chlorophyll and 50% photosystem I and photosystem II energy that cannot be dissipated (*Ex*), quantum yield of unregulated non-photochemical energy loss in photosystem II (ϕ NO) and quantum yield of regulated non-photochemical energy loss in photosystem II (ϕ NPQ) [41].

2.5. Carbohydrate Concentration

Total soluble sugars were extracted from the leaf material obtained from a pool of samples of two plants per replicate (six replicates per treatment), according to Garcia et al. [42], with minor modifications, and starch was extracted according to Clegg [43]. The procedure to determine the concentration of total soluble sugars was performed according to Morris [44]; for starch, it was described by Yemm and Folkes [45]; for reducing sugars, it was determined by Miller [46]; and for sucrose, it was established by Passos [47], with minor modifications.

2.6. Activity of Antioxidant Enzymes, Hydrogen Peroxide and Lipid Peroxidation

The extraction of antioxidant enzymes was performed as described by Kar and Mishra [48] from leaf material obtained from a pool of samples of two plants per replicate (six replicates per treatment). The activities of superoxide dismutase, EC 1.15.1.1 and catalase EC 1.11.1.6 enzymes were determined by the method of Peixoto et al. [49]; the activity of the peroxidase EC 1.11.1.7 enzyme was established according to Teisseire and Guy [50]; and soluble proteins were quantified as described by Bradford [51].

The hydrogen peroxide content was determined by the method of Alexieva et al. [52] and lipid peroxidation was determined according to methodology proposed by Heauth and Packer [53], and both analyses were obtained using a pool of leaf material from two plants per replicate (six replicates per treatment).

2.7. Extraction of Total Alkaloids

Total alkaloids were extracted from the root material of 18 *A. crassiflora* plants (six replicates of 2 plants each); the material was stored in a greenhouse with forced air circulation at 30 °C for ten days, and subsequently ground to obtain 1 g of dry mass for each replicate. Alkaloids were extracted from roots previously dried using the acid–base method. After thorough grinding, the plant material was moistened with a saturated sodium carbonate (Na₂CO₃) solution and left to dry for 48 h at room temperature. Alkaloids were extracted with chloroform (CHCl₃) by constant stirring for 1 h and then filtered and washed with distilled water. The CHCl₃ phases were extracted into a 1 M hydrochlo-ric acid (HCl) solution before being alkalinized to pH 9.5 with a saturated solution of Na₂CO₃. The alkaline solution was then re-extracted with CHCl₃, dried with anhydrous sodium sulfate (Na₂SO₄), filtered and evaporated at approximately 25 °C to obtain total alkaloids [25].

2.8. Quantification of Total Alkaloids and Liriodenine

To determine the total alkaloid content, the 18 samples were stored at room temperature, re-solubilized with CHCl₃ and transferred to quartz cuvettes. The absorbance of each solution was obtained by spectrophotometer at 254 nm wavelength using liriodenine as the standard for the elaboration of the standard curve (y = 0.0881x - 0.0112, $R^2 = 0.9949$).

After obtaining the extract, liriodenine was quantified using ultra-high-performance liquid chromatograph (UHPLC—Thermo Fisher-Scientific[®], Waltham, MA, USA) with a gradient pump and UV-Vis detector using C 18 reverse phase column (150 × 4, 6 mm and 5 µm in particle diameter). The mobile phase was 30:70 water (pH 3.5 with acetic acid) and cratic isomethanol, with a flow rate of 1 mL/min, keeping the column temperature at 30 °C. Detection was carried out in UV at 254 nm. For liriodenine quantification, calibration curves were performed by analyzing the stock solution series (y = 0.3595x - 0.0011; R² = 0.9989 for samples with up to 10 µg of liriodenine in the extract and y = 0.3658x + 1.142; R² = 0.9992 for samples with more than 10 µg [25].

2.9. Statistical Analysis

Data were submitted to analysis of variance (ANOVA) using the SigmaPlot software Version 12 and means were compared by the Tukey test at 5% (p < 0.05) [54]. To present the biochemical variables, a radar chart was used. Input variables were initially standardized as a result of the different units, using the scale command from the basic package of the R computing environment, which centers the mean at zero and changes the scale to standard deviation [55].

3. Results

Annona crassiflora plants showed, in general, that the ability to adapt to water restriction conditions (drought: -1.5 MPa) reflected in the increase in specialized metabolism, unlike what occurred under flooding conditions (Figures 2 and 3, Table 2). In this experiment, an increase in the production of total alkaloids without the occurrence of reductions in the photosynthetic metabolism of plants (gas exchange and chlorophyll *a* fluorescence) was observed when *A. crassiflora* plants were kept under drought stress conditions (Figures 4 and 5).



Figure 2. Biochemical variables: (liriodenine; total alkaloids; hydrogen peroxide (H₂O₂); lipoperoxide, peroxidase (POD); catalase (CAT); superoxide dismutase (SOD); starch; sucrose; reducing sugar; total sugars; trehalose) obtained from young *A. crassiflora* plants submitted to three water condition levels (Field Capacity (-0.01 MPa); Flooding; Drought (-1.5 MPa)) at 18 days after the beginning of treatments. Variables represented in the graph and that showed significant differences in statistical analysis by Tukey tests at 5% are shown in Table 2.



Figure 3. Chromatogram indicating liriodenine obtained from young *A. crassiflora* plants submitted to three water condition levels [Field Capacity (-0.01 MPa); Flooding and Drought (-1.5 MPa)] at 18 days after the beginning of treatments.

