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Original Article

Ecophysiological mechanisms and growth of *Inga vera* Willd. under different water and light availability

Mecanismos ecofisiológicos e crescimento de *Inga vera* Willd. sob diferentes disponibilidades de água e luz

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Abstract

Light and water availability can impact plant survival and growth, making ecophysiological studies crucial for understanding their tolerance and to single and combined stresses. The aimed of this study was to investigate the physiological and growth responses of *Inga vera* Willd. plants induced by different water regimes and light intensities. Three water regimes were implemented based on substrate water retention capacity (WRC) - 50%, 75%, and 100%, along with shading levels (SH) - 0% (full sun), 30%, and 70%. Evaluations were conducted at 25 and 50 days after applying the water regimes, and during a recovery period of 30 days when all treatments were maintained at 75% of WRC. Photochemical efficiency, gas exchange, chlorophylls indices, growth, quality of the seedlings and content proline amino acid were assessed. Overall, *I. vera* plants showed greater sensitivity to increased exposure to light than to low water availability. The interaction of SH + WRC was beneficial for the gas exchange and chlorophylls indices characteristics under SH 70% + WRC 75-100% at 25 and 50 days, with higher results, greater plant growth and higher proline contents for leaves and roots under SH 30% and 70% + WRC 50%, 75% and 100% at 25 and 50 days. There was no recovery effect for seedlings grown in full sun. The plants grown under shade during the recovery period maintained their values for most of the characteristics evaluated. SH 30% + WRC 75% contributed to an increase in photosynthetic metabolism and, as a result, to the quality of the seedlings.

Keywords: shading, chlorophyll-*a* fluorescence, proline, dickson quality index, water deficit.

Resumo

A disponibilidade de luz e água pode afetar a sobrevivência e o crescimento das plantas, tornando os estudos ecofisiológicos cruciais para a compreensão de sua tolerância a estresses individuais e combinados. O objetivo deste estudo foi investigar as respostas fisiológicas e de crescimento das plantas de *Inga vera* Willd. induzidas por diferentes regimes hídricos e de intensidades de luz. Foram implementados três regimes hídricos com base nos níveis de capacidade de retenção de água do substrato (CRA) - 50%, 75% e 100%, juntamente com níveis de sombreamento (SH) - 0% (pleno sol), 30% e 70%. As avaliações foram realizadas aos 25 e 50 dias após a aplicação dos regimes hídricos e durante um período de recuperação (REC) de 30 dias, quando todos os tratamentos foram mantidos em 75% da CRA. Foram avaliados a eficiência fotoquímica, as trocas gasosas, os índices de clorofila, o crescimento, a qualidade das mudas e o conteúdo do aminoácido prolina. De modo geral, as plantas de *I. vera* mostraram maior sensibilidade à exposição aumentada à luz do que à baixa disponibilidade de água. A interação de SH + WRC foi benéfica para as características de trocas gasosas e índices de clorofila sob SH 70% + WRC 75-100% aos 25 e 50 dias, com resultados mais altos, maior crescimento da planta e maiores teores de prolina para folhas e raízes sob SH 30% e 70% + WRC 50%, 75% e 100% aos 25 e 50 dias. Não houve efeito de recuperação para as mudas cultivadas a pleno sol. As plantas cultivadas sob sombra durante o período de recuperação mantiveram seus valores para a maioria das características avaliadas. O SH 30% + WRC 75% contribuiu para o aumento do metabolismo fotossintético, como resultado, melhor qualidade das mudas.

Palavras-chave: sombreamento, fluorescência da clorofila-*a*, prolina, índice de qualidade de dickson, déficit hídrico.

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1. Introduction

Plants exhibit different responses to the availability of water and light, leading to physiological, anatomical, and leaf changes that enhance their survival, growth, and development. These responses are beneficial for management practices during the early stages of growth and development (Cremon et al., 2020; Linné et al., 2021).

Morphophysiological adjustments are reported in the photosynthetic apparatus and metabolism (Terashima et al., 2021), which can alter the synthesis and composition of photosynthetic pigments (Lage-Pinto et al., 2012), tissue and leaf thickness, and increase antioxidant activity and the proportion of proline, an amino acid universally considered a stress marker (Arora and Pardha-Saradhi, 1995; Zhang et al., 1995; Sanchita et al., 2015).

Plants have mechanisms of stress tolerance that respond to changes in the availability of environmental resources, which are characterized as phenotypic plasticity. Plant species with greater phenotypic plasticity exhibit changes in morphology and response to the environment, enabling them to adapt to new conditions to maximize resource acquisition (Esperon-Rodriguez et al., 2020). The greater the degree of plasticity, the greater the ability to acclimate and survive in unfavorable environmental conditions (Barbosa et al., 2021; Bastos et al., 2021; Santos et al., 2023).

