

Ascophyllum nodosum seaweed extract in *Inga edulis* seedlings under drought and the potential of phenotypic plasticity

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Abstract

Studies aiming alleviating the effect of drought on plants have increased, and the use of seaweed extract has been a sustainable and promising management for stress tolerance. Thus, this work aimed to evaluate the role of *Ascophyllum nodosum* (L.) Le Jolis seaweed extract (ANE) in *Inga edulis* Mart. seedlings under drought and post-stress. The seedlings were cultivated under: 1) control (daily irrigation), 2) drought by water restriction – WR (irrigation suspension), 3) WR + 15 mL L⁻¹ ANE, 4) WR + 30 mL L⁻¹ ANE, and 5) WR + 45 mL L⁻¹ ANE, and evaluated in two periods: i) SWD - when the seedlings showed symptoms of water deficit, and ii) REC: recovery (post-stress) - resumption of irrigation for 60 days. The application of 45 mL ANE contributed to the N content of seedlings under drought and post-stress. The order of nutritional requirement was similar for N, Ca, and Mg, but P and K varied according to the evaluation periods under drought. The application of 30 and 45 mL L⁻¹ ANE contributed in the post-stress, favoring the quantum efficiency of photosystem II in response to the higher nutrient content in plant tissue, reflecting on growth in the REC. ANE increased proline content as osmoprotection mechanism during SWD and promoted an efficient recovery. The application of 30 and 45 mL ANE contributes to induction of stress tolerance in *I. edulis* seedlings under drought, and it favors recovery of plants in the post-stress due to phenotypic plasticity, which becomes a promising management for this species.

Introduction

In recent years, drastic reductions in soil water status have been verified due to droughts, resulting from climate changes, which promote increase of atmospheric temperature and changes in precipitation distribution (Beltramin et al. 2020; Santos et al. 2022). Under conditions of water stress, several alterations in the morphophysiological responses and the survival of plants in different phytophysiognomies in the world may occur.

Plants have physiological, biochemical and/or morphological mechanisms of escape or tolerance to drought, which are related to the phenotypic plasticity of the species and the intensity of the stressor. The efficiency of these mechanisms may confer potential of ecological resilience or lead the plant to a state of exhaustion, causing irreversible damage during and/or after the period of drought.

Therefore, under these conditions of water stress, the photosynthetic capacity of the plant is reduced, causing negative effect on its growth and quality (Pilon et al. 2018; Qi et al. 2021; Zhang et al. 2021). In order to mitigate possible damage due to water deficit, some cultural treatments stand out, such as the application of biostimulants, which may increase plant tolerance to disturbances, such as those caused by water stress (Ai et al. 2008; Bajguz and Hayat, 2009; Liu et al., 2011; Wang et al., 2022). Currently, the use of seaweed in agricultural cropping systems has needed special attention, because such organisms, applied exogenously, are source of growth promoters, readily available nutrients, amino acids and they perform protective action (Arioli et al. 2015; Shukla et al. 2015).

Several formulations can be found on the market, however, the efficiency of these products depends on the target species, applied concentration and the composition of the product (Du Jardin 2015). In this sense, different groups of seaweed are used as biostimulants, among them, *Ascophyllum nodosum* (L.) Le Jolis (Fucaceae) is the species that stands out the most for its potential use. Its composition contains phytohormones, proteins, and other compounds that can enhance plant performance through physiological and biochemical changes (Furlan et al. 2020), besides favoring root expansion and initial development (Bernardes et al. 2023).

Considering the need for conservation, environmental legalization and programs of afforestation and reforestation, the demand for native seedlings of forest species has increased. Among the fruit tree species, *Inga edulis* Mart., known as ingá-cipó (Fabaceae), is native from Tropical America, widely distributed in South and Central America (Nichols et al. 2006), in wet areas such as riparian forest or gallery (Garcia and Fernandes 2015). This species is classified as initial secondary according to the ecological succession group and it is considered fast growing (Dechinik-Vázquez et al. 2019), therefore, it is indicated to plant in areas of recomposition of permanent preservation areas, riparian forests and formation of integrated production systems (Veras 2021).