Table 2. Biochemical variables: liriodenine (μ g.g⁻¹), total alkaloids (μ g.g⁻¹), peroxidase (POD, μ mol prupurogallin min⁻¹ mg prot⁻¹), catalase (CAT, μ Kat μ g⁻¹ protein), starch (μ g.g⁻¹ FW) and trehalose (μ g.g⁻¹ FW) obtained from young *A. crassiflora* plants submitted to three water condition levels (Field Capacity (-0.01 MPa), Flooding and Drought (-1.5 MPa) at 18 days after the beginning of treatments.

Water Condition	Total Alk	Liriodenine	CAT	POD	Trehalose	Starch
Field capacity	54.26 B	10.8770 AB	0.0101 B	0.2681 B	83.90 A	69.713 B
Flooding	63.49 AB	8.4098 B	0.1034 A	0.6655 A	12.46 B	104.089 A
Drought	80.87 A	13.4374 A	0.0391 B	0.7656 A	13.16 B	89.381 AB

Averages followed by the same letter not differ based on the Tukey 5% significance test. Mean \pm standard deviation (n = 4).



Figure 4. Carbon assimilation rate (A_{net}) and Rubisco carboxylation efficiency (A_{net}/Ci) in Annona crassiflora plants kept under Field capacity (-0.01 MPa), Flooding and Drought (-1.5 MPa) conditions at 5, 10 and 18 days after the application of treatments, respectively, and 168, 173 and 178 days after transplanting. Capital letters indicate significant differences among treatments (p < 0.05).

At the same time that plants showed adaptation to water restriction conditions characterized by responses observed in primary metabolism, the production of total alkaloids and liriodenine was also increased. Plants kept under drought conditions produced higher concentrations of total alkaloids in relation to those kept in soil with maximum water availability (field capacity), while saturated soil did not cause significant variations in total alkaloids but reduced the liriodenine concentration in relation to drought soils.

In this context, plants kept under water restriction showed greater carboxylation efficiency of the Rubisco enzyme (A_{net}/Ci) compared to plants kept under flooding (Figure 4). However, in both conditions, the carbon assimilation rate (A_{net}) was lower in relation to plants without water restriction (Field Capacity) and without differences in relation to *Ci* (data not shown). The other gas exchange variables did not show significant differences (stomatic conductance (*gs*), transpiration (*E*), vapor pressure deficit (*VPD*) and water use efficiency (WUE).

The chlorophyll *a* fluorescence was also impacted by treatments, and plants kept under drought conditions had higher maximum quantum yield (Fv/Fm), effective quantum yield (ϕ PSII), potential quantum efficiency values (Fv'/Fm') and lower fraction of energy dissipated in the form of heat (*D*) compared to plants kept under flooding conditions (Figure 5). Photosystem II energy that cannot be dissipated and used in the photochemical phase (Ex), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), quantum yield of unregulated non-photochemical energy loss in photosystem II (ϕ NO) and quantum yield of regulated non-photochemical energy loss in photosystem II (ϕ NPQ) did not show significant differences.



Figure 5. Maximum quantum yield (Fv/Fm), potential quantum efficiency (Fv'/Fm'), effective quantum yield (Φ PSII) and energy dissipated in the form of heat (*D*) in *Annona crassiflora* plants kept under Field capacity (-0.01 MPa), Flooding and Drought (-1.5 MPa) conditions at 5, 10 and 18 days after the application of treatments, respectively, at 168, 173 and 178 days after transplanting. Uppercase letters indicate significant differences among treatments; lowercase letters indicate differences among times (p < 0.05).

Starch and trehalose were affected depending on the conditions in which plants were kept, while total sugars, reducing sugars and sucrose did not show significant differences. Starch was found in higher concentrations in plants kept under flooding but without differing from plants kept under drought, and the lowest trehalose concentrations were found both in leaves of plants kept under both drought and flooding, indicating that this sugar may have been translocated to roots and used in order to neutralize the damage caused by stress (Figure 2, Table 2).

In general, the enzymatic system acted satisfactorily, preventing membrane damage, since there was no difference in lipid peroxidation and hydrogen peroxide among treatments, possibly indicating that the antioxidant enzymes inhibited the activity of reactive oxygen species. In plants kept under drought soil and flooding, higher peroxidase activity (POD) was observed, while catalase activity (CAT) was higher only in plants kept under flooding. Thus, flooded plants required greater enzymatic activity (Figure 2, Table 2).

4. Discussion

The increase in alkaloid production in *A. crassiflora* plants kept under drought stress conditions seems to be related to their ability to adapt to the Cerrado conditions, which has well-defined drought periods [56], since the photosynthetic process was preserved, ensuring both primary and specialized metabolism. This ability to adapt to the Cerrado conditions seems to be specific, since under flooding, reductions in primary metabolism were evident, affecting the specialized metabolism, especially the synthesis of alkaloid liriodenine.

The fact that drought stress causes increases in specialized metabolism substances such as alkaloids has been shown by several authors, such as Ghorbanpour and Hatami [57] in work with *Hyoscyamus niger*; Kleinwächter et al. [58] with thyme (*Thymus vulgaris*); Kleinwächter and Selmar [59] with spices and medicinal plants; and Liu et al. [29] with *Catharanthus roseus*. Specifically with the genus *Annona*, Castro-Moreno et al. [60] found the highest liriodenine concentration in *Annona lutescens* roots at the end of the dry season (about 377 μ mol/g), which is the first report of the presence of liriodenine in *Annona* crassiflora roots under water stress (about 80.9 μ g/g of total alkaloids and 13.47 μ g/g of liriodenine). Some periodic collections of *Annona* species tissues in an annual cycle allow us to point out that although the biosynthesis of alkaloids is distributed throughout the plant, the roots generally accumulate the greatest number of alkaloids and produce a higher yield regardless of the phenological stage of plants [61].