Light and water availability can significantly impact plant survival and growth, particularly during the initial growth phase. Therefore, ecophysiological studies are necessary to provide information on their tolerance to single or combined stress (Cremon et al., 2020; Barbosa et al., 2021; Linné et al., 2021). Some plants can show phenotypic plasticity when subjected to different experimental conditions of shading, flooding and water deficit or by the combined effect of stress (Linné et al., 2021, Figueiredo et al., 2023). Another interesting insight is the tolerance mechanism of pioneer species when induced by the combined effect of waterlogging and shading under controlled experimental conditions (Dalmolin et al., 2012). This information may aid in the production and implementation of plants in projects for the recovery of degraded areas using native species (Santos et al., 2017; Wu et al., 2021).

Among the native species with potential for use in the restoration of degraded areas is *Inga vera* Willd., commonly known as “ingá” or “ingá-banana” in the literature. It is classified as a pioneer (Pennington, 1997). It is a large tree, reaching up to 30 meters in height, semideciduous, and selective hygrophilous (Tamashiro and Escobar, 2016; BFG, 2022). The fruit of *I. vera* is highly appreciated by dispersers, fishs, primates, and other animals (Furlan et al., 2017). The specie is also used as food, beekeeping, ornament, and medicinal product and is considered a tree of high value by riverside communities due to its sustainable use (Ubessi-Macarini et al., 2011). In Brazil, *I. vera* occurs in almost all regions, mainly in seasonally flooded areas (Oliveira et al., 2016). It is indicated for covering degraded areas (BFG, 2022), since covering species present fast growth and have the function of shading the soil and controlling invasive species that need full light.

Although *I. vera* is cited as a species that establishes in seasonally flooded areas, it is not known how long it tolerates waterlogging and low water availability, nor how shading may affect its survival and growth, considering that it is described as a pioneer species.

In this study, we aim to investigate the ecophysiological responses of *I. vera* to light and water availability, and address the following research questions: (i) Does shading alleviate the detrimental effects of low soil water availability or does it aggravates the impacts on the characteristics of photosynthetic metabolism, growth, and plant quality? (ii) Can plants recover after normal water supply? (iii) To what extent was the adjustment of plants to the growing conditions aided by the osmoregulatory action of proline?

2. Materials and Methods

In February 2021, seeds of *Inga vera* Willd. were collected from fruits around ten adult individuals within a remaining area of Cerrado in the municipality of Dourados, Mato Grosso do Sul, Brazil. The seeds were selected according to their integrity and uniformity. Subsequently, two seeds were sown in each tube of 290 cm^3, which contained a substrate of Red Dystroferric Latosol and coarse sand at a proportion of 3:1, at a depth of 1 cm. The plants were irrigated twice daily and kept under 70% shading.

In May 2021, the plants were transplanted to 7-kg capacity pots containing the same of substrate. Thereafter, for 2 months, they were irrigated at 75% water retention capacity (WRC) according to Souza et al. (2000) and kept in a greenhouse with a Sombrite screen with 70% shading. Subsequently, the pots were separated for acclimatized to different shading levels (SH) at 0%, 30%, and 70% for 30 days with 75% WRC. In September 2021, following the acclimatization period, the plants (n= 192) were subjected to different water regimes based on the water retention capacity of the substrate (WRC) at 50%, 75%, and 100%. At each shading level, the plants were covered with high-density plastic, 150 microns thick, which provides top and side protection to prevent precipitation.

Three evaluations were performed. Data were collected at 25 and 50 days after the plants were subjected to variations in water and light availability. After this period, all plants were irrigated, maintaining the same water regime of 75% WRC for 30 days, which characterized the recovery period (REC), when the data were collected again, resulting in a total duration of the experiment of 80 days.

3. Evaluated Characteristics

The following evaluations were carried out in both periods of the experiment:

(a) Gas exchanges: the photosynthetic rate (A, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol $m^{-2}s^{-1}$) and internal CO_2 concentration (C_i, μmol mol^{-1}) were measured using a portable infrared gas analyzer (IRGA) LCI PRO - SD (Analytical Development Co. Ltd, Hoddesdon, UK). The evaluations were carried out in the morning, between 8 and 11 hours, in expanded leaves previously marked from the beginning to the end of the experiment. Intermediate mature leaves of similar age were evaluated in one plant in each replication. From the gas exchange data, the instantaneous carboxylation efficiency (A/C_i) was calculated;

(b) Chlorophyll *a* fluorescence: the determination of chlorophyll *a* fluorescence was performed between 8 and11 hours, on the same leaves as used for gas exchange evaluations. The leaves were submitted to dark using leaf clips for 30 minutes in order to induce a complete oxidation of the photosynthetic system of electron transport of reaction centers. Then, they were exposed to a flash of one second with an intensity of 1,500 µmol m^{-2} s^{-1} and the potential photochemical quantum efficiency of PSII (F_v/F_m) was determined using an OS-30p portable fluorometer (Opti-Sciences Chlorophyll Fluorometer, Hudson, USA). From the data of initial and maximum fluorescence was calculated the absorbed energy conversion efficiency (F_v/F_0);

(c) Chlorophyll Index: the determination of the chlorophyll indexes *a*, *b* and total (*a* + *b*) was carried out between 8 a.m. and 11 a.m. on the same leaves as used for the evaluations above using a portable chlorophyll meter (Falker);