Considering this, the production of seedlings from native forest species, whether for purposes of wood exploitation, fruit harvest, enrichment of ecological corridors and/or recovery of degraded areas, has been limited due to the lack of information about management techniques to expand the forest sector (Souza et al. 2020).

Thus, we hypothesize that *I. edulis* seedlings are sensitive to water deficit and the application of *Ascophyllum nodosum* extract (ANE) may contribute to the nutritional status and the accumulation of the amino acid proline, reducing negative impacts on the photochemical processes in the PS II, favoring growth and the vigor of this species during and after the period of stress.

Material e Methods

Fruit collection, seedling production and experiment area

Ripe fruits of *I. edulis* were collected from matrices located in a forest fragment area near a watercourse in Glória de Dourados, Mato Grosso do Sul, Brazil (22°22'39.8" S, and 54°16'06.9" W). Later, fruits were processed manually and seeds were immersed in a solution with 2% sodium hypochlorite for 5 minutes, for sanitization.

Sowing was carried out in black polypropylene tubes with volume of 290 cm³, previously filled with commercial substrate called Tropstrato® composed of pine bark, vermiculite, PG mix 14.16.18, potassium nitrate, simple superphosphate and peat. Then, plants were kept in the nursery with 30% shading, using black nylon screen (Sombrite®), with top and side protection of 150 µm plastic cover to prevent the occurrence of precipitation on the seedlings, which were irrigated daily.

When seedlings reached the average height of 20 cm, they were transplanted into pots with capacity of 7 liters filled with Distroferric Red Latosol (Oxisol) + coarse sand (3:1, v/v) and kept under the same previous conditions for 30 days (acclimatization), at the Faculty of Agricultural Sciences, Federal University of Grande Dourados (UFGD), Dourados – MS, Brazil.

The application of *A. nodosum*, water management and evaluation periods

Initially, the *A. nodosum* extract (ANE) was diluted in distilled water according to the doses established for the experiment: 15, 30 and 45 mL L⁻¹. Two applications were performed, which the first occurred via leaf spraying on the abaxial and adaxial surfaces, in the morning, until drip point (10 mL per plant, based on pre-test) and the second application happened 60 days later via soil, adding the same amount of solution around the seedling collar, maintaining the irrigation for more 30 days and after this period the experiment then started.

The seedlings were submitted to five treatments: 1) control – daily irrigation, maintaining 70% of the water retention capacity (WRC), according to the methodology proposed by Souza et al. (2000); 2) drought by water restriction (WR) – complete suspension of irrigation; 3) WR + 15 mL L⁻¹ ANE; 4) WR + 30 mL L⁻¹ ANE and 5) WR + 45 mL L⁻¹ ANE. The ANE we used was commercially formulated by Acadian®, with the following specifications according to the manufacturer: appearance = dark brown viscous liquid; odor = marine; water solubility = 100%; pH = 7.4–8.2; organic matter = 13–16%; Total N = 0.30–0.60%; available phosphorus (P₂O₅) = < 0.1%; soluble potassium (K₂O) = 5.00–7.00; S = 0.30–0.60%; Mg = 0.05–0.10%; Ca = 0.10–0.20%; Fe = 30–80 ppm; Cu = 1–5 ppm, Zn = 5–15 ppm; Mn = 1–5 ppm; B = 20–50 ppm; carbohydrates (alginic acid, mannitol and fucoidin) and amino acids = 1.01%, produced with 100% fresh seaweed of *A. nodosum*.

Seedlings were evaluated in two periods: 1) SWD = in this period the visual symptoms of water deficit (SWD) were evaluated by monitoring daily, until the experimental units of one of the treatments under water restriction showed curved apex according methodology proposed by Santos et al. (2023), withered and/or yellow leaves /leaf abscission. At this time, part of the seedlings from all treatments was evaluated for non-destructive and destructive characteristics. We kept monitoring plants from other treatments in WR until all of them showed SWD. 2) REC = after the SWD of seedlings from each treatment, these plants were submitted to the resumption of irrigation, maintaining 70% of WRC in the substrate, similar to the control seedlings, during 60 days, characterizing the recovery period (post-stress).