In general, water restriction conditions lead to stomatal closure and reduced CO_2 absorption and, as a consequence, there is a considerable decrease in the consumption of NADPH + H⁺ for CO_2 fixation via the Calvin cycle, which generates an excess supply and accumulation of this equivalent reducer. Thus, metabolic processes are directed towards the synthesis of highly reduced compounds such as isoprenoids, phenols and alkaloids with the use of these accumulated reducing agents [62]. In this context, although there were no significant differences in stomatal conductance in *A. crassiflora*, reductions in CO_2 assimilation rates (A_{net}) were observed due to water restriction (drought) and flooding, which corroborates the results of Simonneau et al. [63] and Oliveira and Gualtieri [64], respectively, and may have led to lower CO_2 fixation in the Calvin cycle [65]. However, only *A. crassiflora* plants kept under drought conditions showed greater synthesis of total alkaloids in relation to those kept under field capacity, in addition to having higher carboxylation efficiency (A_{net}/Ci) and higher liriodenine concentration in relation to flooded plants, which indicates that these responses were evident when plants were under water restriction.

The high carboxylation efficiency in A. crassiflora indicates adaptation for survival in environments with periods of low water availability, which is justified by the fact that the species is native to the Cerrado, unlike the results obtained by Mantoan et al. [66] with A. emarginata, showing a decrease in the carboxylation efficiency under irrigation suspension conditions, which may be related to the fact that A. emarginata is a species present in the Atlantic Forest, an environment with greater water availability. In addition, in plants submitted to water stress events, increased damage to the photosynthetic apparatus is observed, causing changes in chlorophyll a fluorescence patterns, which changes the light energy dissipation pathways and increases plant stress [67]. However, in this experiment, plants kept under drought conditions did not show reductions in the chlorophyll a fluorescence pattern, indicating that there was no significant damage to the photosynthetic apparatus. Under flooding, damage is evidenced by low chlorophyll a fluorescence values (ϕ PSII, Fv/Fm, Fv/Fm') and high energy dissipation in the form of heat (D) (Figure 5), which resulted in lower carboxylation efficiency of the enzyme ribulose 1,5-bisphosphate carboxylase (rubisco) (Figure 4). Thus, flooding directly affected the photosynthetic apparatus in A. crassiflora, reducing its efficiency regardless of stomatal conductance, as proposed by Parolin and Wittmann [68] and Oliveira and Gualtieri [64]. In studies with Annona glabra, this reduction in photosynthetic efficiency did not occur, which reinforces its characteristics of adaptation to restinga, a highly flooded environment [69].

The highest effective quantum yield (\oplus PSII), maximum quantum yield in the dark (Fv/Fm) and potential quantum efficiency (Fv'/Fm') values observed in *A. crassiflora* plants kept under drought conditions (similar to plants kept under field capacity) indicate that the energy generated may have been destined both for the production of carbon skeletons used in primary metabolism and for specialized metabolism [58,70]. On the other hand, in plants submitted to flooding conditions, the low quantum yield in the dark (Fv/Fm), low potential quantum efficiency (Fv'/Fm') and greater heat dissipation of the antenna (*D*) indicate a photoprotection mechanism to minimize damage to the photosystem [65].

Thus, in addition to the increase in NADPH + H that can be used for the synthesis of specialized metabolites such as alkaloids, an increase in the production of free radicals from the energy generated in the system is observed [58], and therefore, in stress situations, increases in the production of hydroxyl radicals (OH), superoxides (O_2^-), hydrogen peroxide molecules (H_2O_2) and singlet oxygen (1O_2) are observed, originating from redox reactions that can be in the form of free radicals or in the molecular form of a non-radical [71,72]. Lipoperoxides are the result of the interaction between free radicals and fatty acids in cell

membranes, and when this process occurs, the cell membrane integrity is compromised, resulting in the production of carboxylic compounds such as monoaldehyde [73]. To protect itself and try redox homeostasis, the plant has antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POD) [74].

The enzyme that acts first is SOD, catalyzing the dismutation of two $O_2^{\bullet-}$ radicals, generating H_2O_2 and O_2 . Then, CAT, which is one of the main enzymes acting in the elimination of H_2O_2 generated during photorespiration and β -oxidation of fatty acids, converts two H_2O_2 molecules into water and molecular oxygen. Subsequently, POD, located in the cytosol and vacuoles, catalyzes reactions that use H_2O_2 as oxidants, so this reactive oxygen species (ROS) is also eliminated, even when SOD activity is low [74,75].

Within this complex enzyme system, no significant superoxide dismutase (SOD) activity was observed in *A. crassiflora*; however, CAT activity was higher in flooded plants, which seems to be a specific characteristic of saturated soils [76] and indicates an attempt to reduce H_2O_2 accumulated by stress in order to avoid damage to lipids, proteins and nucleic acids and ensure flooding tolerance [77]. POD activity was also observed, both in plants kept in flooding and in drought, which indicates the continued elimination of reactive oxygen species (ROS) from the system to avoid damage to cells. In this context, POD seems to have been efficient, since no differences were observed in the hydrogen peroxide or lipoperoxide concentration between treatments and control (Figure 2 heat map), which could mean higher malondialdehyde concentrations, Which, in turn, would represent damage to cell membranes [74].

It is noteworthy that to avoid the deleterious effect caused by reactive oxygen species (ROS) in plant tissues, especially in root regions, plants can show greater activity of antioxidant enzymes, especially POD, and accumulate amino acids in roots. Thus, with the greater allocation of amino acids to roots, plants can increase their nitrogen reserve by synthesizing alkaloids [57], which would explain their higher production in *A. crassiflora* roots, especially when submitted to drought stress.