(d) Initial growth and quality of plants: The root length (RL) was measured with a ruler graduated in millimeters, the leaf area (LA) was measured using an area integrator (LI-COR, 3100 C – Area Meter), stem diameter was measured using a digital caliper (0.05 mm), shoots and roots samples were placed in an oven with forced air circulation at 60 ± 5 ºC for three days, and then the respective masses were measured with an analytical scale with decimal precision (0.0001 g). To assess the quality of the seedlings, the height, diameter and dry biomass of the shoots and roots was evaluated by the Dickson Quality Index - DQI (Dickson et al., 1960). obtained by the mathematical expression: DQI = [total dry matter/ (height/diameter ratio + shoot dry matter/ root dry matter ratio)];

(e) Proline: the extraction of proline in leaves and roots was performed in sulfosalicylic acid according to Colton-Gagnon et al. (2014) and quantification was performed using spectrophotometer at 520 nm according to the methodology of Bates et al. (1973).

4. Experimental Design and Statistical Analysis

The experimental design used for each evaluation period was completely randomized in a 3 x 3 factorial scheme with four replications, and each experimental unit consisted of two plants, with the factors being analyzed in each individual period. For each evaluation period, the data of the isolated factors and their interaction were subjected to analysis of variance (ANOVA), and when significant by F test ($p \leq 0.05$), the means of treatments were compared using the Tukey test ($p \leq 0.05$).

5. Results

Most of the characteristics evaluated in *I. vera* plants were positively influenced by shading levels (Table 1). Water regimes alone influenced the absorbed energy conversion efficiency (F_v/F_0) at 25 and 50 days. Interaction between water regimes and shading levels was observed for some gas exchange (*A*, A/C_i, C_i, and *gs* at 25 days) and growth characteristics (LA and RL at REC and 50 days, respectively, while DQI both at 50 days and in recovery). We verified that the proline content was influenced by the interaction between the treatments, both for the leaves and the roots.

At 25 days, plants grown under 70% SH with 100% WRC had the highest photosynthetic rate (5.74 µmol m^{-2} s^{-1}) (Figure 1a), while at 50 days, with the same shading, they showed a similar effect regardless of water regimes (Figure 1b). In REC, plants under 30 and 70% shading had higher values of *A*, not significantly differing according to the WRC of cultivation with an average of 4.62 µmol m^{-2} s^{-1} (Figure 1b).

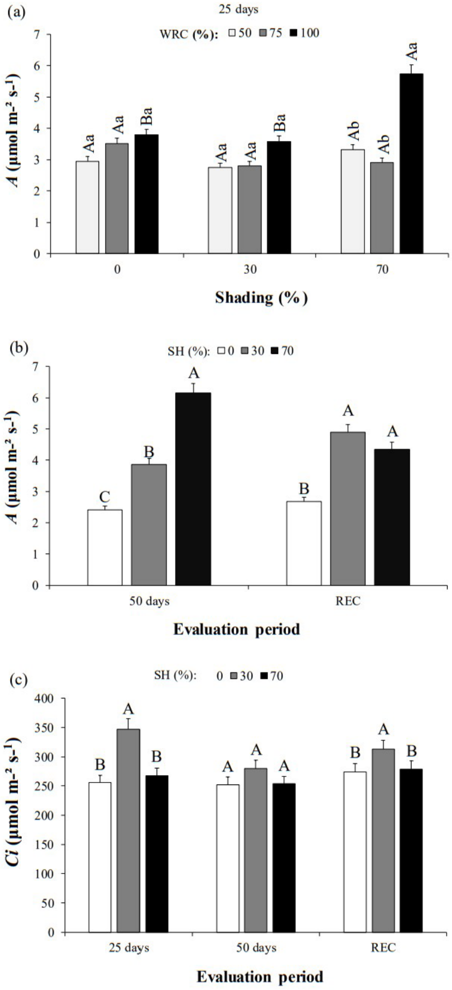

Figure 1. Photosynthetic rate (a-b) and internal CO_2 concentration (c) in *Inga vera* Willd. under different shading (SH) and water retention capacity (WRC) levels at different evaluation periods. (a) Capital letters compare the effects of WRC on each SH and lowercase letters compare the combined effect of WRC + SH (Tukey's test, $p \leq 0.05$). (b, c) Capital letters compare the effects of SH each evaluation periods. Same capital latter and lowercase do not differ by Tukey's test ($p \leq 0.05$).

Table 1. Results of variance analysis of isolated effects and the interaction of shading (S) levels and water regimes (WR) in *Inga vera* Willd. plants in three evaluation periods (25, 50 days and Recovery.