For each evaluation period, the experimental design used was completely randomized with five treatments and four replications and the experimental unit consisted of a vase with two plants.

Values of temperature and average relative humidity were recorded using thermo-hygrometer, during the all the period of experimental development, starting when the irrigation was suspended (Fig. 1).

Assessments

Chlorophyll index and photochemical responses: through fully expanded leaves, located in the middle third part of the plant, the chlorophyll index was performed using portable chlorophyll meter (SPAD 502) - Soil Plant Analysis Development, measured in the morning from 8 to 10 am. Then, leaves were subjected to dark condition for 25 minutes, using leaf-clip holders, so that all the reaction centers in this leaf region acquired the “open” condition, that is, complete oxidation of the photosynthetic electron transport system (Bolhàr-Nordenkamp et al 1989).

Growth, allometric index, and quality: seedlings were collected and roots were washed to remove the excess of substrate, then the length of the largest root was measured using ruler graduated in centimeters. Leaf area was measured with an area integrator (LI-COR, Model 3100 LC). To determine the dry mass values, the material was conditioned in drying oven with forced air circulation at temperature of $60^{\circ} \pm 5$ C for 72 hours and subsequently weighed on a precision scale. Using data of the leaf area and dry mass, we calculated the leaf area ratio and specific leaf area, according to the methodology proposed by Hunt (2017). The seedling quality was calculated as proposed by Dickson et al. (1960).

Nutritional status: dry material of shoots and roots was ground separately in a “Willey” knife mill; then, the total content of nitrogen, phosphorus, potassium, calcium, magnesium was determined, according to the methodology proposed by Malavolta et al. (1997) and determined the order of nutritional requirement.

Proline content: it was determined using 0.5 g of leaf and root separately by the sulfosalicylic acid method by spectrophotometry (520 nm) according to Bates et al. (1973).

Phenotypic Plasticity Index – PPI: was calculated for F_v/F_m and IQD, according to the methodology proposed by Valadares et al. (2006), presenting the results in a characterized way, not applying statistical analysis. The values vary on a scale from 0.00 to 1.00, and as higher the value, more plastic the evaluated characteristic is, depending on the cultivation condition.

Statistical analyzes

For each period, the data were submitted to analysis of variance (ANOVA) and when significant by the F test ($p \leq 0.05$), the means of the treatments were compared by the Tukey test $\pm$ standard error ($p \leq 0.05$), using the statistical program SISVAR. The analysis of the similarity index among the factors under study (treatments) was also performed using the Euclidean distance with a cophenetic correlation coefficient ≥ 0.60 based on the UPGMA group method, using the PAST software.

Results

The symptoms of water deficit

In the SWD phase, seedlings under WR showed yellow leaves and leaf abscission (Fig. 2). We verified the seedlings that first presented SWD were those grown in WR + 15 mL L⁻¹ ANE, while those cultivated under WR and WR + 45 mL L⁻¹ ANE had SWD with similar time (48 days of WR) (Table 1).

Table 1

The appearance of symptoms of water deficit (SWD) in *Inga edulis* Mart. seedlings for the treatments with or without the application of *Ascophyllum nodosum* L. seaweed extract doses in both evaluation periods (SWD and REC)

Treatments	SWD	REC
	(Days after WR)	(Days after resumption of irrigation)
Control (daily irrigation)	—	—
Water Restriction (WR)	48 d	60 d
WR + 15 mL ANE L ⁻¹	21 d	
WR + 30 mL ANE L ⁻¹	45 d	
WR + 45 mL ANE L ⁻¹	48 d	

Nutritional status of plants

In general, the contents of N, P, K, Ca and Mg varied in both evaluation periods (Fig. 3). The highest N contents were 39.66 and 42.46 g kg⁻¹ for control seedlings and under WR + 45 mL L⁻¹ ANE, respectively, differing from the other treatments during the SWD (Fig. 3a). In the REC phase, the highest values remained in these same seedlings and in those with 30 mL L⁻¹ ANE, differing only from seedlings previously cultivated under WR, which presented the lowest value (19.60 g kg⁻¹) (Fig. 3a).

