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ALTERAÇÕES METABÓLICAS EM MUDAS DE ESPÉCIES FLORESTAIS NATIVAS DO CERRADO BRASILEIRO DURANTE O PROCESSO DE RUSTIFICAÇÃO MEDIADO POR TRATAMENTOS COM SINALIZADORES

CAMBIOS METABÓLICOS EN PLÁNTULAS DE ESPECIES FORESTALES NATIVAS EN EL CERRADO BRASILEÑO DURANTE EL PROCESO DE RUSTIFICACIÓN MEDIADO POR TRATAMIENTOS DE BANDERA

METABOLIC CHANGES IN SEEDLINGS OF NATIVE FOREST SPECIES OF BRAZILIAN CERRADO DURING HARDENING PROCESS MEDIATED BY SIGNALIZERS TREATMENTS

Presentation: Oral Communication

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RESUMO

O cerrado é um dos cinco biomas brasileiros, que sofrem constantes episódios de queimadas e desmatamento devido à crescente ocupação por atividades agropecuárias. Para neutralizar os danos e restaurar o meio ambiente, estudos com foco na preservação e requalificação dessas áreas são particularmente importantes, especialmente no sul do Piauí. Assim, a aclimatação de mudas de espécies florestais nativas constitui uma etapa crítica para programas de reflorestamento. Este estudo teve como objetivo determinar o papel dos indutores de sinal no estabelecimento e nas respostas metabólicas das espécies Angico Branco, Faveira, Jatobá e Ipê Roxo durante o processo de rustificação. Aos 150 dias após a germinação, as mudas foram submetidas aos tratamentos com ácido salicílico (10, 100 e 1000 µM), peróxido de hidrogênio - H2O2 (10, 15 e 20 mM), estresse hídrico (75% (leve), 50% (moderado) e 25% (severo) da capacidade de campo) e estresse salino (solução salina com 25, 50 e 75 mM de NaCl). Um grupo de plantas permaneceu sem aplicação de tratamento, constituindo o controle negativo. Os parâmetros de integridade da membrana celular, teor relativo de água e teor de lignina foram avaliados e submetidos a ensaios estatísticos apropriados. As espécies Angico-branco e Ipê-roxo mostraram-se sensíveis ao estresse hídrico e salino, e algumas plantas morreram antes da avaliação. As espécies Faveira e Jatobá não apresentaram alteração nos teores de lignina em resposta aos tratamentos estudados, enquanto o Angico-branco apresentou aumento no teor de lignina quando tratado com 25 mM de H2O2 e 100 µM de ácido salicílico, resposta relacionada ao baixo dano de membrana nas folhas e raízes e acúmulo inalterado de massa seca. Adicionalmente, plantas de Ipê-roxo acumularam maiores teores de lignina quando pulverizadas com 15 e 25 mM de H2O2 e com 10, 100 e 1000 µM de ácido salicílico, mas não apresentaram alterações metabólicas e de crescimento em relação aos tratamentos controle. Nossos dados demonstram claramente que H_2O_2 e ácido salicílico são tratamentos potenciais para intensificar o endurecimento de mudas de Angico-branco e Ipê-roxo.

Palavras-Chave: *Albizia niopoides*, *Handroanthus impetiginosus*, *Parkia platycephalla*, *Hymenea coubaril*, Aclimatação.

RESUMEN

El cerrado es uno de los cinco biomas brasileños, que sufren constantes episodios de incendios y deforestación debido a la creciente ocupación por actividades agrícolas. Para contrarrestar los daños y restaurar el medio ambiente, los estudios que se centran en la conservación y recuperación de estas áreas son particularmente importantes, especialmente en el sur de Piauí. Por lo tanto, la aclimatación de plántulas de especies forestales nativas constituye un paso crítico para los programas de reforestación. Este estudio tuvo como objetivo determinar el papel de los inductores de señales en el establecimiento y las respuestas metabólicas de las especies Angico Branco, Faveira, Jatobá e Ipê Roxo durante el proceso de endurecimiento. 150 días después de la germinación, las plántulas fueron sometidas a tratamientos con ácido salicílico (10, 100 y 1000 µM), peróxido de hidrógeno - H2O2 (10, 15 y 20 mM), estrés hídrico (75% (ligero), 50% (moderado) y 25% (grave) de capacidad de campo) y estrés salino (solución salina con NaCl 25, 50 y 75 mM). Un grupo de plantas permaneció sin aplicación de tratamiento, constituyendo el control negativo. Los parámetros de integridad de la membrana celular, contenido relativo de agua y contenido de lignina se evaluaron y sometieron a ensayos estadísticos apropiados. Se encontró que las especies Angico-branco e Ipê-roxo eran sensibles al estrés hídrico y salino, y algunas plantas murieron antes de la cosecha. Las especies Faveira y Jatobá no mostraron alteración en el contenido de lignina en respuesta a los tratamientos estudiados, mientras que Angico-branco mostró un mayor contenido de lignina cuando se trató con 25 mM H2O2 y 100 µM de ácido salicílico, respuesta relacionada con bajo daño de membrana en hojas y raíces y acumulación de masa seca inalterada. Además, las plantas de Ipê-roxo acumularon mayores contenidos de lignina cuando se rociaron con 15 y 25 mM de H_2O_2 y con 10, 100 y 1000 µM de ácido salicílico, pero no mostraron alteraciones metabólicas y de crecimiento en comparación con los tratamientos de control. Nuestros datos demuestran claramente que el H2O2 y el ácido salicílico son tratamientos potenciales para intensificar el endurecimiento de las plántulas de Angico-branco e Ipê-roxo.
Palabras clave: *Albizia niopoides*, *Handroanthus impetiginosus*, *Parkia platycephalla*, *Hymenea coubaril*, Aclimatación.

