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TRATAMENTOS DE ACLIMATIZAÇÃO NO DESEMPENHO DE CRESCIMENTO E TEOR DE LIGNINA DE MUDAS DE ESPÉCIES DE ÁRVORES NATIVOS

TRATAMIENTOS DE ACLIMATIZACIÓN EN RENDIMIENTO DE CRECIMIENTO Y CONTENIDO DE LIGNINA DE PLÁNTULAS DE ESPECIES DE ÁRBOLES DEL BOSQUE NATIVO

ACCLIMATIZATION TREATMENTS IN GROWHT PERFORMANCE AND LIGININ CONTENT OF SEEDLINGS FROM NATIVE FOREST TREE SPECIES

Presentation: Oral Communication

Renato Oliveira de Sousa[1]; Flávia Marques de Brito [2]; Milena Rodrigues Luz [3] Manderson Nunes da Silva[4]; Rafael de Sousa Miranda[5]

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RESUMO

O cerrado vem passando por constantes mudanças climáticas, incluindo um crescente processo de desmatamento. Para recuperar esse bioma, o reflorestamento com espécies nativas é uma atividade fundamental. Durante o processo de produção de mudas, o uso de técnicas de aclimatação é fundamental para reduzir os impactos ambientais antes do plantio. O presente estudo teve como objetivo investigar o papel dos tratamentos de aclimatação no crescimento e teor de lignina em quatro espécies florestais nativas. Sementes de *Albizia niopoides* (Angico-branco), *Parkia platycephala* (Faveira), *Handroanthus impetiginosus* (Ipê-roxo) e *Hymenaea courbaril* (Jatobá) foram semeadas em substrato comercial e submetidas a tratamentos com estresse hídrico (75, 50 e 25% da capacidade de campo), estresse salino (NaCl a 25, 50 e 75 mM), ou pulverizado com ácido salicílico (10, 100 e 1000 µM) e peróxido de hidrogênio – H2O2 (10, 15 e 25 mM). Aos 60 dias após a imposição dos tratamentos, avaliou-se a área foliar, massa fresca e seca da folha, caule, raiz e total, e teor de lignina. Para todos os casos, o desempenho das plantas varia de acordo com o tratamento estudado. O déficit hídrico promoveu diminuição no crescimento de todas as espécies, enquanto o estresse salino foi letal em altas concentrações para *A. niopoides* e *H. impetiginosus*. O ácido salicílico e H_2O_2 não influenciaram negativamente a produção de biomassa de *P. platycephala*, *H. courbaril e H. impetiginosus*. O teor de lignina não foi significativamente alterado em *P. phatycephala e H. courbaril*, enquanto plantas de *A. niopoides e H. impetiginosus* apresentaram aumento significativo no teor de lignina quando tratadas com H2O2 (dose de 25 mM). Em conclusão, o pré-tratamento com H2O2 a 25 mM é uma estratégia eficaz para promover o acúmulo de lignina sem inibir o crescimento

[1] Mestrando em Ciências Agrárias, Universidade Federal do Piauí, renato.sousa@ufpi.edu.br
[2] Graduanda em Engenharia Florestal, Universidade Federal do Piauí, flavia.marquesbrito@hotmail.com
[3] Graduanda em Engenharia Florestal, Universidade Federal do Piauí, milenaluz@gmail.com
[4] Graduando em Engenharia Florestal, Universidade Federal do Piauí, mandersonnunes@ufpi.edu.br
Doutor em Bioquímica, Universidade Federal do Piauí, rsmiranda@ufpi.edu.br

de Angico-branco e Ipê-roxo, que está despontando como uma estratégia poderosa para o endurecimento de plântulas de espécies nativas do semiárido.
Palavras-chave: *Albizia niopoides, Parkia platycephala, Handroanthus impetiginosus, Hymenaea courbaril,* Rustificação de mudas, Reflorestamento.

RESUMEN

El cerrado ha sufrido cambios climáticos constantes, incluido un proceso de deforestación creciente. Para recuperar este bioma, la reforestación con especies autóctonas es una actividad clave. Durante el proceso de producción de plántulas, el uso de técnicas de aclimatación es fundamental para reducir los impactos ambientales antes de la siembra. El estudio actual tuvo como objetivo investigar el papel de los tratamientos de aclimatación sobre el crecimiento y el contenido de lignina en cuatro especies forestales nativas. Semillas de *Albizia niopoides* (Angico-branco), *Parkia platycephala* (Faveira), *Handroanthus impetiginosus* (Ipê-roxo) e *Hymenaea courbaril* (Jatobá) fueron sembradas en sustrato comercial y sometidas a tratamientos con estrés hídrico (75, 50 y 25% capacidad de campo). estrés salino (NaCl a 25, 50 y 75 mM), o rociados con ácido salicílico (10, 100 y 1000 μM) y peróxido de hidrógeno – H2O2 (10, 15 y 25 mM). A los 60 días de la imposición de los tratamientos se evaluó área foliar, masa fresca y seca de hoja, tallo, raíz y total, y contenido de lignina. Para todos los casos, el rendimiento de las plantas varía según el tratamiento estudiado. El déficit hídrico promovió una disminución en el crecimiento de todas las especies, mientras que el estrés salino fue letal en altas concentraciones para *A. niopoides* y *H. impetiginosus*. El ácido salicílico y el H2O2 no influyeron negativamente en la producción de biomasa de *P. platycephala*, *H. courbaril* y *H. impetiginosus*. El contenido de lignina no se alteró significativamente en *P. phatycephala* y *H. courbaril*, mientras que las plantas de A. niopoides y H. impetiginosus mostraron un aumento significativo en el contenido de lignina cuando se trataron con H2O2 (dosis de 25 mM). En conclusión, el pretratamiento con H2O2 a 25 mM es una estrategia eficaz para promover la acumulación de lignina sin inhibir el crecimiento de Angico-branco e Ipê-roxo, que se perfila como una poderosa estrategia para el endurecimiento de plántulas de especies nativas semiáridas.
Palabras clave: *Albizia niopoides, Parkia platycephala, Handroanthus impetiginosus, Hymenaea courbaril,* endurecimiento de plántulas, reforestación.