Regarding P during SWD phase, we verified that control seedlings the best value (6.18 g kg⁻¹), differing from the other treatments, while in the REC period the best contents presented were 3.53, 3.78 and 3.99 g kg⁻¹ for control seedlings and those previously cultivated under WR with 30 and 45 mL L⁻¹ ANE, respectively, and the lowest value (2.54 g kg⁻¹) occurred in those seedlings under WR without the application of ANE (Fig. 3b).

The highest contents of K in SWD were 3.98 and 3.69 g kg⁻¹ for control seedlings and those cultivated under WR + 30 mL L⁻¹ ANE and the lowest occurred in seedlings under WR and WR + 15 mL L⁻¹ ANE (Fig. 3c). In REC, we verified the lowest values for the same treatments mentioned before, i.e., the seedlings previously cultivated under WR and WR + 15 mL L⁻¹ ANE (Fig. 3c).

Ca had the lowest contents for control seedlings and those cultivated under WR + 45 mL L⁻¹ ANE, differing only from the seedlings of WR + 15 mL L⁻¹ ANE, presenting values of 6.03, 5.55 and 7, 35 g kg⁻¹, respectively (Fig. 3d). Seedlings previously cultivated under WR + 45 mL L⁻¹ ANE had the best content of Ca (9.63 g kg⁻¹) in the REC, differing from the control (Fig. 3d). The highest contents of Mg occurred in seedlings under WR + 15 mL L⁻¹ ANE (2.68 g kg⁻¹) during SWD, while in REC the worst values were 1.76 and 1.62 g kg⁻¹ for control and seedlings with WR + 45 mL L⁻¹ ANE, respectively (Fig. 3e).

We observed that the descending order of nutritional requirement for *I. edulis* seedlings showed the same trend with a little variation between evaluation periods (Table 2). The nutrients required the most by seedlings were N and Ca for all the treatments, except for the control in the SWD phase, which showed higher requirement of P than Ca, while the nutrient required in lower concentration was Mg for both evaluation periods. In the REC, seedlings with 30 and 45 mL L⁻¹ ANE presented greater requirement for P than K compared to the other treatments.

Table 2
Nutritional requirement of *Inga edulis* Mart. seedlings under water regimes – control (daily irrigation) and drought by water restriction (WR) – and with or without the application of *Ascophyllum nodosum* L. seaweed extract (ANE) doses in both evaluation periods (SWD and REC)

Treatments	SWD	REC
Control	N > P > Ca > K > Mg	N > Ca > K > P > Mg
Water Restriction (WR)	N > Ca > P > K > Mg	N > Ca > K > P > Mg
WR + 15 mL L ⁻¹ ANE	N > Ca > P > K > Mg	N > Ca > K > P > Mg
WR + 30 mL L ⁻¹ ANE	N > Ca > P > K > Mg	N > Ca > P > K > Mg
WR + 45 mL L ⁻¹ ANE	N > Ca > K > P > Mg	N > Ca > P > K > Mg

Proline content, chlorophyll index and photochemical responses

We verified better accumulation of proline in leaves (> 5.00 µg/mL) of seedlings that received the application of ANE, especially with 45 mL L⁻¹ in the SWD (Fig. 4a). In the REC, values decreased for all seedlings compared to SWD, but those previously cultivated under WR showed higher values (1.98 µg/mL) than other treatments (Fig. 4a). Regarding the proline content in roots, the highest values were 4.22 and 5.12 µg/mL for seedlings that received 30 and 45 mL L⁻¹ ANE, respectively, during SWD (Fig. 4b), while in the REC, these seedlings showed values that did not differ from those observed for the control (< 1.50 µg/mL).

Chlorophyll index and initial fluorescence of *a* chlorophyll (F_0) were not significantly influenced by treatments in the SWD phase ($p > 0.05$). In the REC phase, the values of F_0 for seedlings that received ANE were lower, but the WR treatment had increase (0.076), differing from the other ones, and the lowest value (0.035) of F_0 occurred in seedlings with 45 mL L⁻¹ ANE (Fig. 4c).