ABSTRACT

The cerrado is one of the five Brazilian biomes, which suffer from constant episodes of fires and deforestation due to increasing occupation by agricultural activities. To counteract damage and restore the environment, studies focusing on preserving and refallowing these areas are particularly important, especially in southern Piauí. Thus, the acclimatization of seedlings of native forest species constitutes a critical step for reforestation programs. This study aimed to determine the role of signal inducers in establishing and metabolic responses of the species Angico Branco, Faveira, Jatobá and Ipê Roxo during the hardening process. 150 days after germination, the seedlings were subjected to treatments with salicylic acid (10, 100 and 1000 µM), hydrogen peroxide - H_2O_2 (10, 15 and 20 mM), water stress (75% (light), 50% (moderate) and 25% (severe) of field capacity) and salt stress (saline solution with 25, 50 and 75 mM NaCl). A plant group remained without treatment application, constituting the negative control. The parameters of cell membrane integrity, relative water content, and lignin contents were evaluated and subjected to appropriate statistical assays. The species Angico-branco and Ipê-roxo were found to be sensitive to water and saline stress, and some plants died before harvest. The Faveira and Jatobá species did not show any alteration in lignin contents in response to the studied treatments, whereas Angico-branco displayed increased lignin content when treated with 25 mM H_2O_2 and 100 µM salicylic acid, a response related to low membrane damage in leaves and roots and unaltered dry mass accumulation. Additionally, Ipê-roxo plants accumulated higher lignin contents when sprayed with 15 and 25 mM H_2O_2 and with 10, 100 and 1000 µM salicylic acid but did not show any metabolic and growth alterations compared to control treatments. Our data clearly demonstrate that H_2O_2 and salicylic acid are potential treatments to intensify the hardening of Angico-branco and

Ipê-roxo seedlings.
Keywords: *Albizia niopoides, Handroanthus impetiginosus, Parkia platycephalla, Hymenea coubaril*, Acclimatization.

INTRODUCTION

The Cerrado biome has been occupied for decades. The south of Piauí is considered by Rufo and Sobrinho (2018) one of the last agricultural frontiers within the biome, with emphasis on grain production. The overexploitation of forest resources in this biome, fires and deforestation for agricultural purposes are a challenge in regard to sustainable exploitation (ANDRADE et al, 2019). In view of this, the Brazilian Forest Code emphasizes the importance of adhering to reforestation and recovery of areas, with the purpose of reversing the degradation process (EMBRAPA, 2012). of the ecosystem (AQUINO et al., 2009).

Among the forest species with potential for the Brazilian semiarid region and relative adaptation to the different annual climatic conditions, we highlight *Handroanthus impetiginosus* (Ipê Roxo), *Hymenaea courbaril* (Jatobá), *Parkia platycephala* (Faveira), *Albizia niopoides* (Angico Branco), among others (NASCIMENTO et al., 2012; AFONSO et al., 2017; OLIVEIRA 2018).

Hardening or acclimatization is the final stage of seedling production and is an extremely important process for adapting to the environment in which they will be implanted (HOFFMANN, 2002). During this process, a set of techniques and procedures are used to adjust to the environmental adversities of the field, attributing important characteristics, which will be fundamental for the high survival rate of the seedlings after planting (D'AVILA et al., 2011).

Several parameters demonstrate the hardening process in seedlings, with emphasis on the lignin content. Lignin is a substance that makes plant tissues rigid, giving plants mechanical resistance and a greater ability to deal with stressors of both biotic and abiotic origin (SCHULZ et al., 2021).

However, there is a scarcity of studies related to hardening inducers in native forest species of the Brazilian Cerrado, as well as techniques that induce this process. Given this fact, further research is needed to identify agents capable of improving the tolerance of seedlings to stress factors.

THEORETICAL FOUNDATION

Relationship between deforestation and reforestation in the Brazilian Cerrado

The Cerrado is one of the five Brazilian biomes, comprising the North, Northeast and Midwest regions of Brazil. The Cerrado do Piauí, called the last agricultural frontier, comprises 24 municipalities, with 70% of its territorial extension covered by Cerrado vegetation, which has experienced an accelerated occupation in the last three decades, intensified in the 1990s through grain production (EMBRAPA, 2021).

From 2002 to 2009, the deforestation rate in the Cerrado of Piauí was 14.49%, mainly due to the advance of agricultural activity in legal reserves, permanent protection areas (APPs) and other portions of restricted use (ROCHA, 2011). According to INPE (2018), there was a 9% increase in deforestation in 2017, totaling 7,408 km², emphasizing the existence of only 50% of the original vegetation of the ecosystem today.

Due to the high incidence of deforestation, the conservation of the Cerrado is currently supported by current environmental legislation as permanent preservation areas and Legal Reserve areas, established by the Brazilian Forest Code, according to the Civil House of Legal Affairs (2012). In addition, as a way of minimizing the impacts of deforestation of the flora in the Cerrado of Piauí, the act of replanting forests becomes highly appropriate, with the purpose of recovering devastated areas. In view of this, new alternatives for forest exploitation are implemented through sustainable forest management (IBAMA, 2018).

However, the development of studies on the production of seedlings of native species is still low, as it is also known that there is a great need to recover areas destined for environmental preservation. Thus, the use of woody species from native forests is extremely important for reforestation projects or recovery of degraded areas, with the production of quality seedlings being an essential activity (OLIVEIRA, 2018).

The seedling hardening process

According to Hoffmann (2002), hardening is the final stage of seedling production, which involves a set of techniques that progressively reduce environmental impacts of a biotic and abiotic nature, avoiding losses and contributing to the establishment of seedlings. When transferred to a new environment, seedlings are subjected to biotic and abiotic stresses, which can compromise their survival (MARTINS et al., 2009).

According to Alone et al. (2016), hardening consists of the gradual exposure of plants to the conditions of an uncontrolled environment, being capable of causing changes in the behavior of plants, exposing them to different types of stress. The authors also emphasize that plant mortality usually occurs due to water stress, pests and diseases in an uncontrolled external environment.