ABSTRACT

The cerrado has been undergoing constant climate changes, including an increasing deforestation process. To recover this biome, reforestation with native species is a key activity. During the seedling production process, the use of acclimatization techniques is critical to reduce environmental impacts before planting. The current study aimed to investigate the role of acclimatization treatments on growth and lignin content in four native forest species. Seeds from *Albizia niopoides* (Angico-branco), *Parkia platycephala* (Faveira), *Handroanthus impetiginosus* (Ipê-roxo) and *Hymenaea courbaril* (Jatobá) were sown in commercial substrate and subjected to treatments with water stress (75, 50 and 25% of field capacity), salt stress (NaCl at 25, 50 and 75 mM), or sprayed with salicylic acid (10, 100 and 1000 μM) and hydrogen peroxide – H_2O_2 (10, 15 and 25 mM). At 60 days after the imposition of treatments, leaf area, fresh and dry mass of leaf, stem, root and total, and lignin content were evaluated. For all cases, the performance of plants varies depending on the studied treatment. Water deficit promoted a decrease in the growth of all species, while salt stress was lethal at high concentrations for *A. niopoides* and *H. impetiginosus*. Salicylic acid and H_2O_2 did not have a negative influence on the biomass production of *P. platycephala*, *H. courbaril* and *H. impetiginosus*. The lignin content was not significantly altered in *P. phatycephala* and *H. courbaril*, whereas *A. niopoides* and *H. impetiginosus* plants displayed a significant increase in lignin content when treated with H_2O_2 (25 mM dose). In conclusion, pretreatment with H_2O_2 at 25 mM is an effective strategy to promote lignin accumulation without inhibiting the growth of Angico-branco e Ipê-roxo, which is emerging as a powerful strategy for seedling hardening of semiarid native species.
Keywords: *Albizia niopoides, Parkia platycephala, Handroanthus impetiginosus, Hymenaea courbaril,* seedling hardening, reforesting.

INTRODUCTION

The high rate of conversion of areas covered by native forest to areas of agropastoral production in the cerrado biome has generated problems related to environmental impacts from inappropriate land use. These changes tend to fragment areas of natural vegetation and trigger erosion processes, silt up water courses and lower water tables, causing not only environmental problems but also economic and social losses (ROCHA et al., 2011). Sano et al. (2010) stated that the accelerated expansion of agriculture converted an area of approximately 80 million hectares into pasture and areas for agriculture by 2002, equivalent to approximately 5 times the extension of the wetland biome.

The use of native species for the recovery of degraded areas for the implementation of human activities has been increasingly used. Taking into account the high mortality of seedlings of native species after planting, it is necessary to improve production techniques to facilitate field planting, providing cost reduction and success in the establishment of seedlings in restoration areas (BARBOSA; RODRIGUES; COUTO, 2013). To mitigate seedling mortality, there has been an increasing number of studies aimed at the use of hardening inducers at the end of the production cycle. This practice aims to increase tolerance to adverse edaphoclimatic conditions found in the place of establishment of seedlings in the field (LIMA et al., 2014).

The hardening process can be carried out by both physical and chemical stimuli exerted by the application of plant regulators. Given the above, it is extremely important to know the native forest species and the elucidation of seedling hardening inducers for the success of reforestation plantations, especially in the southern region of the state of Piauí.

THEORETICAL FOUNDATION

Logging

Klink and Machado (2005) emphasize that despite the cerrado biome being part of a certain area of ecological relevance for having differentiated vegetation, called the ecological hotspost, being part of the 36 world biogeographic regions of biodiversity that are destined for world conservation, its rates of deforestation are shown to be high for a low conservation effort when compared, for example, with the Amazon rainforest.

Since the 1970s, several factors have led to an increase in deforestation, with emphasis on the characteristics of flat relief, population growth and the implementation and development of infrastructure. All this has promoted the advancement of agriculture and livestock (Rocha et al. 2011). In 2002, approximately 40% of the total area of the biome was converted into pasture, urbanization and agriculture areas (SANO et al., 2008).