Roots are the main alkaloid production organ in plants of the genus *Annona* [25] and alkaloid accumulation in certain situations, as observed in *A. crassiflora* roots, may indicate osmotic adjustment due to the accumulation of precursor osmolytes, such as amino acids, carbohydrates and sugars such as starch and trehalose [78]. The accumulation of osmolytes can alter the water potential and favor water absorption even in soils with water restriction and generate greater alkaloid [79], which may have occurred with *A. crassiflora*. Thus, even under reduced soil water conditions, gas exchange and fluorescence were not negatively affected, and alkaloid production still occurred.

Regarding the presence of osmolytes, starch interconversion into other sugars that act as osmoprotectors can be considered, with the ability to influence the carbon allocation for the entire plant, mitigating the negative effect of stress caused by water restriction. Furthermore, in photosynthetic cells, starch can be synthesized and temporarily stored in chloroplasts, and this "transient" starch is synthesized and degraded within a 24-hour period [80–82]. Flooding caused an increase in starch concentration in *A. crassiflora* leaves, as reported in wheat (*Triticum* spp.) [83,84], which may be a result of the rapid inhibition of plant growth at the beginning of flooding, leading to lower consumption of sugars produced by photoassimilation, in addition to reduction in photosynthesis [85–87].

Another aspect to be observed regarding the capacity of *A. crassiflora* plants to tolerate abiotic stress was the presence of trehalose both in flooded plants and in those under drought stress. As this non-transport-reducing disaccharide acts as a biostimulant in stress tolerance, its lower concentrations in leaves may indicate its translocation to roots and use in order to neutralize the damage that could be caused by stress, since two glucose molecules are generated from the hydrolysis of trehalose [88]. In addition, trehalose synthesis is induced from some stress condition in order to protect enzymes, proteins and lipid membranes against denaturation under stress situations, also playing an osmoprotective role [78,89].

In summary, when *A. crassiflora* plants are under stress situations, e.g., due to lack of water, CO₂ assimilation tends to decrease and as a consequence, a smaller amount of NADPH₂ is consumed within the Calvin cycle. Thus, much of the energy produced should be dissipated, and, despite the action of non-photochemical mechanisms, such as photorespiration and the xanthophyll cycle, these are potentiated in this situation, and numerous electrons are still transferred to molecular oxygen, generating ROS. Under this situation, plants activate their antioxidant system (SOD, POD and CAT), thus blocking the harmful effect of ROS, leading to a strong increase in the reduction potential of the reducing equivalent (NADPH₂), which can be directed to the synthesis of specialized metabolites [62], which seems to have occurred with *A. crassiflora* plants. Furthermore, starch and trehalose play a role in mitigating the effects of reduced soil water availability to ensure root water absorption (osmoregulation). Thus, *A. crassiflora* plants under drought stress showed increases in the content of total alkaloids, specifically liriodenine.

5. Conclusions

A. crassiflora plants are affected by flooding and drought conditions. Drought generates a stimulus signal for the synthesis of total alkaloids and liriodenine without reducing primary productivity, which denotes rusticity and adaptation of the species to the Cerrado conditions. On the other hand, flooding stress is harmful to the photosynthetic apparatus, which does not result in increased alkaloid production and reduces liriodenine production.

Author Contributions: A.B.M.H.: Conceptualization, data collection, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. I.D.-I.-C.-C.: Investigation, methodology, resources, writing—review. M.M.-V.: Investigation, methodology. M.R.d.S.: investigation, methodology, resources, writing—review. F.G.C.: Data collection, data curation, formal analysis, investigation, methodology, writing—original draft, writing—review and editing. B.C.M.: Data collection, investigation, methodology. G.C.d.S.: Data collection, investigation, methodology. C.S.F.B.: Formal analysis, investigation, methodology, resources, writing—original draft, writing—original draft, writing—review and editing, supervision. G.F.: Conceptualization, data curation, validation, investigation, methodology, resources, writing—original draft, writing—review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Acknowledgments: To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil— CAPES, for the doctoral scholarship granted to the first author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Prado, L.G.; Arruda, H.S.; Araujo, N.M.P.; Braga, L.E.D.O.; Banzato, T.P.; Pereira, G.A.; Figueiredo, M.C.; Ruiz, A.L.T.G.; Eberlin, M.N.; de Carvalho, J.E.; et al. Antioxidant, antiproliferative and healing properties of araticum (*Annona crassiflora* Mart.) peel and seed. *Food Res. Int.* 2020, 133, 109168. [CrossRef]
- Rodrigues, J.A.M.; de Oliveira Andrade, A.C.; Viola, M.R.; Ferreira, D.D.; de Mello, C.R.; Thebaldi, M.S. Hydrological modeling in a basin of the Brazilian Cerrado biome. *Rev. Ambient. Agua* 2021, *16*, 2–18. [CrossRef]
- 3. Lima, J.E.F.W. Situação e perspectivass sobre as águas do Cerrado. Cienc. Cult. 2011, 63, 27–29. [CrossRef]
- 4. Caldas, J.; Cerrados, E. Sustentabilidade Águas do Cerrado: Questão estratégica para o país. Manejo racional dos recursos hídricos é preocupação da pesquisa agropecuária uma vez que Cerrado é origem de grandes bacias hidrográficas. Sustentabilidade 2014, 9–11. Available online: http://www.diadecampo.com.br/zpublisher/materias/Newsletter.asp?id=28947&secao=Agrotemas (accessed on 16 October 2021).
- 5. Lee, J.-E.; Lintner, B.R.; Boyce, C.K.; Lawrence, P.J. Land use change exacerbates tropical South American drought by sea surface temperature variability. *Geophys. Res. Lett.* **2011**, *38*. [CrossRef]
- 6. Debortoli, N.S.; Dubreuil, V.; Funatsu, B.; Delahaye, F.; De Oliveira, C.H.; Rodrigues-Filho, S.; Saito, C.; Fetter, R. Rainfall patterns in the Southern Amazon: A chronological perspective (1971–2010). *Clim. Chang.* **2015**, *132*, 251–264. [CrossRef]
- Penereiro, J.C.; Badinger, A.; Maccheri, N.A.; Meschiatti, M.C. Distribuições de Tendências Sazonais de Temperatura Média e Precipitação nos Biomas Brasileiros. *Rev. Bras. Meteorol.* 2018, 33, 97–113. [CrossRef]
- 8. Aghajanlou, F.; Mirdavoudi, H.; Shojaee, M.; Mac Sweeney, E.; Mastinu, A.; Moradi, P. Rangeland Management and Ecological Adaptation Analysis Model for *Astragalus curvirostris* Boiss. *Horticulturae* **2021**, *7*, 67. [CrossRef]