Among the treatments and techniques used for the hardening phase of seedlings, according to Teixeira (2012), water restriction, reduction of nitrogen fertilization, and exposure of seedlings to sunlight and wind stand out. Hardening should be started 15 days before the seedlings are planted, resulting in an increase in the seedling establishment capacity in the field (TEIXEIRA, 2012).

In general, hardened seedlings present physiological (gas exchange) and molecular (enzymatic activity) adjustments, as well as deposition of starch and lignin in the stem during hardening, which can improve the response of the seedlings after planting.

Seedlings of woody species that have a high lignin content become more rustic because they have greater mechanical strength, better architecture, water circulation through the xylem and lower rates of herbivory (TAIZ; ZEIGER, 2013).

However, in the case of forest species endemic to the Brazilian Cerrado, there are few studies that clarify the biochemical and physiological aspects of the hardening process (WENDLING, DUTRA, GROSSI, 2006).

Potential native species in the Cerrado Piauiense

Among the various forest species found in the Piauí savanna, we can highlight *Handroanthus impetiginosus* (Ipê-roxo), *Hymenaea courbaril* (Jatobá), *Parkia platycephala* (faveira) and *Albizia niopoides* (Angico-branco).

Ipê Roxo belongs to the Bignoniaceae family and is characterized as a tree species native to Brazil that is endemic to the Cerrado and can reach 20 to 35 m in height. Due to its rapid growth and high adaptation, it has become an interesting species for reforestation, but it has a short seed viability period, which represents difficulties in establishing degraded areas and seedling production throughout the year (MARTINS; LAGO; ANDRADE, 2011).

The species *Albizia niopoides*, popularly known as Angico Branco and belonging to the Fabaceae-Mimosoideae family, is a native, nonendemic, dispersed, irregular and discontinuous species found in several South American countries that can be used in the reforestation of degraded areas due to its rapid growth and ability to acclimatize in different environments (KISSMANN et al., 2009; AFONSO et al., 2017).

Faveira (*Parkia platycephalla*) is a native species belonging to the Fabaceae family, endemic to Brazil (ALVES et al., 2016), whose crown favors the development of late secondary species in reforestation programs (PEREIRA, 2011). In addition, due to its tolerance to drought and good adaptation to soils poor in nutrients and with high levels of aluminum, it has become a very interesting species for reforestation (SILVA, 2011).

Finally, Jatobá (*Hymenea coubaril*), belonging to the Fabaceae family, is characterized as a large tree, with emphasis on its adaptive characteristics to different soil and climate conditions and low nutritional and water requirements, which reinforces its potential to be a viable alternative to reforestation (NASCIMENTO et al., 2011).

METHODOLOGY

The experiments were carried out in a greenhouse, and the analyses were performed at the Laboratory for Propagation of Plants, both from the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas (CPCE), located in the city of Bom Jesus, Piauí, Brazil (9°04' south latitude, 44°21' west longitude, with an altitude of approximately 650 m). The environmental conditions of the region include a photoperiod of approximately 12 h and maximum and minimum temperatures of 36 and 22°C, respectively.

Seed collection and storage

Seeds from the species *H. impetiginosus* (Ipê-roxo), *H. courbaril* (Jatobá), *Parkia platycephala* (Faveira) and *Albizia niopoides* (Angico-branco) were collected in three different cities, Colônia do Gurguéia-PI, Bom Jesus-PI and Formosa do Rio Preto-BA. For Jatobá and Faveira, the fruits were collected from two matrices, and their seeds were extracted after 24 h of soaking in water. Then, the material was sanitized in running water for surface cleaning and dried in open air at room temperature. For Angico-branco and Ipê-roxo seeds, the fruits were randomly removed, followed by manual removal of the seeds.

The seeds were stored in individual packages with temperatures varying between 4 and 8 °C. Jatobá seeds were stored in Kraft paper bags, according to Gomes, Garcia and Sousa (2012), while Angico-branco, Faveira and Ipê-roxo seeds were packed in polyethylene plastic bags (SILVA et al., 2017).

Treatments to break dormancy

Except for Ipê-roxo, seed dormancy was broken before sowing. To overcome tegumentary dormancy in Angico-branco and Faveira, the process of mechanical scarification of the seeds was carried out with sandpaper number 80 in the region adjacent to the micropyle until slight visible wear of the tegument, according to Nascimento et al. (2009). For the Jatobá species, after initial disinfestation in 2% sodium hypochlorite for 5 minutes, the seeds were subjected to chemical treatment with 80% sulfuric acid diluted in water for 10 min, according to Sousa et al. (2020). Finally, they were again immersed in sodium hypochlorite for 5 minutes to remove sulfuric acid residues.

Sowing

After previous germination tests, the installation of definitive experiments was carried out to select the doses/concentrations of the potential agents inducing hardening. Initially, 176 seeds of Faveira were sown on December 30, 2020, while on January 4, 2020, and January 7, 2021, Angico-branco, Ipê-roxo and Jatobá seeds were sown, respectively, with 176 seeds sown for each species. All 704 seeds of the four species were sown in 120 cm³ tubes containing the Carolina Soil substrate, consisting of sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum and NPK fertilizer (traces). In this case, the tubes were kept in the Forest Nursery with automatic irrigation four times a day for 30 minutes for a period of 74 days. The germination process and the morphological aspects of the seedlings were evaluated daily until the formation of the aerial part of the same parts, which was the criterion used to evaluate germination.

The treatments were applied at 151 days after sowing (DAS) on 5-month-old seedlings, where the seedlings from all forest species were subjected to five treatments with five replications. Each treatment was analyzed individually for each species as follows:

Negative control - spraying with deionized water + nonionic surfactant, Tween 20;

Saline stress - nonionic surfactant Tween 20 and irrigation with saline solution containing NaCl at 25, 50 and 75 mM;

Hydrogen peroxide (H_2O_2) - Spraying with deionized water + surfactant nonionic Tween 20 at 10 mM, 15 mM and 25 mM;

Salicylic Acid (SA) – Spraying with deionized water + nonionic surfactant, Tween 20, doses of 10, 100 and 1000 µM;

Water stress – reduction of irrigation water level by 75%, 50% and 25% of the substrate's water holding capacity (CRA).