Oliveira, Oliveira Filho and Eisenlohr (2020) explained that the Cerrado region is a biodiversity hotspot, corresponding to approximately 25% of the Brazilian territory, being the largest savanna on the planet. It presents levels of xerophytic vegetation, cerradão, gallery forest (riverine forests) and seasonal forests. The Cerrado is located in central Brazil and has a seasonal tropical climate, with annual rainfall between 696 and 2,443 mm and an average annual temperature between 17 and 27.9°C. The Cerrado has lost 50% of its original area, and the main threat to its biodiversity is agriculture and livestock.

Reforestation

Planting seedlings to restore degraded areas is a widely used technique. Among the models or strategies of restoration of degraded areas, the planting of seedlings stands out based on the concept of ecological restoration and is also the most used method. This strategy accelerates the process of natural succession, quickly protects the soil against erosion and guarantees the success of recovery (GUIMARÃES, 2019).

The native species of the semiarid region, despite having a low average annual increment (IMA) when compared with fast-growing species, are advantageous for the purpose of reconstituting degraded areas due to their relative adaptation to the edaphic and climatic conditions of the region. which they are inserted (MOREIRA et al., 2019). In association with the correct choice of species, other factors, such as seedling quality, planting time and properly established cultural practices, directly influence the success of forest plantations (GOMES et al., 2002).

Rustification Inducers

Hardening or acclimatization refers to the set of cultural practices adopted during seedling formation to increase tolerance to the shock of field planting (DRANSKI; MALAVASI; MALAVASI, 2017). Therefore, the use of preconditioning methods is advantageous for obtaining plants with greater tolerance to biotic and abiotic stress factors (DUARTE, 2017). Exposure to full sun, water restriction, and, more recently, the application of plant hormones and exposure to adverse conditions constitute the main management techniques employed for hardening processes (SARAIVA, SOUZA, RODRIGUES, 2014).

The application of water stress in the seedling phase as a form of acclimatization allows plants to develop strategies to effectively control physiological processes, such as transpiration and photosynthesis, to provide their survival with minimal water loss (LIMA et al., 2014). Due to the increase in the salinization process caused by water scarcity in northeastern soils, salinity stress can be an important inducer of hardening, as it significantly reduces plant yield, affecting the physiological, biochemical and morphological processes of plants (LOPES et al., 2019).

Salicylic acid has been cited as effective in activating plant hardening, which may result in plant protection, adaptation and resistance to water and saline stress (MAZZUCHELLI; SOUZA; PACHECO, 2014; LOPES et al., 2019). This hormone acts in the signaling and expression of genes that activate plant defense mechanisms, providing a reduction in planting and management costs (MAZZUCHELLI; SOUZA; PACHECO, 2014).

The prior supply of hydrogen peroxide-H_2O_2 may also induce the acclimatization of plants under stress conditions, reducing the deleterious effects on their growth and physiology (DA SILVA et al., 2019).

Native Species of Cerrado from Piauí State with Potential for Reforestation

Albizia niopoides (Angico-branco), *Parkia platycephala* (Faveira), *Handroanthus impetiginosus* (Ipê-roxo) and *Hymenaea courbaril* (Jatobá) include common forest species found in the Cerrado of Piauí. Ipê-roxo, belonging to the Bignoniaceae family, is a tree species native to Brazil that is endemic to the Cerrado and can reach a height of 20 to 35 m. This species is critical for reforestation because it presents a short period of seed viability and presents difficulties in establishing degraded areas and producing seedlings throughout the year (MARTINS; LAGO; ANDRADE, 2011).

Angico-branco belongs to the Fabaceae-Mimosoideae family, a native, nonendemic species of irregular and discontinuous dispersion found in several countries in South America, displaying the ability to acclimate to different environments (KISSMANN et al. 2009).

Faveira is a native species belonging to the Fabaceae family, endemic to Brazil (ALVES et al., 2016). In reforestation programs, the crown of this species favors the development of late secondary species (PEREIRA 2011). 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, 2014).

Jatobá, belonging to the Fabaceae family, is a large tree, highlighting its adaptive characteristics to different soil and climate conditions, with low nutritional and water requirements, and it is a viable alternative to reforestation (NASCIMENTO et al., 2020).

METHODOLOGY

The experiments were carried out from April to October 2021 at the Forest Nursery and Plant Propagation Laboratory, both located at the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas (CPCE), in the city of Bom Jesus, Piauí, Brazil. According to the Köppen climate classification (1948), the climate is Aw, hot and semihumid. The region has two well-defined seasons, the dry winter and the rainy summer, with a maximum temperature of 36° and a minimum of 20° and an average annual precipitation volume between 800 and 1000 mm.

Collection, Treatment and Storage of Seeds.