- Palhares, D.; Franco, A.C.; Zaidan, L.P. Respostas fotossintéticas de plantas do cerrado nas estações seca e chuvosa. *Rev. Bras. Biociências* 2010, 8, 213–220.
- Hoffmann, W.A.; Adasme, R.; Haridasan, M.; De Carvalho, M.T.; Geiger, E.L.; Pereira, M.A.B.; Gotsch, S.G.; Franco, A.C. Tree topkill, not mortality, governs the dynamics of savanna-forest boundaries under frequent fire in central Brazil. *Ecology* 2009, 90, 1326–1337. [CrossRef]
- 11. Hoffmann, W.A.; Franco, A.C. The importance of evolutionary history in studies of plant physiological ecology: Examples from cerrados and forests of central Brazil. *Braz. J. Plant Physiol.* **2008**, *20*, 247–256. [CrossRef]
- 12. Yousefi, A.R.; Rashidi, S.; Moradi, P.; Mastinu, A. Germination and seedling growth responses of *Zygophyllum fabago*, *Salsola kali* L. and *Atriplex canescens* to peg-induced drought stress. *Environments* **2020**, *7*, 107. [CrossRef]
- Justino, A.B.; Pereira, M.N.; Vilela, D.D.; Peixoto, L.G.; Martins, M.M.; Teixeira, R.R.; Miranda, N.C.; da Silva, N.M.; de Sousa, R.M.F.; de Oliveira, A.; et al. Peel of araticum fruit (*Annona crassiflora* Mart.) as a source of antioxidant compounds with α-amylase, α-glucosidase and glycation inhibitory activities. *Bioorg. Chem.* 2016, 69, 167–182. [CrossRef]
- Roesler, R.; Malta, L.G.; Carrasco, L.C.; Pastore, G.C. Food Chemistry and Toxicology Evaluation of the Antioxidant Properties of the Brazilian Cerrado Fruit Annona crassiflora (Araticum). Food Chem. Toxicol. 2006, 71, 102–107.
- 15. Joca, T.A.C.; Oliveira, D.C.; de Zotz, G.; Winkler, U.; Moreira, A.S.F.P. The velamen of epiphytic orchids: Variation in structure and correlations with nutrient absorption. *Flora Morphol. Distrib. Funct. Ecol. Plants* **2017**, 230, 66–74. [CrossRef]
- 16. Cardoso, L.D.M.; Oliveira, D.D.S.; Bedetti, S.D.F.; Martino, H.S.D.; Pinheiro-Sant'Ana, H.M. Araticum (*Annona crassiflora* Mart.) from the Brazilian Cerrado: Chemical composition and bioactive compounds. *Fruits* **2013**, *68*, 121–134. [CrossRef]
- 17. Modesto, E.C.; Santos, G.T.; dos Vilela, D.; Gonçalvez, G.D.; Matsushita, M. Efeitos nutricionais de dietas ricas em ácidos graxos poliinsaturados para os ruminantes e alguns benefícios para o homem. *Arq. Ciências Veterinárias E Zool. UNIPAR* **2002**, *5*, 119–134.
- 18. Luzia, D.M.M.; Jorge, N. Bioactive substance contents and antioxidant capacity of the lipid fraction of *Annona crassiflora* Mart. seeds. *Ind. Crops Prod.* 2013, 42, 231–235. [CrossRef]
- 19. Debnath, B.; Singh, W.S.; Das, M.; Goswami, S.; Singh, M.K.; Maiti, D.; Manna, K. Role of plant alkaloids on human health: A review of biological activities. *Mater. Today Chem.* **2018**, *9*, 56–72. [CrossRef]
- Gonçalves, M.A.; Lara, T.A.; Pimenta, L.P.S. Oxaporphynic alkaloids of *Annona crassiflora* wood Mart. *Annu. Meet. Braz. Chem.* Soc. 2006, 2, 1–2.
- Silva, M.A.; Da Silva, G.A.; da Marques, M.J.; Bastos, R.G.; da Silva, A.F.; Rosa, C.P.; Espuri, P.F. Triagem fitoquímica, atividade antioxidante e leishmanicida do extrato hidroetanólico 70% (v/v) e das frações obtidas de (*Annona crassiflora* Mart.). *Rev. Fitos* 2017, *10*, 505–517. [CrossRef]