The values of relative humidity and air temperature during the experiments were obtained daily with the aid of a digital thermohygrometer.

Cell membrane integrity

The percentage of membrane damage (MD) was estimated using the electrolyte leakage method with the following relationship: MD (%) = (L1/L2) x 100, as described by Lima et al. (2011).

Lignin content

Frozen stem samples were initially macerated, washed with 1.5 mL of 80% ethanol and centrifuged at 12,000 x g for 15 minutes. The supernatant was discarded and replaced with 1.5 mL of deionized water. The precipitate was dried in an oven at 65°C overnight. After washing, the material was subjected to lignin extraction using the thioglycolic acid method. After cooling, the samples were centrifuged for 10 min at 12,000 x g, and the supernatant was discarded. A fraction of 1.5 ml of sodium hydroxide (NaOH) was added to the precipitate and stirred at 150 rpm. After centrifugation again for 10 minutes at 12,000 x g, the supernatant was transferred to a new tube. Then, 200 μL of concentrated HCl was added and taken to the cold chamber for 4 h and centrifuged for 10 min, and the supernatant was discarded. In the precipitate, 2 mL of NaOH was added, and the lignin was measured by spectrophotometric readings at 280 nm.

Biomass determination

The determination of the biomass of roots, stems and leaves was determined from the drying of the plant material in an oven at a temperature of 65°C and subsequent weighing.

Statistical assays

Four independent experiments for each species were carried out in a completely randomized design (DIC), with 5 replications. In each experiment, the data were submitted to analysis of variance (ANOVA) using the F test at 5% probability, and the means were compared using Tukey's test ($P \leq 0.05$). Thereafter, the treatments and plant species more responsive to lignin accumulation were analyzed again by one-way ANOVA. Statistical analyses were performed using the SISVAR program.

RESULTS AND DISCUSSION

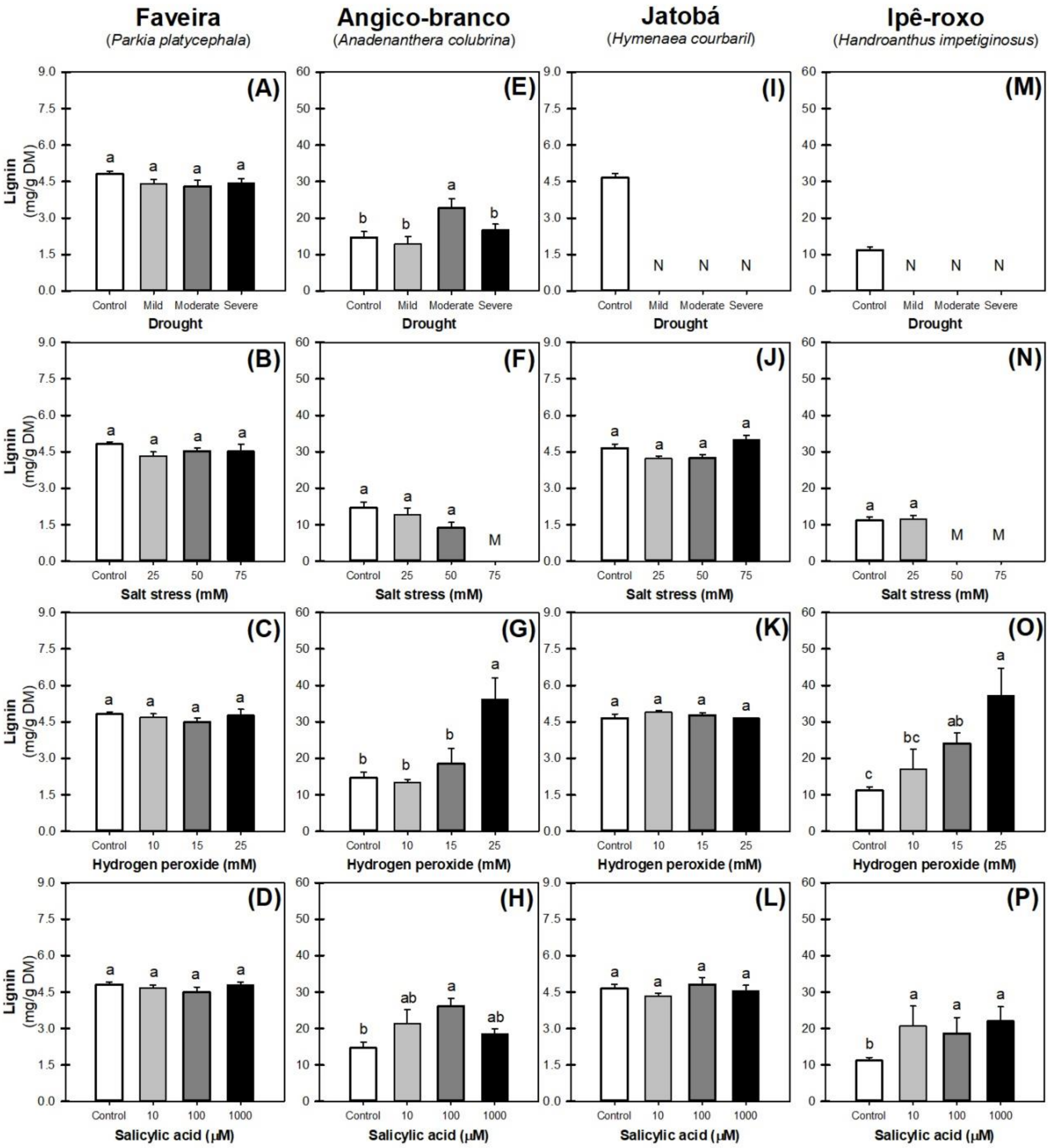
Lignin contents in the stems of native cerrado forest species as a function of different treatments

Cellulose and lignin are the most abundant organic substances in plants. Lignin is a phenolic macromolecule that presents several functions, such as rigidity and maintenance of stem verticality, water circulation through the xylem, mechanical support and plant defense against herbivores (TAIZ; ZEIGER, 2013). Herein, the treatments did not stimulate an increase in the production of lignin in Faveira and Jatobá, suggesting that these species are not responsive to the tested conditions (Figure 01). On the other hand, Angico-branco plants showed significant increases in lignin content under moderate water stress at 50%, as well as when sprayed with 25 mM hydrogen peroxide and 100 μM salicylic acid (Figure 01 E, G and H). However, Ipê-roxo displayed a significant increase when treated with 15 and 25 mM hydrogen peroxide and 10, 100 and 1000 μM salicylic acid, showing to be effective treatments for the hardening of the species (figure 01 O and P).