The first stage of the project consisted of collecting seeds from the four native species. Thus, seeds of *H. impetiginosus, A. niopoides, H. courbaril* and *P. platycephala* were collected between October and November 2020 in the rural area of the city of Colônia do Gurguéia, south of the state of Piauí, Brazil (coordinates geographical areas: 8° 10' 57" south latitude, 43° 47' 32" west longitude, situated at an altitude of approximately 233 m); in the city of Bom Jesus, also located in the south of Piauí (geographical coordinates: Latitude: 9° 4' 30" south, Longitude: 44° 21' 26" west, established at an altitude of 273 meters); and in Formosa do Rio Preto, municipality in the state of Bahia (located at an altitude of 498 meters, with the following geographical coordinates: latitude: 11° 2' 19" south, longitude: 45° 10' 43" west).

After collection, the treatment was carried out to extract the seeds from the fruits of *H. courbaril* and *P. platycephala*, which consisted of the extraction of the thick skin and subsequent incubation of the fruits for approximately 24 h in water. At the end of the period, the seeds were removed from the pulp by friction and physical methods, followed by washing in running water and laying on canvas under the sun for three days for complete drying. The fruits of *P. platycephala* also went through the soaking stage to soften the resin, but their drying was done in excess for a period of 24 h. Finally, the selection and storage of the seeds of each species was carried out in plastic bags (*H. impetiginosus*, *A. niopoides* and *H. courbaril*) and in PET bottles (*P. platycephala*), which were allocated to environments with controlled temperature (between 4 and 8 °C) and low humidity until sowing.

Growth Conditions

The seeds of the four forest species were sown in substrate Carolina soil, which contains sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural gypsum and NPK fertilizer (traces), pH 5.5. The experiment was initially carried out in a greenhouse with 50% shading for the entire time and was initially submitted to an irrigation of 30 min at 4 times a day at preestablished times. Treatments with hardening inducers were started 150 days after sowing. Each treatment was analysed individually for each species, as specified below:

Negative control – spraying with deionized water + nonionic surfactant (TWEEN20) and irrigation with 100% of the substrate's field capacity;

Saline stress – - Irrigation with saline water with NaCl at concentrations of 25, 50, 75 mM;

Hydrogen peroxide (H_2O_2) – Spraying with H_2O_2 diluted in deionized water + TWEEN 20 at 10, 15 and 25 mM;

Salicylic acid (SA) – Spraying in SA diluted in deionized water + Tween 20 at 10, 100 and 1000 μM;

Water stress – water retention capacity, mild, moderate and severe, at 75 and 50 and 25%, respectively.

The application of the treatments took place over a period of two months, where the seedlings received irrigation twice a day, except for water stress, in which the seedlings received irrigation only once a day according to treatments. For treatments with H_2O_2 and salicylic acid, six applications were carried out, being divided into two groups of three applications in a row at an interval of 1 month between them, exercised through foliar spraying manually. The saline stress treatment was performed through irrigation with saline solution throughout the entire period of application of the treatments.

Determination of the leaf area

The leaves were removed, and the leaf area values were determined by means of an LI-3100 leaf area meter at the Plant Propagation Laboratory of the Federal University of Piauí/UFPI-CPCE. accordingly, Gobbi et al. (2011).

Determination of biomass

The fresh leaves, stems and roots were placed in an oven at 65°C for 72 hours to determine their dry weight, according to Gobbi et al. (2011).

Measurement of 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 minutes at 12,000 x g, and the supernatant was discarded. Sodium hydroxide (NaOH, 1.5 ml) 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 Eppendorf tube. Then, 200 µL of concentrated HCl was added to the cold chamber for 4 hours and centrifuged for 10 minutes, and the supernatant was discarded. In the precipitate, 2 mL of NaOH was added, and the absorbance of lignin was read at 280 nm.