The maximum quantum efficiency of photosystem II (F_v/F_m) in the SWD period reduced in those seedlings grown in WR with the application of ANE (Fig. 4d). In REC, there was increase of F_v/F_m in seedlings with 45 mL L⁻¹ ANE (0.707) similar to seedlings with 30 mL L⁻¹ ANE (Fig. 4d). Regarding the

chlorophyll index in the REC period, the control seedlings had the best value (49.42) compared to the other treatments, presenting magnitude similar to seedlings with 30 and 45 mL L⁻¹ ANE (42.08 and 47.42 respectively) (Fig. 4e).

Initial growth, allometric index, and quality

The leaf area of *I. edulis* seedlings was influenced by the treatments for both evaluation periods (Fig. 5). In the SWD phase, seedlings in WR showed the highest leaf area (412.75 cm²), which did not differ statistically from the control (350.50 cm²) and plants with 30 and 45 mL L⁻¹ ANE (391.75 and 389.75 cm², respectively), with the exception of WR + 15 mL L⁻¹ ANE which showed the lowest value (Fig. 5a). In the REC, seedlings previously grown under WR + 45 mL L⁻¹ ANE showed the highest leaf area (830.75 cm²), not differing from the control treatment (Fig. 5a).

Seedlings cultivated in WR with 15 and 45 mL L⁻¹ ANE in the SWD phase had the greatest root length (81.75 and 68.50 cm, respectively), while those under WR, which did not receive the application of ANE, presented the lowest value (38.50 cm) (Fig. 5b). In the REC, the seedlings previously cultivated in WR presented lower value (44.25 cm) compared to the control and seedlings with 30 and 45 mL L⁻¹ ANE (77.65, 69.00 and 82.50 cm, respectively) (Fig. 5b).

The leaf area ratio (LAR) of seedlings under WR + 15 mL L⁻¹ ANE in the SWD period was lower (11.51 cm² g⁻¹) compared to the other treatments, but it did not differ significantly from seedlings with 30 and 45 mL L⁻¹ ANE (18.27 and 15.05 cm² g⁻¹, respectively) (Fig. 5d). In the REC phase, the seedlings that received the application of 15 and 45 mL L⁻¹ ANE showed the highest values (30.56 and 31.28 cm² g⁻¹ respectively) (Fig. 5d).. The seedlings with 30 mL L⁻¹ ANE had the lowest specific leaf area (SLA), differing from all the other treatments (Fig. 5c). In the REC period, treatments did not differ statistically for SLA (Fig. 5c).

The seedlings cultivated with 15 mL L⁻¹ ANE had the lowest shoot/root ratio (SRR) (0.650) during the SWD period, however the control seedlings and those cultivated with 30 mL L⁻¹ ANE had the highest SRR (1.34 and 1.38 respectively) in this period (Fig. 5e). In the REC, seedlings with 30 and 45 mL L⁻¹ ANE presented the highest SRR (1.01 and 1.02, respectively) (Fig. 5e).

In the SWD, the seedlings with the highest Dickson quality index (DQI) (3.47 and 3.75) were those that received 30 and 45 mL L⁻¹ ANE, respectively (Fig. 5f). In the REC, the control seedlings had the best DQI (4.96) compared to the other seedlings treated with the biostimulant and the WR treatment (without ANE) (Fig. 5f). Although the control presented the best value in the REC phase, it is important to highlight that the seedlings previously cultivated with 30 and 45 mL L⁻¹ ANE in the SWD maintained higher DQI for the next period (REC).

Phenotypic plasticity and cluster analysis

Regarding to the phenotypic plasticity index (PPI) for F_v/F_m , the highest values were observed in those seedlings that received different ANE doses during the SWD (Table 3). The highest PPI values for IQD occurred in seedlings with 30 and 45 mL L⁻¹ ANE when analyzed in the SWD. In the REC, these same seedlings had good development, while those under WR and WR + 15 mL L⁻¹ ANE showed higher PPI.