The saline stress treatment was found to be less effective, as it did not induce the production of lignin in any species (Figure 01) and was even lethal to Angico-branco and Ipê-roxo species by promoting the death of plants at the highest levels (Figure 01 F and N). However, the species Faveira and Jatobá were tolerant to stress because even without stimulation of lignin production, the plants adapted, and their growth was stimulated (Figure 01 B and J).

By analyzing the lignin content data, two species were found to be responsive to the studied treatments, Angico-branco and Ipê-roxo, which displayed a significant increase in lignin content. Thus, the plant material of these responsive treatments was selected to carry out physiological assays in plants exposed to rusting agents.

Figure 01. Lignin content in the stems of Angico-branco, Faveira, Jatobá and Ipê-roxo species submitted to different conditioning treatments (negative control, saline stress at 25, 50 and 75 mM NaCl, water stress at 25, 50 and 75%, hydrogen peroxide at 10, 15 and 25 mM, and salicylic acid at 10, 100 and 1000 µM). In each treatment, different lowercase letters represent significance due to treatment level, according to Tukey's test ($p \leq 0.05$). M - plant death and N - No data.

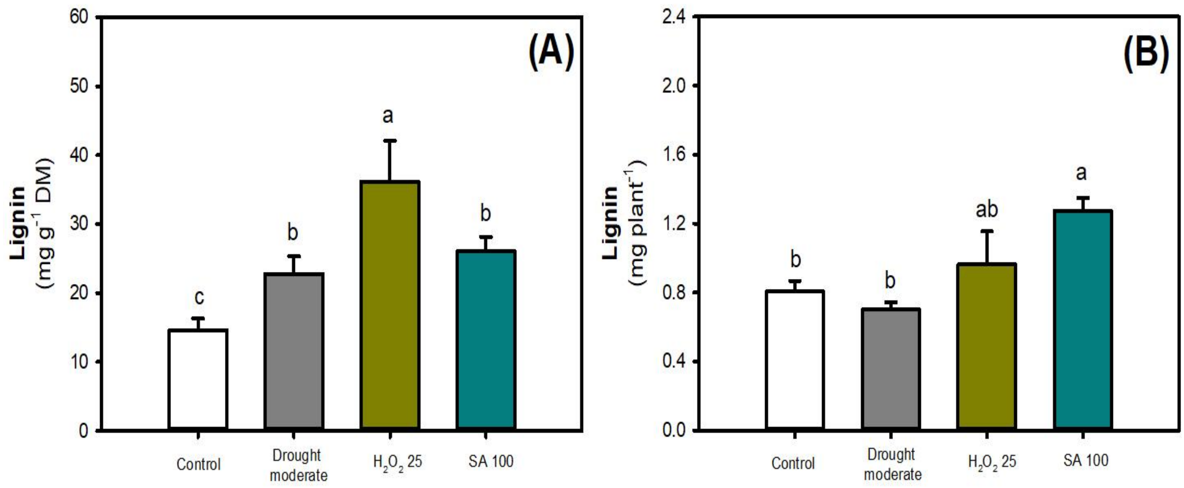


Source: own (2022).

Lignin content and accumulation in Angico-branco

In Angico-branco plants, the spray with hydrogen peroxide at 25 mM (higher dose) was found to be more effective, providing high levels of lignin in mg g^{-1} MS, higher than in the control plants (Figure 02 A). However, by analyzing the total accumulation of lignin per plant, only the salicylic acid treatment promoted significant increases compared to the control (Figure 02 B).

Figure 02. Lignin contents in mg g^{-1} of dry matter (A) and lignin accumulation in mg/plant of Angico-Branco seedlings submitted to different conditioning treatments (negative control, water stress at 25% FC, hydrogen peroxide at 25 mM and salicylic acid at 100 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).