- 22. Egydio, A.P.M.; Valvassoura, T.A.; Santos, D.Y.A.C. Geographical variation of isoquinoline alkaloids of *Annona crassiflora* Mart. from cerrado, Brazil. *Biochem. Syst. Ecol.* 2013, 46, 145–151. [CrossRef]
- 23. Ferraz, C.R.; Silva, D.B.; Prado, L.C.D.S.; Canabrava, H.A.N.; Bispo-Da-Silva, L.B. Antidiarrhoeic effect and dereplication of the aqueous extract of *Annona crassiflora* (Annonaceae). *Nat. Prod. Res.* **2019**, *33*, 563–567. [CrossRef] [PubMed]
- Costa, E.V.; Da Cruz, P.E.O.; De Lourenço, C.C.; De Souza Moraes, V.R.; De Lima Nogueira, P.C.; Salvador, M.J. Antioxidant and antimicrobial activities of aporphinoids and other alkaloids from the bark of *Annona salzmannii* A. DC. (Annonaceae). *Nat. Prod. Res.* 2013, 27, 1002–1006. [CrossRef] [PubMed]
- 25. De La Cruz Chacón, I.; González-Esquinca, A.R. Liriodenine alkaloid in *Annona diversifolia* during early development. *Nat. Prod. Res.* **2012**, *26*, 42–49. [CrossRef] [PubMed]
- 26. González-Esquinca, A.R.; De-La-Cruz-Chacón, I.; Castro-Moreno, M.; Orozco-Castillo, J.A.; Riley-Saldaña, C.A. Alkaloids and acetogenins in Annonaceae development: Biological considerations. *Rev. Bras. Frutic.* **2014**, *36*, 01–16. [CrossRef]
- Sousa, M.C.; Bronzatto, A.C.; González-Esquinca, A.R.; Campos, F.G.; Dalanhol, S.J.; Boaro, C.S.F.; Martins, A.L.; da Silva Almeida, J.R.G.; Costa, E.V.; De-la-Cruz-Chacón, I.; et al. The production of alkaloids in *Annona cacans* seedlings is affected by the application of GA4+7 + 6-Benzyladenine. *Biochem. Syst. Ecol.* 2019, *84*, 47–51. [CrossRef]
- Chen, C.-Y.; Wu, H.-M.; Chao, W.-Y.; Lee, C.-H. Review on pharmacological activities of liriodenine. *Afr. J. Pharm. Pharmacol.* 2014, *8*, 364–371. [CrossRef]
- 29. Liu, Y.; Meng, Q.; Duan, X.; Zhang, Z.; Li, D. Effects of PEG-induced drought stress on regulation of indole alkaloid biosynthesis in *Catharanthus roseus*. J. Plant Interact. 2017, 12, 87–91. [CrossRef]
- Costa, E.V.; Pinheiro, M.L.B.; Xavier, C.M.; Silva, J.R.A.; Amaral, A.C.F.; Souza, A.D.L.; Barison, A.; Campos, F.R.; Ferreira, A.G.; Machado, G.M.C.; et al. A pyrimidine-β-carboline and other alkaloids from *Annona foetida* with antileishmanial activity. *J. Nat. Prod.* 2006, 69, 292–294. [CrossRef]
- Cota, L.G.; Vieira, F.A.; Melo Júnior, A.F.; Brandão, M.M.; Santana, K.N.O.; Guedes, M.L.; Oliveira, D.A. Genetic diversity of Annona crassiflora (Annonaceae) in northern Minas Gerais State. Genet. Mol. Res. 2011, 10, 2172–2180. [CrossRef] [PubMed]
- 32. De Lima, J.P.S.; Pinheiro, M.L.B.; Antonio, A.M.; José, J.L.; Santos, D.M.F.; Barison, A.; Silva-Jardim, I.; Costa, E.V. In Vitro Atileishmanial and Cytotoxic Activities of *Annona mucosa* (Annonaceae). *Rev. Virtual Quim.* **2012**, *4*, 692–702. [CrossRef]
- Khan, T.M.; Gul, N.S.; Lu, X.; Wei, J.H.; Liu, Y.C.; Sun, H.; Liang, H.; Orvig, C.; Chen, Z.F. In vitro and in vivo anti-tumor activity of two gold(III) complexes with isoquinoline derivatives as ligands. *Eur. J. Med. Chem.* 2019, 163, 333–343. [CrossRef] [PubMed]
- 34. De-la-Cruz-Chacón, I.; López-Fernández, N.Y.; Riley-Saldaña, C.A.; Castro-Moreno, M.; González-Esquinca, A.R. Antifungal activity in vitro of *Sapranthus microcarpus* (Annonaceae) against phytopathogens. *Acta Bot. Mex.* **2019**, 126, 1420. [CrossRef]