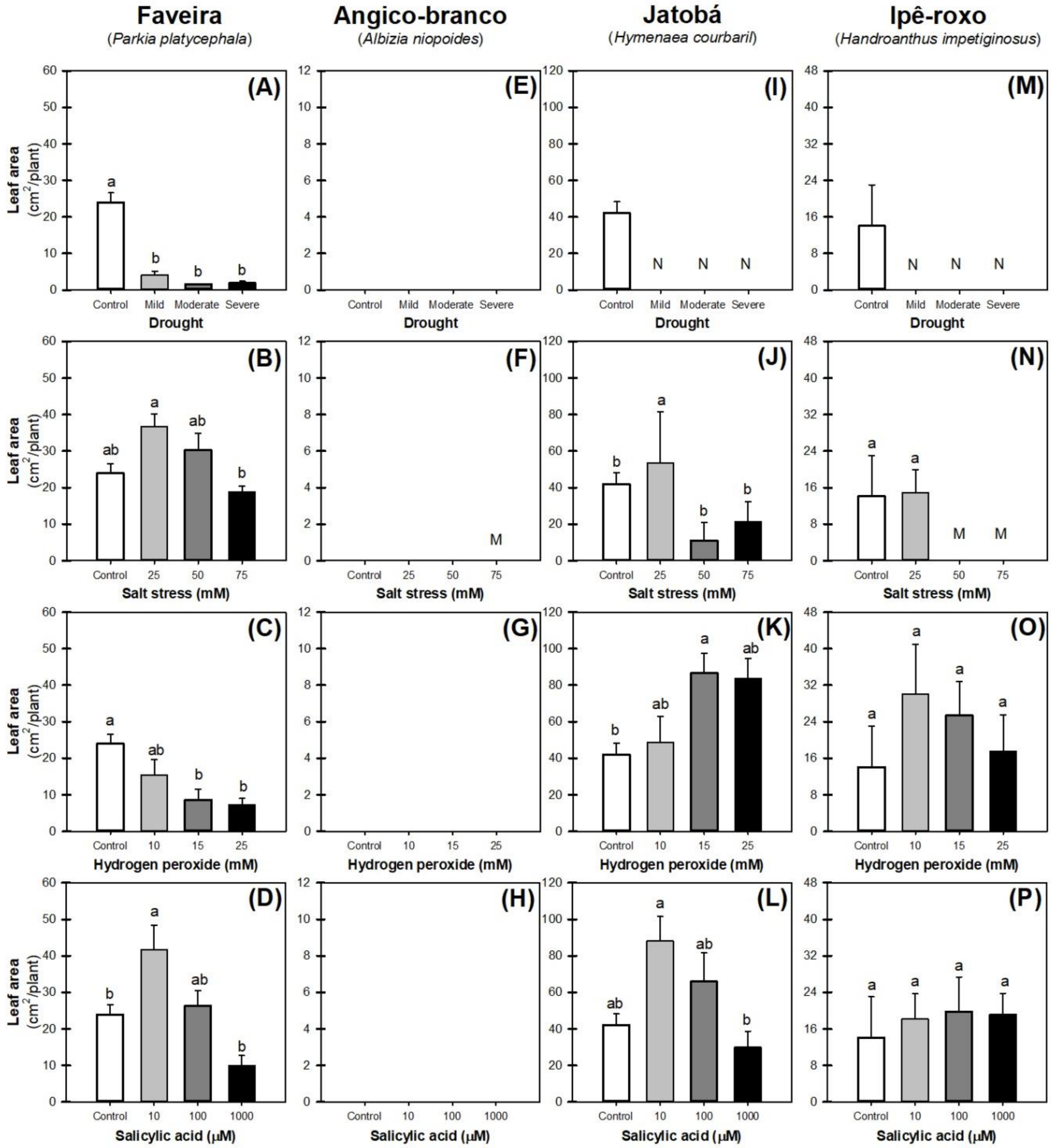
Data analysis

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 Scott–Knott or Tukey's test ($P \leq 0.05$). Additionally, for each plant species, the data were normalized using the values of control plants as a reference and employed to generate a heatmap. Statistical analyses were performed using the SISVAR program (FERREIRA, 2011).

RESULTS AND DISCUSSION

Leaf area analysis was not performed for the species *A. niopoides*, as the leaflet size was insufficient for accurate measurement by the leaf area measuring equipment. For the other species, *P. platycephala* showed an increase in leaf area when subjected to treatments with saline water at 25 mM NaCl (Figure 01 B) and 10 µM salicylic acid (Figure 01 D), while it showed drastic reductions when exposed to water stress and subjected to foliar spraying with hydrogen peroxide (Figure 01 A and C). The species *H. courbaril* also showed a significant difference with the application of treatments, as evidenced by the increase in leaf area as a function of saline stress treatments with 25 mM NaCl, 15 mM hydrogen peroxide and 10 µM salicylic acid (Figure 01 J, K and L).

Figure 01. Leaf area of native species of the Cerrado subjected to different conditioning treatments (negative control; saline stress at doses of 25, 50 and 75 mM NaCl; water stress of 25, 50 and 75 water retention capacity; hydrogen peroxide at 10, 15 and 25 mM; and salicylic acid doses of 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).

Water stress promoted a decrease in root fresh mass in Angico-branco plants sprayed with salicylic acid and hydrogen peroxide at 25 mM (Figure 02). In addition, there was a reduction in total fresh mass in plants exposed to all levels of water stress and those sprayed with salicylic acid and hydrogen peroxide at 25 mM. It is important to emphasize that the species Angico-branco is sensitive to salt stress and dies when treated with NaCl at 50 and 75 mM.

In addition, Angico-branco also exhibited a decrease in leaf dry mass under mild water stress (75% water retention capacity) and hydrogen peroxide at 10 and 25 mM and with all doses of salicylic acid. The same was observed for the dry mass of the stem sprayed with hydrogen peroxide at 25 mM and with salicylic acid at 100 μM and for the fresh mass of the root when treated with water stress, sprayed with salicylic acid, at all doses, and with peroxide of hydrogen at 25 mM. The total dry mass decreased as a function of severe water stress (25% of water retention capacity), spraying with hydrogen peroxide at 25 mM and spraying with salicylic acid.

Treatments with mild and severe water stress resulted in increases in stem fresh mass. Treatment with hydrogen peroxide at 10 and 15 mM increased the stem dry mass. Importantly, the species showed sensitivity to saline stress at doses with NaCl at 50 and 75 mM.