	Evaluation periods								
	25 days			**50 days**			**Recovery**		
	S	**WR**	**S x WR**	**S**	**WR**	**S x WR**	**S**	**WR**	**S x WR**
A	**0.0066**	**<0.0001**	**0.0073**	**<0.0001**	0.1915	0.4715	**0.0001**	0.8756	0.2832
C_i	**0.0004**	0.3519	0.2847	0.7197	0.8267	0.1313	**0.0469**	0.8715	0.0788
A/C_i	**0.0005**	**0.0018**	0.0554	**<0.0001**	0.4074	0.1302	**0.0006**	0.6037	0.5299
Gs	**0.0007**	**0.0272**	**0.0072**	**0.0001**	0.1066	0.2212	**0.0007**	0.7118	0.2111
F_v/F_m	**<0.0001**	0.3462	0.8037	**<0.0001**	0.2828	0.9971	**<0.0001**	0.3419	0.4088
F_v/F_0	**<0.0001**	**0.0009**	0.3092	**<0.0001**	**0.0002**	0.1609	**<0.0001**	0.1488	0.2650
Chl_a	**<0.0001**	0.2389	0.4675	**<0.0001**	0.1538	0.0645	**<0.0001**	0.8475	0.5609
Chl_b	**<0.0001**	0.3099	0.9234	**<0.0001**	0.0647	0.0712	**<0.0001**	0.6917	0.6246
Chl t	**<0.0001**	0.0709	0.9724	**<0.0001**	0.1275	**0.0261**	**<0.0001**	0.0908	**0.0335**
LA	**0.0067**	0.7233	0.2169	0.0931	0.3739	0.1279	**<0.0001**	**0.0109**	**0.0003**
RL	0.7519	0.1427	0.8504	**<0.0001**	**<0.0001**	**0.0006**	0.0713	0.1098	0.2422
DQI	**<0.0001**	0.0916	0.4931	0.6013	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0012**
P_{leaf}	**<0.0001**	**<0.0001**	**<0.0001**	**0.0189**	**<0.0001**	**0.0225**	**<0.0001**	**0.0005**	**<0.0001**
P_{root}	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

A = photosynthetic rate; C_i = internal CO_2 concentration; A/C_i = instantaneous carboxylation efficiency; G_s = stomatal conductance; F_v/F_m = potential photochemical quantum efficiency of PSII; F_v/F_0; absorbed energy conversion efficiency; *Chla* = Chlorophyll *a*; Chl_b = Chlorophyll *b*: Chl_t = Chlorophyll total; LA = leaf area; RL = Root length; DQI = Dickson Quality Index; P_{leaf} = Proline leaves; P_{root} = Proline roots.

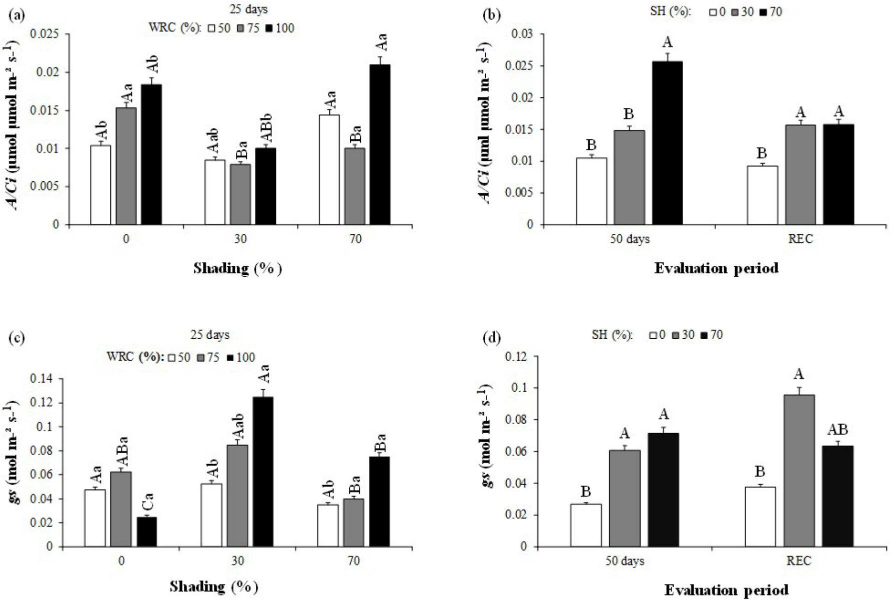

Figure 2. Rubisco carboxylation efficiency (a, b) and stomatal conductance (c, d) in leaves of *Inga vera* Willd. (a-c) Capital letters compare the effects of WRC on each SH and lowercase letters compare the combined effect of WRC + SH (Tukey's test, p ≤ 0.05). (b-d) Capital letters compare the effects of SH each evaluation periods. Same letters do not differ by Tukey's test (p ≤ 0.05).

The internal CO_2 concentration (C_i) was higher in plants under 30% SH at 25 days and in the REC, regardless of the water regime (Figure 1c). The highest values of Rubisco carboxylation efficiency – A/C_i occurred in plants under 100% WRC under 0% e 70% shading at 25 days (Figure 2a) and at 50 days under 70% SH (Figure 2b). In REC, the highest values of A/C_i were observed in shaded plants regardless of water regimes (Figure 2b).