PPI (0.00 to 1.00)					
Characteristics	Times	WR	WR + 15 ANE	WR + 30 ANE	WR + 45 ANE
F_v/F_m	SWD	0.01	0.18	0.16	0.19
	REC	0.05	0.02	0.05	0.17
DQI	SWD	0.06	0.02	0.33	0.38
	REC	0.52	0.70	0.21	0.24
SWD: symptoms of water deficit; REC: recovery (post-stress)					

We verified by the cluster analysis (cophenetic correlation: 0.653) that the control seedlings and those under WR + 30 mL L⁻¹ ANE had higher similarity, with distance of 3.07, and they were grouped with those under WR + 45 mL L⁻¹ ANE, all in the REC phase (Fig. 6). On the other hand, the seedlings cultivated under WR in both evaluation periods were grouped with WR + 15 mL L⁻¹ ANE, while the seedlings under WR that received the application of 30 and 45 mL L⁻¹ ANE, both treatments in the SWD, formed another group with distance of 4.31.

Discussion

I. edulis seedlings are sensitive to water restriction (WR), since the plants showed symptoms of water deficit (SWD), although the most pronounced symptoms in seedlings under WR without the application of *Ascophyllum nodosum* L. extract (ANE) occurred only after 48 days of WR, which may be attributed to the high relative humidity of the air during the period of the experiment. Under drought, there is reduction in the turgor of the plant tissue, and it affects the hormonal balance, with increase in the synthesis of ethylene and abscisic acid (Malaga et al. 2020; Bastos et al. 2022), promoting yellowing and leaf abscission, as observed in our study with *I. edulis*. On the other hand, in the REC, the seedlings recovered their visual appearance due to the species' ability to regrow, especially when they received 45 mL L⁻¹ ANE, suggesting morphophysiological plasticity under these conditions.

Considering the period that the SWD started, we suggest that the application of 15 mL L⁻¹ ANE accentuated the effect of WR. By our results, we raised the question: does the application of ANE

contribute to the production of *I. edulis* seedlings under water deficit? Yes! ANE contributes in the nutritional status, proline content, photochemical activities, and growth but in specific doses, here represented by 30 and 45 mL L⁻¹.

The effects of stress on seedlings under WR and WR + 15 mL L⁻¹ ANE reflected more expressively in the post-stress period compared to SWD, because even when irrigation was resumed, most of the shoot and root characteristics of the seedlings and the contents of N, P and K were lower than the control treatment and those seedlings that received 30 and 45 mL L⁻¹ ANE. These responses demonstrate the gradual and positive effect of the seaweed extract at these doses in the post-stress period by contributing to the nutritional status and physiological responses, which reflected in the production of photoassimilates.

The increase of nutrient content in those seedlings that received ANE is due to the fact that the biofertilizer, besides containing readily available nutrients and organic matter in its composition, it also contains mannitol, alginic acid and fucoidins. These carbohydrates are complex carriers that perform antioxidant action and contribute to the use of nutrients for plants (Khan et al. 2009; Holdt and Kraan 2011; Moreira et al. 2017; Yuan and Macquarrie 2015ab), since they are substrates that favor the production of energy and the electrochemical gradient for nutrient absorption (Fagan et al. 2016). Our results confirm this information because of the lower values of N, P and K in *I. edulis* seedlings under WR without the application of ANE in both evaluation periods.

The lowest N content in seedlings under WR is possibly due to the reduction in the activity of enzyme, the nitrate reductase, which is negatively affected by low water availability in the soil and by water potential in the plant, affecting N assimilation (Marur et al. al., 2000; Oliveira et al., 2005), and consequently harming the synthesis of other compounds and vegetative growth.

According to Malavolta et al. (1997) Ca in lower concentration in the plant tissue presents a synergistic relationship with K, this fact was verified in our study with *I. edulis* during the stress condition (WR), while there was increase of K for control seedlings resulting in competitive inhibition for Ca and Mg. In addition, we suggest that the lowest K contents in seedlings grown under WR and WR + 15 mL L⁻¹ ANE indicate that these plants are less resistant to drought, since this nutrient participates in the regulation of water absorption by osmotic pressure (Ávila et al. 2022; Javed et al. 2024). We also highlight that the K influences the absorption of other nutrients by having transporters with high or low affinity (Fagan et al. 2016; Xu et al. 2020) and its reduction in the plant tissue reflected in a lower P content in *I. edulis* seedlings.