Figure 02. Cluster analysis for the biometric variables of Angico-branco (*Albizia niopoides*) after 60 days of submission to treatments with water stress, saline stress, hydrogen peroxide and salicylic acid. Each row represents an individual analysis. Variations in color intensity refer to changes (red - reduction; blue - increase) resulting from treatment with inducers, using control data as a reference. The letter inside the box and the different intensities of red and blue express the extent of the change according to the increase/decrease in relation to the reference, where the same lowercase letters on the same line do not differ from each other by Scott–Knott test at 5% probability.

Angico-branco	Control	WS_{light}	WS_{medium}	WS_{severe}	SS_{light}	SS_{medium}	SS_{severe}	AS_{low}	AS_{medium}	AS_{high}	HP_{low}	HP_{medium}	HP_{high}
Leaf fresh mass	b	b	b	b	a	b	dead	b	b	b	b	b	b
Stem fresh mass	a	a	b	a	a	a	dead	b	b	b	a	a	a
Root fresh mass	b	c	c	c	a	b	dead	c	c	c	c	c	c
Total fresh mass	a	b	b	b	a	b	dead	b	b	b	b	b	b
Leaf dry mass	a	b	b	b	a	b	dead	b	b	b	b	b	a
Stem dry mass	a	a	a	a	a	a	dead	a	b	a	a	a	b
Root dry mass	a	a	a	b	a	b	dead	b	b	b	b	b	b
Total dry mass	a	b	b	b	b	c	dead	b	b	b	b	b	b

Source: won (2022)

The species Faveira (*P. platycephala*) showed an increase in the fresh mass of leaves when treated with salicylic acid at 100 mM but did not show any significant difference between the control and saline stress treatments at 50 mM NaCl, salicylic acid at 100 and 1000 μM, and spraying with hydrogen peroxide (Figure 03). For the stem fresh mass parameters, there were no significant differences between the control treatment and the treatment of mild water stress, mild and moderate salt stress, or all dosages of salicylic acid and hydrogen peroxide. A similar response was observed for root fresh mass, with no difference in accumulation when plants received the control treatment, mild salt stress, or all doses of salicylic acid and hydrogen peroxide at 10 and 15 mM. For the total accumulation of fresh mass, the same situation was verified.

Similarly, the mild and severe water stress treatments, all salt levels and H_2O_2 at 1000 μM were found to promote significant decreases in the dry mass of leaves, stems, roots and total Angico-branco compared to the control (Figure 03).

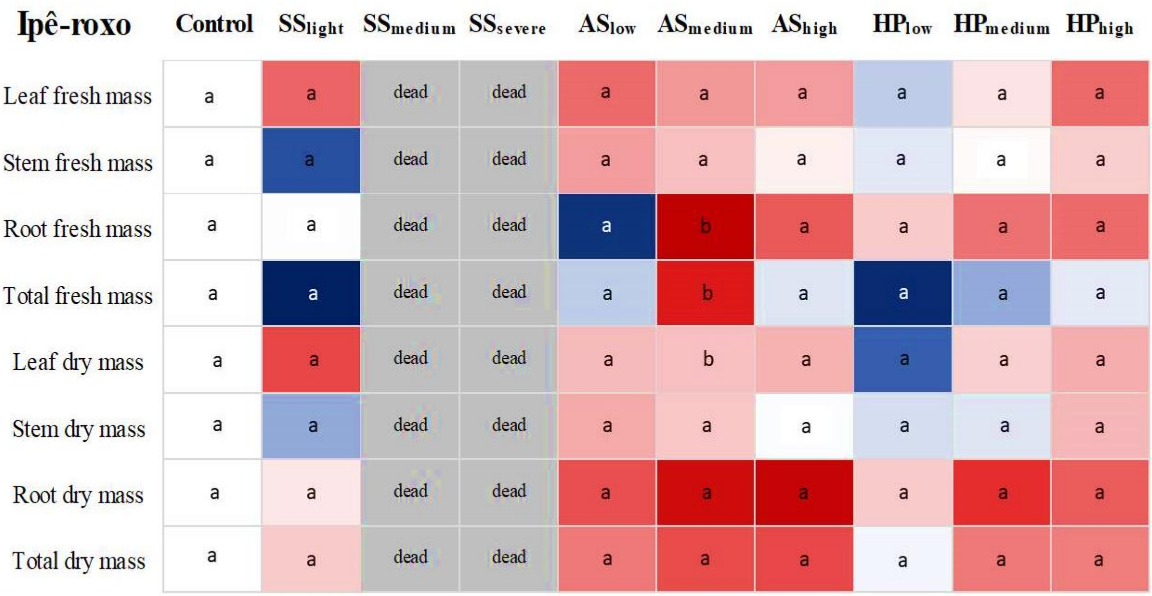
Figure 03. Cluster analysis for biometric variables of Faveira (*Parkia platycephala*) after 60 days of submission to treatments with water stress, saline stress, hydrogen peroxide and salicylic acid. Each row represents an individual analysis. Variations in color intensity refer to changes (red - reduction; blue - increase) resulting from treatment with inducers, using control data as a reference. The letter inside the box and the different intensities of red and blue express the extent of the change according to the increase/decrease in relation to the reference, where the same lowercase letters on the same line do not differ from each other by Scott–Knott test at 5% probability.