The highest values of stomatal conductance (*gs*) were observed in plants under 30% SH + 100% WRC at 25 days (Figure 2c), and, at 50 days, the highest values were observed under shading of 30 and 70% shading regardless of WRC (Figure 2d). In REC, plants grown under 30% shading regardless of WRC had the highest means compared to the 0% SH, but did not varying to 70% SH (Figure 2d).

The potential photochemical quantum efficiency of the photosystem II (F_v/F_m) in all evaluation periods was lower in plants cultivated under 0% shading regardless of water regime (Figure 3a). In general, the absorbed energy conversion efficiency (F_v/F_0) remained lower in plants under higher light availability (0% and 30% SH) in all evaluation periods (Figure 3b and 3d). As for the water regimes, at 25 days F_v/F_0 was higher under 100% WRC while at 50 days it was higher under 50 and 75% WRC (Figure 3c).

Chlorophyll *a* content (Chl_a) increased with shading intensity regardless of water regime (Figure 4a). Chlorophyll *b* (*Chl b*) was highest under 70% SH in all evaluation periods (Figure 4b). Total chlorophyll (*Chl tot*) was higher under 70% SH at 25 days (Figure 4c) and overall, at 50 days and in REC the highest values were observed under 70% SH regardless of WRC (Figure 4d).

Root length (RL) did not vary between treatments at any time evaluated, with an average value of 39.68; 46.11 and 53.67 cm at 25, 50 and REC, respectively.

The leaf area (LA) of plants at 25 days was higher in plants under 30% SH regardless of WRC and did not vary significantly from plants grown under 70% shading (Figure 5a). At 50 days and in REC, the highest LA values were observed in plants shaded in all water regimes evaluated compared to cultivation under 0% SH (Figure 5b). The quality of plants evaluated based on the Dickson quality index (DQI) at 25 days it was higher in plants grown under 30% SH (Figure 5c). At 50 days and in REC, the highest DQI values were observed, in general, in plants under 30% shading + 75 and 100% WRC (Figure 5d).

In the leaves, the highest proline content at 25 days were observed in plants grown under 30% SH regardless of water regime (Figure 6a). At 50 days, visually there was sharp increase in the proline content in plants of all treatments, except for those cultivated under 30% SH which remained similar to the values observed at 25 days, regardless of water regimes (Figure 6b). In REC, the proline content decreased in all treatments, except for plants grown under 0% and 30% light and previously receiving only 50% WRC (Figure 6c). In roots, at 25 days the highest proline value occurred in plants under 0% SH + 100% WRC followed by plants under 30% SH + 75% WRC (Figure 6d). At 50 days, all plants showed an increase in proline content; however, in plants grown under 30% shading, the increase was significantly greater regardless of water regime (Figure 6e). In REC, plants grown under 0% and 30% SH showed reduced levels of proline, except for those grown under 0% SH + 100% WRC (Figure 6f).

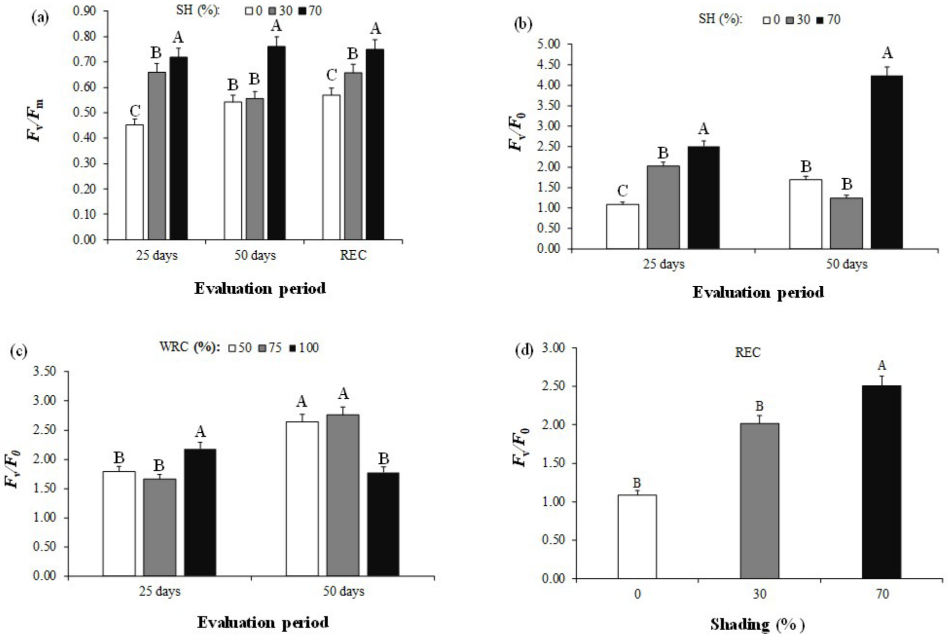

Figure 3. PSII potential photochemical quantum efficiency (a), absorbed energy conversion efficiency (b, c, d) in leaves of *Inga vera* Willd. in response to variations of light (SH) and water retention capacity (WRC). (a, b, d) Capital letters compare the effects of SH on each evaluation periods. (c) Capital letters compare the effects of WRC each evaluation periods. Same letters do not differ by Tukey's test ($p \leq 0.05$).