Although the control seedlings and those under WR with 30 and 45 mL L⁻¹ ANE had lower Mg content, this nutrient was the one with the lowest requirement by *I. edulis*, indicating that the amount present in the plant was enough for the needs of this species, as observed by the chlorophyll index and photosynthetic processes, suggesting good nutrient use efficiency. On the other hand, the seedlings under WR with 15 mL L⁻¹ ANE, even with higher Mg content, directed energy for absorption, but they had low use efficiency in the metabolism.

The reduction of F_v/F_m in the seedlings that received ANE during the SWD can be explained by the fact that these plants present higher energy consumption than the productive capacity due to the limited absorption of nutrients and the synthesis of protective compounds during the WR condition. Under drought, there is the reduction of turgor and water potential, affecting the osmotic and electrochemical gradient, with active absorption standing out, thus, the cell spends more energy than under ideal water conditions (Rodriguez-Iturbe et al. 2001; Zhou et al. 2022).

It is important to note that energy consumption is more evidenced under water restriction, because the low transport of electrons in the reaction centers is affected by the low soil moisture (Dalal and Tripathy 2018), reducing H^+ pumping and ATP production (Siddiqui et al. 2021). As consequence, in the REC we verified increase of F_0 in seedlings previously stressed under WR, indicating that the recovery period for these plants was not enough, different from those seedlings with 30 and 45 mL L⁻¹ ANE.

The increase of F_v/F_m and the reduction of F_0 in the post-stress period demonstrate the potential for stimulation of physiological processes conferred by ANE, especially at the dose of 45 mL L⁻¹, in contributing to the maintenance of activities in photosystem II, that is, plants showed less dissipation of absorbed light energy in the form of thermal energy, which contributes to the production of chemical energy in the form of ATP and NADPH (Bassi and Dall'Osto 2021; Santos et al. 2022) in the leaf metabolism and plant growth.

We emphasize that the highest K requirement in seedlings with 45 mL L⁻¹ ANE during the SWD demonstrates strategy for maintaining the water balance of plant tissue by participating in the regulation of water absorption by osmotic adjustment and membrane stability (Munsif et al. 2022; Mostofa et al. 2022; Rostampour et al. 2023; Fang et al. 2023) even under WR condition. On the other hand, the inversion of requirement of P and K in the REC indicates that when the plant is re-irrigated, i.e., it is cultivated in ideal water conditions in the post-stress period, *I. edulis* seedlings invest in the production of energy for the biochemical stage of photosynthesis as a compensatory mechanism.

The increase of proline in leaves and roots during the SWD phase when seedlings received 30 and 45 mL L⁻¹ ANE shows the induction of stress tolerance by mechanism of osmoprotection and homeostasis of the photochemical and biochemical apparatus of photosynthesis under water restriction. This response can be attributed to the fact that ANE contains amino acids (1.01%) and other compounds with antioxidant function. However, seedlings under WR and WR + 15 mL L⁻¹ ANE did not accumulate enough proline in these organs to alleviate oxidative stress.

Proline acts in the balance of osmotic pressure, maintaining the stability of protein and cell membrane, besides minimizing the negative effects of reactive oxygen species (ROS) (Furlan et al. 2020; Wang et al. 2022), eliminating free radicals and buffering the redox potential of the cell (Ashraf and Harris 2004; Ashraf and Foolad 2007; Shukla et al. 2015). The reduction of proline in leaves and roots of seedlings with 30 and 45 mL L⁻¹ ANE in the REC, statistically similar to the control seedlings, indicates that these seedlings resumed their metabolism in normalized way.

The ANE improved the development of the leaf area (LA) and root system, because the extract contains phytohormones and compounds, such as betaine, that are directly linked to the growth of these plants (Seager et al. 2020), besides the content of N, P, K and Ca in seedlings that received the product application, especially when compared to seedlings under WR without ANE.