Faveira	Control	WS_{light}	WS_{medium}	WS_{severe}	SS_{light}	SS_{medium}	SS_{severe}	AS_{low}	AS_{medium}	AS_{high}	HP_{low}	HP_{medium}	HP_{high}
Leaf fresh mass	b	c	c	c	c	b	c	b	a	b	b	b	c
Stem fresh mass	a	a	b	b	a	a	b	a	a	a	a	a	a
Root fresh mass	a	b	b	b	a	b	b	a	a	a	a	a	b
Total fresh mass	a	b	b	b	a	b	b	a	a	a	a	a	b
Leaf dry mass	a	a	b	b	b	b	b	a	a	a	a	a	b
Stem dry mass	a	a	b	b	b	b	b	a	a	a	a	a	a
Root dry mass	a	a	b	b	b	b	b	a	a	a	a	a	a
Total dry mass	a	a	b	b	b	b	b	a	a	a	a	a	b

Source: won (2022)

Handroanthus impetiginosus (Ipê-roxo) was sensitive to salinity, showing plant death when subjected to treatments with 50 and 75 mm of NaCl (Figure 04). The data showed that there was no significant difference in any analysed parameter of fresh and dry mass, irrespective of the studied treatment. Additionally, the water stress treatment was not applied to Ipê-roxo because of the low number of seedlings in the period of research development.

Figure 04. Cluster analysis for biometric variables of Ipê-roxo (*Handroanthus impetiginosus*) after 60 days of submission to treatments with water stress, saline stress, hydrogen peroxide and salicylic acid. Each row represents an individual analysis. Variations in color intensity refer to changes (red - reduction; blue - increase) resulting from treatment with inducers, using control data as a reference. The letter inside the box and the different intensities of red and blue express the extent of the change according to the increase/decrease in relation to the reference, where the same lowercase letters on the same line do not differ from each other by Scott–Knott test at 5% probability.

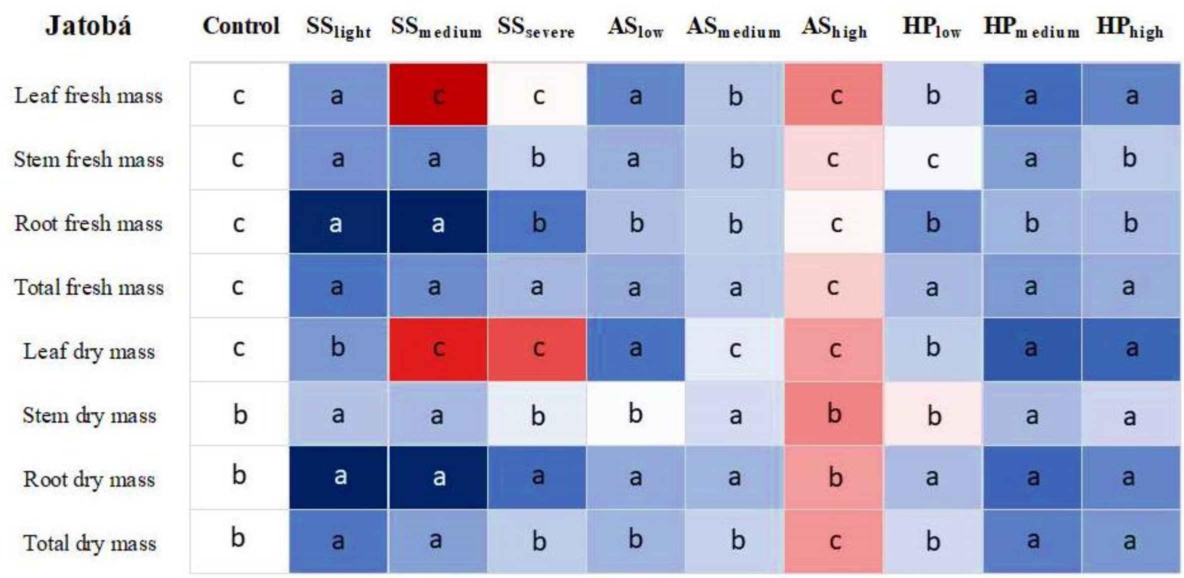


Source: won (2022)

The species *H. impetiginosus* (Jatobá) showed a significant increase in all tested treatments (Figure 05). The accumulation of leaf fresh mass was increased in plants treated with mild salt stress (25 mm of NaCl), salicylic acid at 10 µM, and hydrogen peroxide at 15 and 25 mM (Figure 05). Similar behavior occurred for fresh mass in the stems of plants treated with mild and moderate salt stress, salicylic acid at 10 µM, and hydrogen peroxide at 15 mM. Conversely, the root fresh mass was more stimulated by mild and moderate saline treatments than by the control. An increase in the total fresh mass was registered by all levels of salt stress, salicylic acid at 10 and 100 µM, as well as by hydrogen peroxide at all tested dosages.