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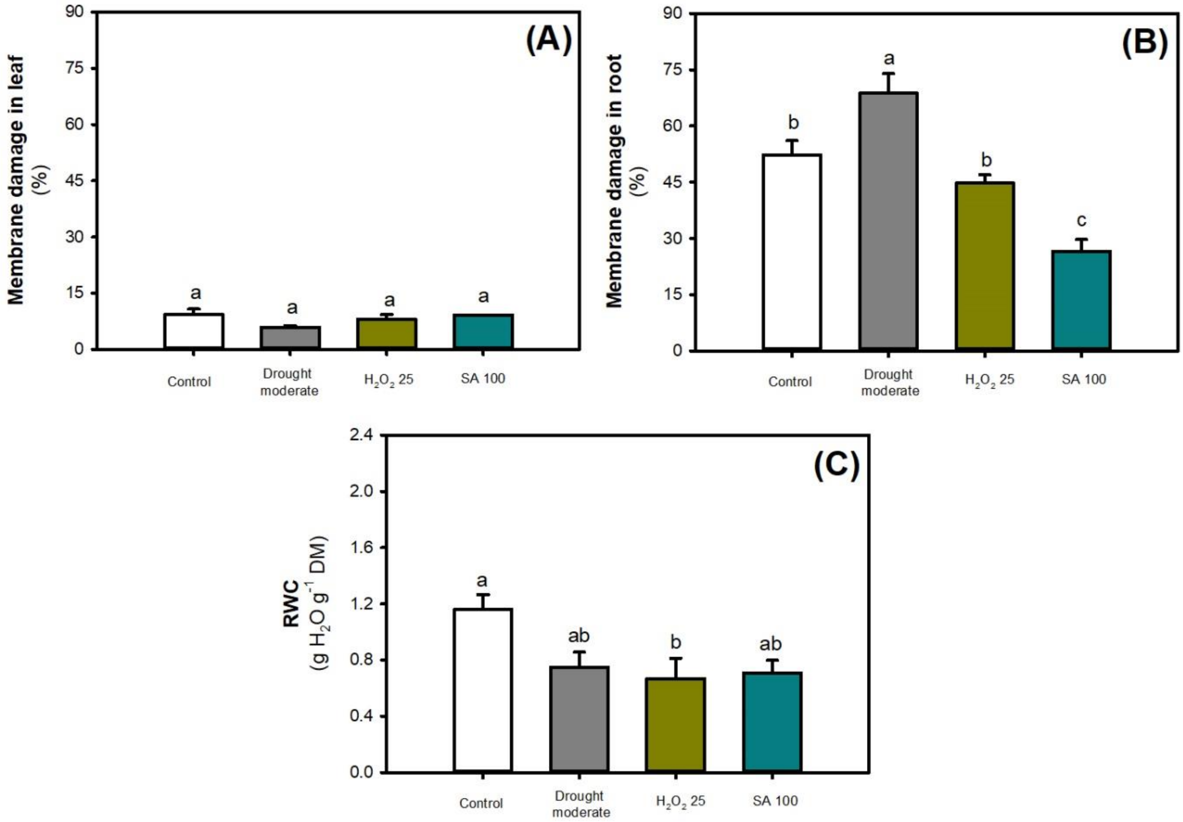
Membrane damage in leaves and roots and relative water content (RWC) of Angico-branco plants

Significant changes in membrane damage were recorded only in the roots of Angico-branco subjected to moderate water stress (Figure 03). On the other hand, treatment with salicylic acid at 100 µM reduced membrane damage in plant roots compared to the control (Figure 03 B). In general, there were no significant variations in the RWC of plants (g H2O g^{-1} DM) submitted to treatments with moderate water stress and salicylic acid, whereas the treatment with hydrogen peroxide significantly reduced the water content of Angico-branco tissues (Figure 03 C).

Figure 03. Membrane damage in leaves (A) and roots (B) of Angico-branco plants and relative water content (C) in tissues of angico-branco plants submitted to different conditioning treatments (negative control, water stress at 25% FC, hydrogen peroxide at 25 mM and salicylic acid at 100 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).

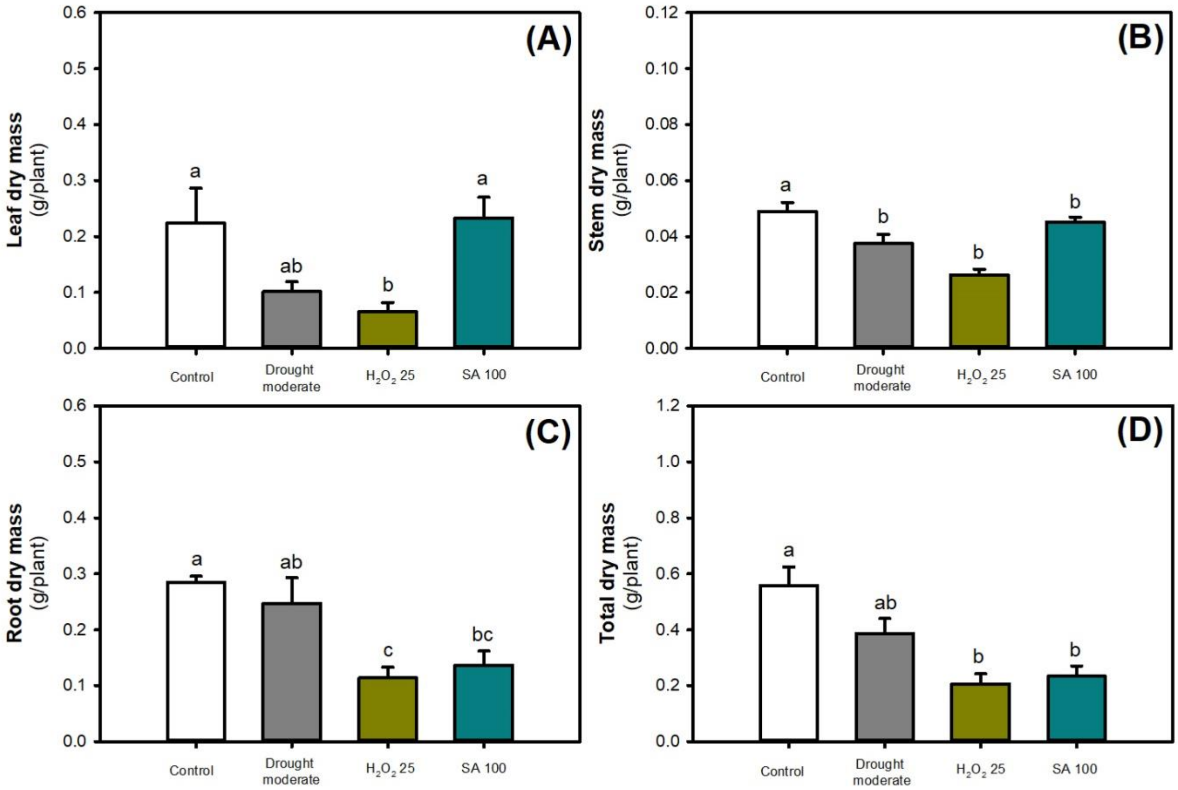


Source: own (2022).

Dry mass of Angico-branco plants

The 100 µM salicylic acid treatment induced an accumulation of leaf dry mass similar to the control treatment but promoted reductions in the dry mass of stems, roots and total Angico-branco (Figure 04). Under water stress, reductions in biomass production were observed only in the stem compared to the control, while the treatment with H_2O_2 promoted reductions in the biomass of all the plant organs and in the total biomass (Figure 04).