- 35. Sousa, M.A.D.L.B.; Cavalheiro, F. Planejamento paisagístico do Campus Universitário da Faculdade de Ciências Agronômicas, UNESP, Botucatu, S.P. *Acta Bot. Bras.* **1987**, *1*, 155–163. [CrossRef]
- 36. Santos, H.G.; dos Jacomine, P.K.T.; Anjos, L.H.C.; dos Oliveira, V.Á.; de Lumbreras, J.F.; Coelho, M.R.; de Almeida, J.A.; de Araujo Filho, J.C.; de Oliveira, J.B.; Cunha, T.J.F. *Sistema Brasileiro de Classificação de Solos*, 5th ed.; EMBRAPA: Brasília, DF, Brazil, 2018; pp. 195–212.
- 37. Kitajima, M.; Butler, W.L. Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *BBA-Bioenerg*. **1975**, *376*, 105–115. [CrossRef]
- 38. Genty, B.; Briantais, J.M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta-Gen. Subj.* **1989**, *990*, 87–92. [CrossRef]
- 39. Schreiber, U.; Schliwa, U.; Bilger, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* **1986**, *10*, 51–62. [CrossRef]
- 40. Bilger, W.; Björkman, O. Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* **1990**, *25*, 173–185. [CrossRef]
- 41. Klughammer, C.; Schreiber, U. Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method. *PAM Appl. Notes* **2008**, *1*, 27–35.
- Garcia, I.S.; Souza, A.; Barbedo, C.J.; Dietrich, S.M.C.; Figueiredo-Ribeiro, R.C.L. Changes in soluble carbohydrates during storage of *Caesalpinia echinata* LAM. (Brazilwood) seeds, an endangered leguminous tree from the Brazilian atlantic forest. *Braz. J. Biol.* 2006, *66*, 739–745. [CrossRef] [PubMed]
- 43. Clegg, K.M. The application of the anthrone reagent to the estimation of starch in cereals. *J. Sci. Food Agric.* **1956**, 7, 40–44. [CrossRef]
- 44. Morris, D.L. Quantitative Determination of Carbohydrates with *Dreywood's* Anthrone Reagent. American Association for the Advancement of Science Stable. *Am. Assoc. Adv. Sci.* **1948**, 107, 254–255.
- 45. Yemm, E.W.; Folkes, B.F. The Estimation of Carbhydrates in Plant Extracts by Anthrone. *Int. J. Pharm. Pract.* **1954**, *57*, 508–514. [CrossRef]
- 46. Miller, G.L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 1959, 31, 426–428. [CrossRef]
- 47. Passos, L.P. Métodos Analíticos e Laboratoriais em Fisiologia Vegetal, 1st ed.; EMBRAPA: Coronel Pacheco, MG, Brazil, 1996; p. 223.
- 48. Kar, M.; Mishra, D. Catalase, Peroxidase and Polyphenoloxidase Activities during Rice Leaf Senescence. *Plant Physiol.* **1976**, 57, 315–319. [CrossRef]
- 49. Peixoto, P.H.P.; Cambraia, J.; Sant'Anna, R.; Mosquim, P.R.; Moreira, M.A. Aluminum effects on lipid peroxidation and on the activities of enzymes of oxidative metabolism in sorghum. *Rev. Bras. Fisiol. Veg.* **1999**, *11*, 137–143.
- Teisseire, H.; Guy, V. Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (*Lemna minor*). *Plant Sci.* 2000, 153, 65–72. [CrossRef]
- 51. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizin the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, 248–254. [CrossRef]
- 52. Alexieva, V.; Sergiev, I.; Mapelli, S.; Karanov, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat V. *Trop. Ecol.* **2001**, *45*, 315–325. [CrossRef]
- 53. Heatuh, R.L.; Packer, L. Photoperoxidation in Isolated Chloroplasts. *Archives of Biochemistry and Biophysics* **1968**, *125*, 850–857. [CrossRef]
- 54. Costa, J.R. Técnicas Experimentais aplicadas às Ciências Agrárias. *Embrapa Agrobiol.* **2003**, 7–102.
- 55. Becker, R.A.; Chambers, J.M. Auditing of Data Analyses. SIAM J. Sci. Stat. Comput. 1988, 9, 747–760. [CrossRef]
- 56. Nascimento, D.T.F.; Novais, G.T. Clima do Cerrado: Dinâmica atmosférica e características, variabilidades e tipologias climáticas. *Élisée-Rev. De Geogr. Da UEG* **2020**, *9*, e922021.
- 57. Ghorbanpour, M.; Hatami, M. Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid. *Turk. J. Biol.* **2013**, *37*, 350–360. [CrossRef]
- 58. Kleinwächter, M.; Selmar, D. New insights explain that drought stress enhances the quality of spice and medicinal plants: Potential applications. *Agron. Sustain. Dev.* **2015**, *35*, 121–131. [CrossRef]
- 59. Kleinwächter, M.; Paulsen, J.; Bloem, E.; Schnug, E.; Selmar, D. Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). *Ind. Crops Prod.* **2015**, *64*, 158–166. [CrossRef]
- 60. Castro-Moreno, M.; Tinoco-Ojangurén, C.L.; Cruz-Ortega, M.D.R.; González-Esquinca, A.R. Influence of seasonal variation on the phenology and liriodenine content of *Annona lutescens* (Annonaceae). *J. Plant Res.* **2013**, *126*, 529–537. [CrossRef]
- De-la-Cruz-Chacón, I.; Riley-Saldaña, C.A.; Arrollo-Gómez, S.; Sancristóbal-Domínguez, T.J.; Castro-Moreno, M.; González-Esquinca, A.R. Spatio-Temporal Variation of Alkaloids in *Annona purpurea* and the Associated Influence on Their Antifungal Activity. *Chem. Biodivers.* 2019, 16, 1–14. [CrossRef]
- 62. Selmar, D.; Kleinwächter, M. Stress enhances the synthesis of secondary plant products: The impact of stress-related overreduction on the accumulation of natural products. *Plant Cell Physiol.* **2013**, *54*, 817–826. [CrossRef]
- 63. Simonneau, T.; Lebon, E.; Coupel-Ledru, A.; Marguerit, E.; Rossdeutsch, L.; Ollat, N.; Soni, A.; Kumari, P.; Dhakar, S.; Kumar, N.; et al. Physiological behavior of *Annona muricata*, *Dipteryx odorata* and *Copaifera langsdorffii* in response to water and light stress. *Front. Plant Sci.* **2017**, *62*, 1–8. [CrossRef]