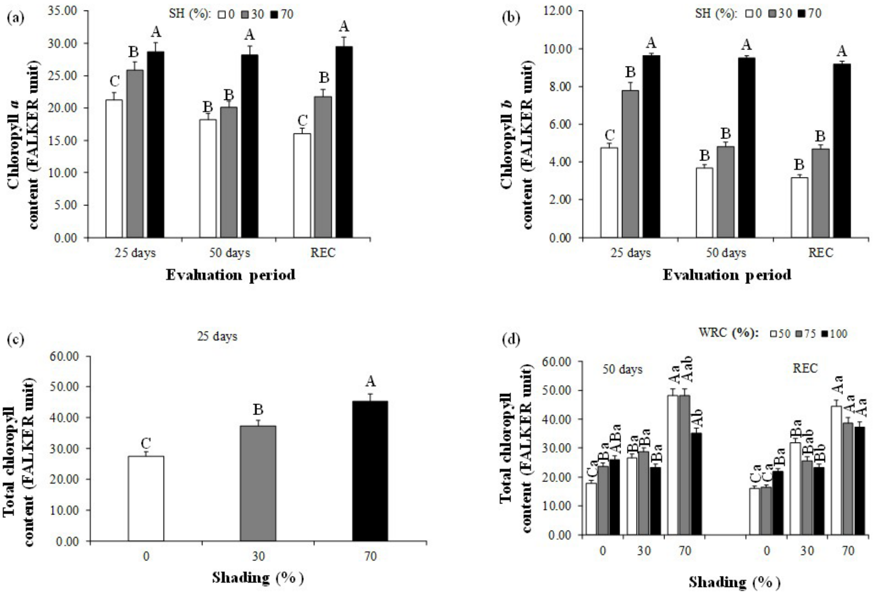

Figure 4. Content of chlorophyll *a* (a), chlorophyll *b* (b) and chlorophyll total (c, d) in leaves of *Inga vera* Will. in response to variations of light (SH) and water retention capacity (WRC). (a, b, c) Capital letters compare the effects of SH in each evaluation periods. (d) Capital letters compare the effects of WRC between SH and lowercase letters compare the combined effect of treatments. Same letters do not differ by Tukey test ($p \leq 0.05$).

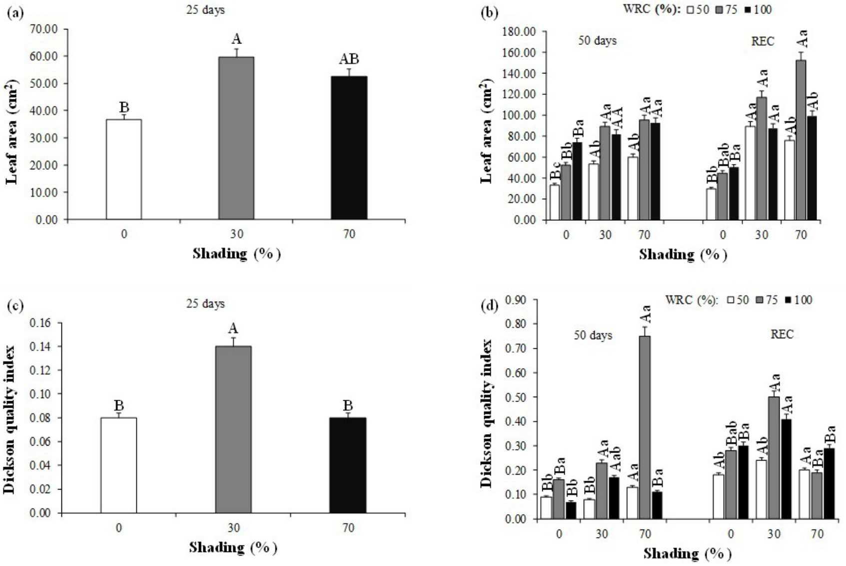

Figure 5. Leaf area (a, b) and Dickson quality index (c, d) of *Inga vera* Willd. during the experimental period. (a, c) Capital letters compare the effects of SH each evaluation periods. (b, d) Capital letters compare the effects of WRC between SH and lowercase letters compare the combined effect of treatments. Same letters do not differ by Tukey test ($p \leq 0.05$).

6. Discussion

In this study, both low water availability and high light levels (full sun) limited the growth and quality of *I. vera* seedlings. It was found that the effect of shading resulted in better performance in the photochemical and biochemical relations of photosynthetic metabolism, suggesting a mitigating effect under low soil water availability. The interaction between shading and water