Figure 04. Dry mass in g/plant of leaves (A), stems (B), roots (C) and total dry mass (D) of Angico Branco plants submitted to different conditioning treatments (negative control, water stress at 25% FC, hydrogen peroxide at 25 mM and salicylic acid at 100 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).

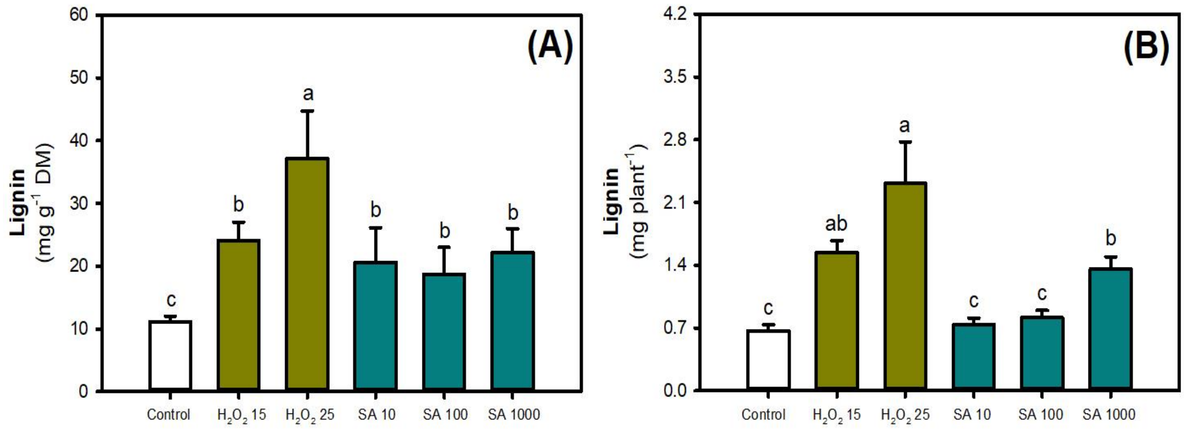


Source: own (2022).

Lignin content in Ipê-roxo

In Ipê Roxo plants, all treatments analyzed intensified the levels of lignin in the stem compared to the control, with the highest increases recorded for the treatment with H_2O_2 at 25 mM (Figure 05 A). This treatment also stimulated the highest total accumulation of lignin per plant, followed by treatment with hydrogen peroxide at 15 mM and treatment with salicylic acid (SA) at 1000 µM (Figure 05 B). It is worth mentioning that the plants treated with salicylic acid at 10 and 100 µM presented total lignin accumulation similar to that of the control (Figure 05 B).

Figure 05. Lignin contents in mg g^{-1} of dry mass (A) and lignin accumulation in mg/plant (B) in the stems of Ipê-roxo plants submitted to different conditioning treatments (negative control, hydrogen peroxide at 15 and 25 mM and salicylic acid at 10, 100 and 1000 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).

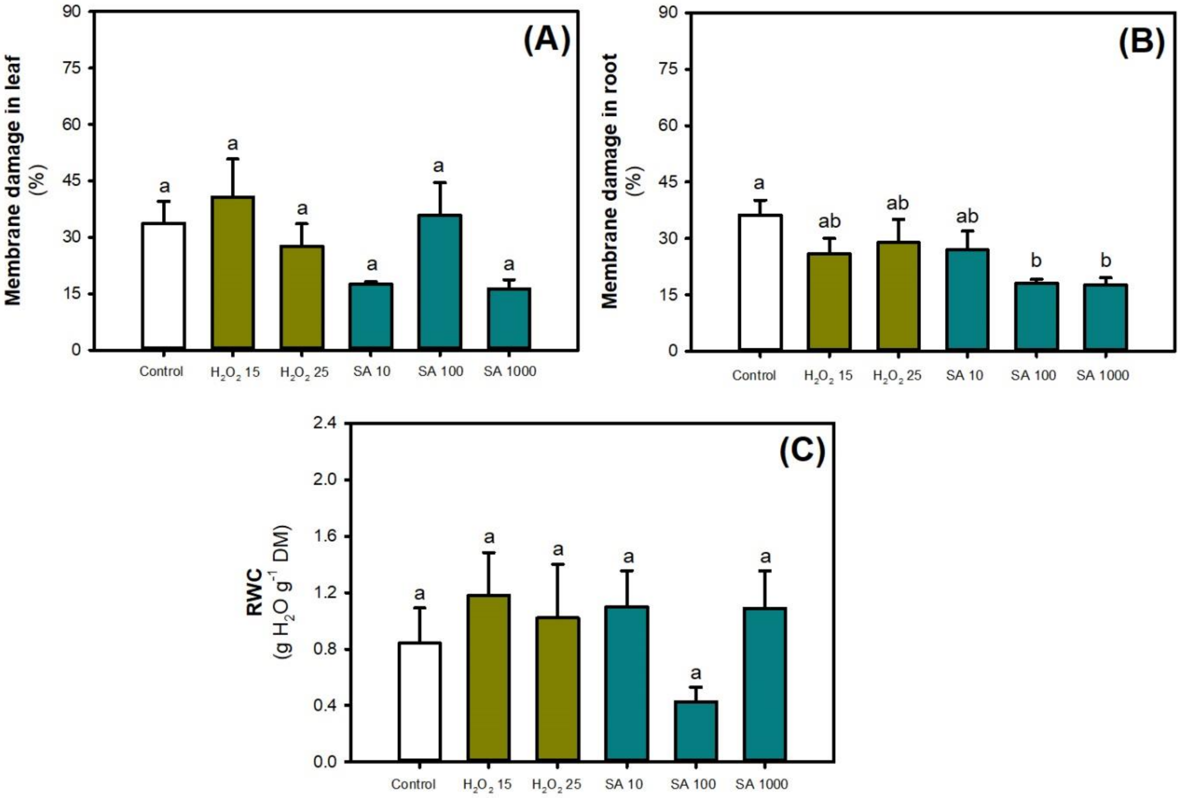


Source: own (2022).