- 64. de Oliveira, A.K.M.; Gualtieri, S.C.J. Trocas gasosas e grau de tolerância ao estresse hídrico induzido em plantas jovens de *Tabebuia aurea* (Paratudo) submetidas a alagamento. *Ciência Florest.* **2017**, *27*, 181–191. [CrossRef]
- Selmar, D.; Kleinwächter, M. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Ind. Crops Prod.* 2013, 42, 558–566. [CrossRef]
- 66. Mantoan, L.P.B.; Rolim de Almeida, L.F.; Macedo, A.C.; Ferreira, G.; Boaro, C.S.F. Photosynthetic adjustment after rehydration in *Annona emarginata. Acta Physiol. Plant.* **2016**, *38*, 1–11. [CrossRef]
- Kalaji, H.M.; Jajoo, A.; Oukarroum, A.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Łukasik, I.; Goltsev, V.; Ladle, R.J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol. Plant.* 2016, *38*, 102. [CrossRef]
- 68. Parolin, P.; Wittmann, F. Struggle in the flood: Tree responses to flooding stress in four tropical floodplain systems. *AoB Plants* **2010**, 2010, 1–19. [CrossRef] [PubMed]
- 69. Mielke, M.S.; Matos, E.M.; Couto, V.B.; Almeida, A.-A.F.; de Gomes, F.P.; Mangabeira, P.A.O. Some photosynthetic and growth responses of *Annona glabra* L. seedlings to soil flooding. *Acta Bot. Bras.* **2005**, *19*, 905–911. [CrossRef]
- Nowak, M.; Kleinwächter, M.; Manderscheid, R.; Weigel, H.J.; Selmar, D. Drought stress increases the accumulation of monoterpenes in sage (*Salvia officinalis*), an effect that is compensated by elevated carbon dioxide concentration. *J. Appl. Bot. Food Qual.* 2010, *83*, 133–136.
- Junior, U.O.B.; Lima, M.D.R.; Barbosa, M.A.M.; Batista, B.L.; Lobato, A. Biochemical Responses of Two Species of Eucalyptus Exposed to Aluminium Toxicity: Oxidative Stress and Antioxidant Metabolism. *Not. Bot. Horti Agrobot. Cluj-Napoca* 2016, 44, 107–115. [CrossRef]
- 72. Sataloff, R.T.; Johns, M.M.; Kost, K.M.; Arndt, S.K.; Irawan, A.; Sanders, G.J.; Boanares, D.; Kozovits, A.R.; Lemos-Filho, J.P.; Isaias, R.M.S.; et al. Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O3. *J. Exp. Bot.* 2014, 69, 907–912. [CrossRef]
- 73. Silva, A.A.; da Gonçalves, R.C. Espécies reativas do oxigênio e as doenças respiratórias em grandes animais. *Ciência Rural* **2010**, 40, 994–1002. [CrossRef]
- 74. Barbosa, M.R.; de Araújo Silva, M.M.; Willadino, L.; Ulisses, C.; Camara, T.R. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. *Cienc. Rural* **2014**, *44*, 453–460. [CrossRef]
- 75. Lignan, C.; Suberin, L.; Stilbenes, C.; Tannin, F. Phenylpropanoids comprise a multitude of plant secondary metabolites and cell wall components. In *Plant Biochemistry*, 4th ed.; Elsevier Academic Press: Amsterdam, The Netherlands, 2011; pp. 435–454.
- 76. Møller, I.M.; Jensen, P.E.; Hansson, A. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* 2007, 58, 459–481. [CrossRef]
- Hossain, Z.; López-Climent, M.F.; Arbona, V.; Pérez-Clemente, R.M.; Gómez-Cadenas, A. Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. *J. Plant Physiol.* 2009, 166, 1391–1404. [CrossRef]
- Cortina, C.; Culiáñez-Macià, F.A. Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. *Plant Sci.* 2005, 169, 75–82. [CrossRef]
- Jaleel, C.A.; Manivannan, P.; Kishorekumar, A.; Sankar, B.; Gopi, R.; Somasundaram, R.; Panneerselvam, R. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. *Colloids Surf. B Biointerfaces* 2007, 59, 150–157. [CrossRef] [PubMed]
- Krasensky, J.; Jonak, C. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J. Exp. Bot. 2015, 63, 1593–1608. [CrossRef] [PubMed]
- 81. Liu, B.; Liang, J.; Tang, G.; Wang, X.; Liu, F.; Zhao, D. Drought stress affects on growth, water use efficiency, gas exchange and chlorophyll fluorescence of Juglans rootstocks. *Sci. Hortic.* **2019**, 250, 230–235. [CrossRef]
- 82. Thalmann, M.; Santelia, D. Starch as a determinant of plant fitness under abiotic stress. *New Phytol.* **2017**, *214*, 943–951. [CrossRef] [PubMed]
- 83. Ahmed, F.; Ra, M.; Ismail, M.R.; Juraimi, A.S.; Rahim, H.A.; Asfaliza, R.; Latif, M.A. Waterlogging Tolerance of Crops: Breeding Mechanism of Tolerance Molecular Approaches and Future Prospects. *BioMed Res. Int.* **2013**, 2013. [CrossRef]
- 84. Malik, A.I.; Colmer, T.D.; Lambers, H.; Setter, T.L.; Schortemeyer, M. Short-term waterlogging has long-term effects on the growth and physiology of wheat. *New Phytol.* 2002, *4*, 225–236. [CrossRef]
- 85. Huang, B.; Johnson, J.W. Root respiration and carbohydrate status of two wheat genotypes in response to hypoxia. *Annals of Botany* **1995**, *75*, 427–432. [CrossRef]
- 86. Malik, A.I.; Colmer, T.D. Changes in physiological and morphological traits of roots and shoots of wheat in response to different depths of waterlogging. *Funct. Plant Biol.* 2001, 28, 1121–1131. [CrossRef]
- Setter, T.L.; Waters, I.; Sharma, S.K.; Singh, K.N.; Kulshreshtha, N.; Yaduvanshi, N.P.S.; Ram, P.C. Review of wheat improvement for waterlogging tolerance in Australia and India: The importance of anaerobiosis and element toxicities associated with different soils. *Ann. Bot.* 2008, 103, 221–235. [CrossRef]
- Fernandez, O.; Béthencourt, L.; Quero, A.; Sangwan, R.S.; Clément Christophe, C. Trehalose and plant stress responses: Friend or foe? *Trends Plant Sci.* 2010, 15, 409–417. [CrossRef]
- Paul, M.J.; Primavesi, L.F.; Jhurreea, D.; Zhang, Y. Trehalose metabolism and signaling. *Annu. Rev. Plant Biol.* 2008, 59, 417–441. [CrossRef] [PubMed]