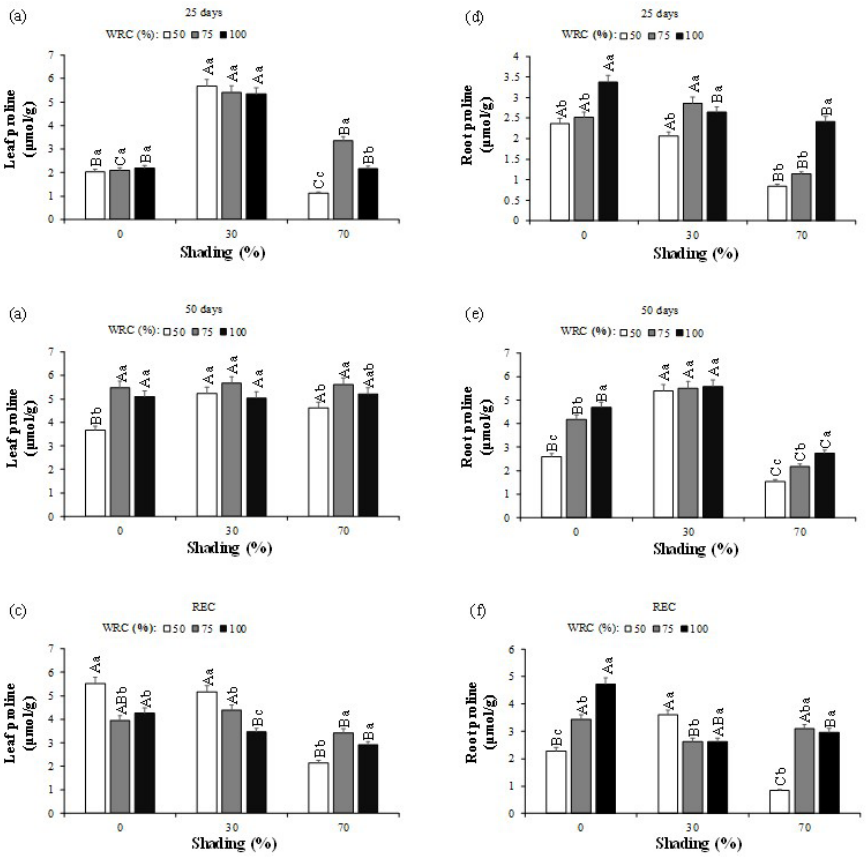

Figure 6. Proline in leaves (a, b, c) and roots (d, e, f) in *Inga vera* Willd. plants during the experimental period. Capital letters compare the effect of WRC in different SH and lower case letters the combinatorial effect. Same letters do not differ by Tukey test ($p \leq 0.05$).

availability was beneficial for most of the physiological characteristics, without synergism of stress was observed due to the combinatorial effect, except under conditions of high solar intensity and low water availability, suggesting that the species is sensitive to cultivation under full light associated with lower WRC.

The reference values for photosystem II potential quantum efficiency (F_v/F_m) observed in full sun (0% shading) indicate that this cultivation condition was unfavorable for *I. vera* plants. Suggested reference values, when reaction centers are intact, range from 0.75 to 0.85 (Baker and Rosenqvist, 2004). Values below this reference value suggest photoinhibition (Terashima et al., 2021). Therefore, considering this characteristic and the other physiological responses observed in this research, *I. vera* plants under full sun, regardless of water availability in the substrate, show signs of dynamic photoinhibition (Taiz et al., 2017; Guidi et al., 2019).

In addition to the F_v/F_m responses, the maximum efficiency of the photochemical process of photosystem II (F_v/F_0) also suggests stressful conditions when plants are cultivated under greater light availability. The values observed in this study were below the reference values, which typically range between 4-6 for most plants (Roháček, 2002). The decrease in the F_v/F_0 ratio in plants kept in full sun may be attributed to damage to the PS-II reaction centers, due to instability in the photosynthetic apparatus. Excess radiation in full sun may have caused excessive excitation of PS-II reaction centers, leading to damage or inactivation (Maxwell and Johnson, 2000). Another factor that may have contributed to a lower efficiency of absorbed energy conversion (F_v/F_0) is the smaller leaf area in full sun.

The increase in the chlorophyll indexes *a*, *b*, and total under shading resulted in adjustment responses to low light availability, optimizing energy interception in conditions of low irradiance. Variations in the proportions of photosynthetic pigments imply plastic responses of adjustment to light conditions in the plant's environment (Tang et al., 2015). In our study, plants grown in full sun had lower levels of chlorophyll, and the reason for this response may be the degradation of these photosynthetic pigments since, in conditions where excess light occurs, it implies the degradation of these molecules (Ferreira et al., 2012). This leads us to suggest that the successional classification as initial secondary would be more appropriate, corroborating existing studies in the literature (Prado Júnior et al., 2012; Magalhães et al., 2017), to the detriment of its classification as a pioneer species (Pennington 1997; Nascimento et al., 2003).

Different environmental conditions to which plants are submitted may act synergistically, easing or increasing the pressure on plant growth. In our study, shading acted as a mitigator under low water availability on total chlorophyll. Similar results of the positive effect of shading occur in *Inga marginata* Willd. plants (Mokochinski et al., 2014) cultivated under 100% light, and in young plants of *Cupania vernalis* Cambess. (Lima Junior et al., 2005) and *Caesalpinia ferrea* Mart. ex Tul. (Lenhard et al., 2013) under 70% shading.