Membrane damage and RWC on leaves and roots of Ipê-roxo plants.

There were no significant changes in membrane damage in leaves of Ipê-roxo plants as a function of treatments with H_2O_2 and AS (Figure 06 A); however, in the roots, spraying with salicylic acid at 100 and 1000 µM promoted significant reductions in membrane damage when compared to the control treatment (Figure 06 B). The data suggest that the treatment with salicylic acid at 1000 µM has the potential for hardening purposes because, in addition to stimulating the production of lignin in the plants, it reduced the damage to the roots of Ipê-roxo (Figures 06 A and 06 B). There were no significant variations in the water contents of the Ipê-roxo plants when the treatments were applied, not differing from the control plants (Figure 06 C).

Figure 06. Membrane damage in leaves (A) and roots (B) and water content (g H_2O g^{-1} DM) in the tissues of Ipê-roxo plants submitted to different conditioning treatments (negative control, hydrogen peroxide at 15 and 25 mM and salicylic acid at 10, 100 and 1000 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).

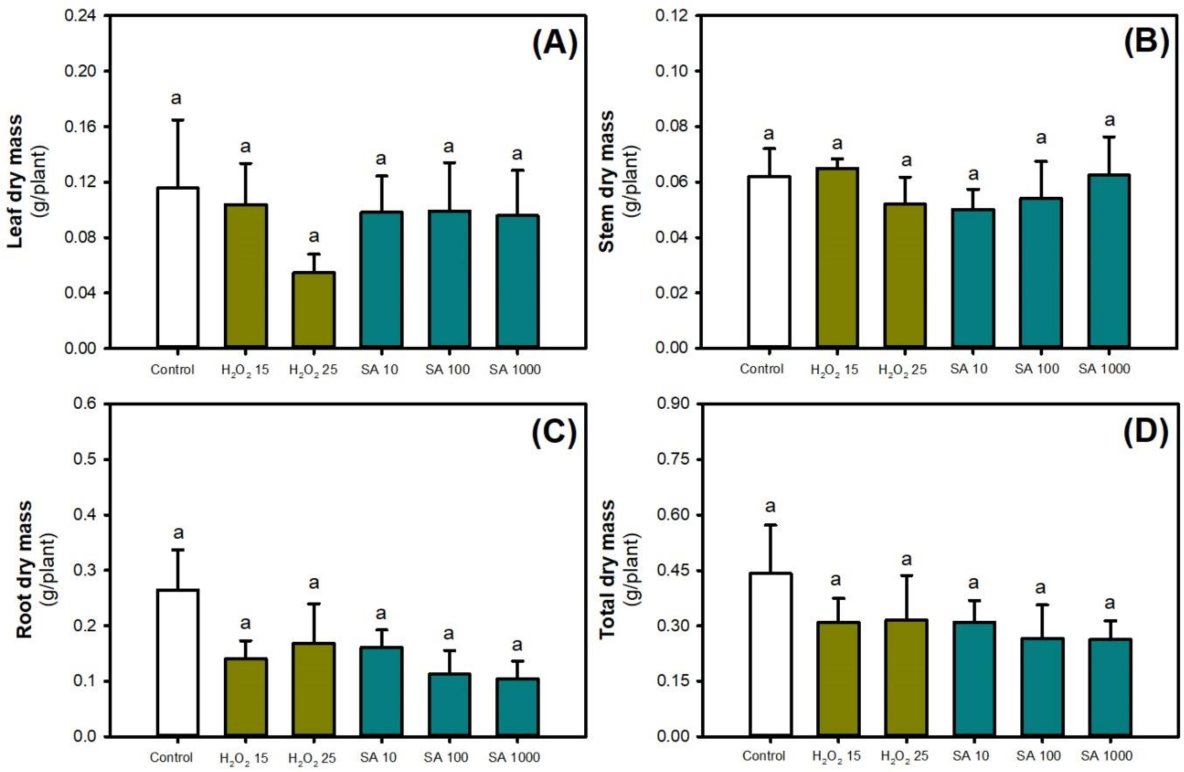


Source: own (2022).

Dry biomass of Ipê-roxo plants

In general, there were no significant variations in the production of dry mass of leaves, stem, roots and total dry mass of Ipê-roxo plants when the different treatments with hydrogen peroxide and salicylic acid were applied in relation to the values of the control plants. (Figure 07).

Figure 07. Dry mass of leaf (A), stem (B), root (C) and total (D) of Ipê plants submitted to different conditioning treatments (negative control, hydrogen peroxide at 15 and 25 mM and salicylic acid at 10, 100 and 1000 µM). Different lowercase letters represent significant differences due to the studied treatment according to Tukey's test ($p \leq 0.05$).



Source: Própria (2022).

CONCLUSIONS

Faveira and Jatobá species were not responsive to treatments with the tested hardening agents, and treatments with water and salt stress were lethal for Angico-branco and Ipê-roxo species under severe conditions.

The conditioning of Angico-branco plants with H_2O_2 at 25 mM, salicylic acid at 100 µM, and moderate water stress promotes an increase in lignin contents, highlighting salicylic acid at 100 µM as the strategy with the greatest potential for Angico-branco hardening.

The Ipê-roxo plants accumulate high levels of lignin when sprayed with H_2O_2 at 15 and 25 mM and salicylic acid at 10, 100 and 1000 µM, but treatment with salicylic acid at 1000 µM is the main potential strategy for hardening purposes because it stimulates the production of lignin and alleviates membrane damage to Ipê-roxo roots.

Further studies are needed to investigate the potential of the inducing agents in the hardening of the Angico-branco and Ipê-roxo species, especially for a longer period of conditioning with salicylic acid and hydrogen peroxide, together with validation and performing assays under field conditions.

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