The leaf dry mass of Jatobá was increased by salicylic acid at 10 µM and hydrogen peroxides at 15 and 25 mM compared to the control. However, the stem dry mass was increased when the plants were treated with mild and moderate saline stress (25 and 50 mM NaCl), salicylic acid at 50 mM and hydrogen peroxide at 15 and 25 mM, whereas the root dry mass was improved by salt stress at all doses tested, salicylic acid at 10 and 100 µM and all doses of hydrogen peroxide. We should comment that the water stress treatment was not applied to Jatobá due to the low number of seedlings during the research development.

Figure 05. Cluster analysis for biometric variables of Jatobá (*Hymenaea courbaril*) after 60 days of submission to treatments with water stress, saline stress, hydrogen peroxide and salicylic acid. Each row represents an individual analysis. Variations in color intensity refer to changes (red - reduction; blue - increase) resulting from treatment with inducers, using control data as a reference. The letter inside the box and the different intensities of red and blue express the extent of the change according to the increase/decrease in relation to the reference, where the same lowercase letters on the same line do not differ from each other by Scott–Knott test at 5% probability.

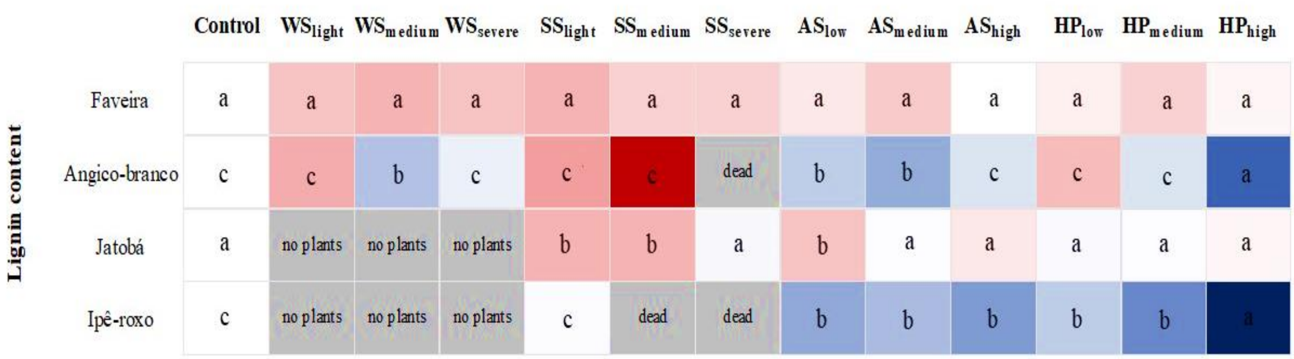


Source: won (2022)

The lignin content from the 4 forest species studied here is presented in Figure 06. The species Faveira did not show any significant change in the studied treatments. However, the Angico-branco plants treated with hydrogen peroxide at 25 mM, moderate water stress and salicylic acid at 10 and 100 μM showed higher lignin contents than the control plants. Conversely, Jatobá displayed a significant decrease in lignin content under mild and moderate salinity and salicylic acid at 10 μM compared to the respective control. On the other hand, Ipê-roxo presented a higher lignin content when sprayed with hydrogen peroxide at a dosage of 25 mM, followed by other treatments with hydrogen peroxide and salicylic acid.

Taken together, the data show that the treatment with hydrogen peroxide at 25 mM proved to be advantageous for the species *H. impetiginosus* (Ipê-roxo) and *A. niopoides* (Angico-branco), once it was able to increase the lignin content, a compound related to greater resistance of the plant tissues, without altering plant growth. Thus, the data suggest that Ipê-roxo and Angico-branco treated with hydrogen peroxide might display better support and greater mechanical resistance to adverse environmental factors.

Figure 06. Cluster analysis for the variable lignin content in the species Faveira (*Parkia platycephala*), Angico branco (*Albizia niopoides*), Jatobá (*Hymenaea courbaril*) and Ipê-roxo (*Handroanthus impetiginosus*) after 60 days of imposition to treatments with water stress, salt stress, hydrogen peroxide and salicylic acid. Each row represents an individual analysis. Variations in color intensity refer to changes (red - reduction; blue - increase) resulting from treatment with inducers, using control data as a reference. The letter inside the box and the different intensities of red and blue express the extent of the change according to the increase/decrease in relation to the reference, where the same lowercase letters on the same line do not differ from each other by Scott–Knott test at 5% probability.



Source: won (2022)

CONCLUSIONS

- ✓ Acclimatization with water stress restricts the growth of all forest species studied, and saline stress is lethal at high concentrations for Angico-branco and Ipê-roxo;
- ✓ Salicylic acid and hydrogen peroxide have no negative influence on biomass production in

all studied species;

- ✓ Parkia and Jatobá species do not present alterations in lignin content in the studied acclimatization treatments;
- ✓ All investigated native species display potential for reforestation processes in degraded areas;
- ✓ Treatment with hydrogen peroxide at 25 mM intensified lignin accumulation in Angico-branco and Ipê-roxo and constituted a potential strategy for the acclimatization/hardening of forest plant species;

More studies are needed to investigate and validate the potential role of the conditioners in seedling hardening of the studied species, particularly through exposure to a longer period of treatment with salicylic acid and hydrogen peroxide and testing the implantation of hardened seedlings in the field.

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