In addition to chlorophyll *a*, we also observed an increase in Chl_b with increasing shading. This response has been considered very important for the photosynthetic metabolism, since Chl_b, in addition to capturing energy of different wave lengths and transferring this energy to reaction centers, also works as a way to dissipate excess energy, optimizing photochemical reactions. Another factor that contributes to the increase in chlorophyll levels under shading, according to Mebrahtu and Hanover (1991), is the development of a greater number of grana in chloroplasts of shaded leaves, where the *a*/*b*-protein complex is located.

The relationship between total chlorophyll content and low soil water availability is a complex variable, as it depends on the phenotypic plasticity of the plant to tolerate the low availability of water in the substrate. However, water deficit generally leads to low chlorophyll content (Freitas et al., 2020; Bastos et al., 2021). Thus, this relationship may be reversible through physiological mechanisms that prevent or minimize the degradation of these photosynthetic pigments, such as shading.

We emphasize that shading acted in a compensatory way and mitigated the effects of low water availability on the levels of these photosynthetic pigments of *I. vera* plants since, even when cultivated under lower WRC, they kept high values. Similar responses were observed in Bartieres et al. (2020) when *Campomanesia xanthocarpa* (Mart.) O. Berg. seedlings were submitted to 30 and 70% shading with water suspension and substrate rehydration.

The larger leaf areas observed in *I. vera* plants under 30% and 70% shading are consistent with the literature. Plants exposed to higher irradiance tend to decrease their leaf area, which is associated with a decrease in transpiration rates (data observed in *I. vera* but not sampled in this study), as there is a reduction in the evapotranspiration surface area (Lenhard et al., 2013).

The increase in leaf area under conditions of lower solar radiation can be considered phenotypic plasticity, as the increase in leaf area optimizes the interception of light rays and thus results in more efficient production of photoassimilates (Poorter et al., 2019; Reis et al., 2022; Silva et al., 2022). Furthermore, during the evaluations, we observed that plants with low water availability showed a reduction in leaf area, which remained stable even after supplying a greater amount of water, as observed in REC. The reduction in leaf area is a strategies response to water deficit or low soil water availability and represents a water-saving resource, as it reduces the evapotranspiration surface area (Morison and Morecroft, 2006; Campello et al., 2015).

The superior quality of plants under 30% shading, regardless of WRC, can be explained by the combined effect of abiotic factors (light and water) and osmoprotectants such as proline, as according to Sanchita et al. (2015), in stress conditions proline acts both as a metabolite and as a signaling molecule. The responses of proline levels are complex and require analysis of treatment effects, organ characteristics and specific species (Lin et al., 2019). In the results, even for underground organs, proline levels in roots vary under different light conditions, suggesting a synergistic response to factors.

The accumulation of solutes is one of the physiological mechanisms against stress, as plants adjust osmotically to tolerate abiotic stresses such as soil acidity, drought, cold, high temperatures or under excessive light exposure (Matysik et al., 2002; Sarvajeet and Narendra, 2010), although plants can show specific differences between species, such as proline accumulation and stress tolerance. In the present study, proline levels varied due to internal plant transport; for example, proline can be transported to the roots to help with osmotic adjustment in response to low water availability (Zhang et al., 1995; Sanchita et al., 2015) or transported to the shoots to protect against excess reactive oxygen species (ROS), which are highly damaging to photosynthetic metabolism and tissue and membrane integrity (Sarvajeet and Narendra, 2010).

In contrast to the findings in the literature (Zhang et al., 1995; Matysik et al., 2002; Sarvajeet and Narendra, 2010; Sanchita et al., 2015), we found an increase in proline content in moderate shading conditions (30% SH) for both leaves and roots, regardless of the WRC, suggesting that these results were not influenced by the WRC conditions until 50 days into the experiment, although there was an increase in proline content in the leaves in full light conditions during the recovery period. Higher proline levels in moderate shading conditions cannot be characterized mechanisms adjustments, contributed to the other positive characteristics found for this light condition, such as gas exchange and seedling quality (Figure 1a-b, 3 and 5c-d). The increase in proline in moderate shading conditions has been reported in *Torreya grandis* Fortune ex Lindl. (Lin et al., 2019), although it negatively influenced growth in deep shading conditions. Therefore, we cannot rule out the positive influence of proline, mainly due to its joint action with other physiological parameters and its osmoprotective action in removing ROS through its active oxygen sequestration system.

7. Conclusions

Inga vera Willd. plants were more sensitive to cultivation under higher light intensity (0% shading) than to different water regimes. Cultivation under 30% shading + 75% water retention capacity was found to promote optimal seedling quality, gas exchange and the adjustment mechanism. There was no recovery effect for seedlings grown in full sun. The plants grown under shade during the recovery period maintained their values for most of the characteristics evaluated, especially for 30% shade. The increase in proline content under moderate shading conditions was not characterized as an osmoregulatory adjustment, as the plants were more sensitive to shading than to WRC, but it did have a positive influence, mainly due to its joint action with other physiological parameters. The results suggest that *I. vera* shows high phenotypic plasticity for tolerance when grown under shading, demonstrating early secondary succession development.

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