The positive response of seedlings to the application of ANE is due to the enrichment of N, which favors vegetative growth (Santos et al. 2020) and the contents of P, K, Ca, which act on the structure of plant tissue and biomass production (Costa et al. 2020; Rodrigues Neto et al. 2021). Thus, these elements acting together increased the development of LA, DQI, chlorophyll index, F_v/F_m and proline content in *I. edulis* seedlings, contributing to greater physiological efficiency even under stress and post-stress conditions, which favored leaf metabolism, promoting better energy production of photochemical processes and subsequent biochemical metabolism of photosynthesis, ensuring the production of photoassimilates.

We verified that the application of 15 mL L^{-1} ANE in the REC affected negatively on some characteristics evaluated in *I. edulis* seedlings, especially in comparison with the seedlings in WR without the application of ANE, indicating a stressful condition. We noticed that the responses of different species to ANE may vary, since at this same dose, ANE contributed positively to the increase of leaf area and root system in *Alibertia edulis* Rich. seedlings (Bernardes et al. 2023).

The values and the way of interpreting the phenotypic plasticity vary according to the pattern of responses in seedlings. As lower the phenotypic plasticity index (PPI) value is as closer the values are of an specific characteristic compared to the control seedlings, suggesting the possible explanations: i) adaptation mechanisms, ii) the time was not enough to exposure the stressor or iii) the species is not able or has a little potential to adjust to adverse conditions due to its intrinsic characteristics. On the other hand, a higher value indicates accentuated discrepancies between the seedlings under stress and the control, reflecting in higher plasticity and potential for inducing tolerance.

By the way, Hayat et al. (2012) describe that, naturally, plants have several physiological, biochemical and/or morphological adaptation strategies to prevent or escape from oxidative damage under water deficit stress, among them, e.g., the most significant ones are osmotic adjustment and an effective antioxidant system. In our study, the increase of proline, N, P, K and other possible structural metabolites with the application of ANE in order to maintain homeostasis and improve plant functioning under water restriction are parameters that indicate osmotic adjustments.

Thus, the highest values found for the indexes of PPI and F_v/F_m in the SWD with the application of ANE helped to promote a better working of the photosynthetic apparatus, because although the photochemical activity was lower, the energy was directed towards the accumulation of osmoprotective compounds, here represented by proline, increasing the potential for inducing drought tolerance due to a higher phenotypic plasticity.

In addition, the highest values of PPI for IQD for seedlings with 30 and 45 mL L⁻¹ ANE indicate that the application of these doses resulted in greater vigor of seedlings in both evaluation periods due to a better stabilization of the metabolic processes, presenting grouping in the hierarchical groups to control seedlings, in the REC phase.

In the REC, the seedlings under WR and WR + 15 mL L⁻¹ ANE had lower growth and morphophysiological quality in response to the acute and chronic damages of exposure to water restriction, result that is highlighted in the cluster analysis, demonstrating that it was an unfavorable condition for the cultivation of *I. edulis* seedlings.

In our study, we could verify that the early application of *A. nodosum* seaweed extract on *I. edulis* seedlings is a promising alternative for nutritional and physiological management of this native tree species, contributing more efficiently to the induction of drought tolerance, increasing plasticity and favoring physiological recovery in the post-stress. In addition, the methodology of visual diagnosis based on the water deficit symptoms is a promising technique because it has low cost and is simple and versatile for nurserymen and/or those responsible for monitoring plant in field conditions.

The use of seaweed biomass as resource for a sustainable agricultural production, although being a challenge, it promotes many opportunities for innovation and generation of scientific knowledge, especially aiming to achieve Sustainable Development Goals (SDGs). For future perspectives, new works should be carried out aiming to study the modes of action of ANE regarding nutritional state, dynamics of absorption, translocation and use of nutrients for *I. edulis*.

Conclusions

In the water deficit symptom phase, *I. edulis* seedlings that received the application of 30 and 45 mL of *A. nodosum* extract (ANE) showed higher content and demand for nitrogen, potassium, besides the accumulation of the amino acid, proline in the leaf and root as osmoprotection mechanisms and physiological adjustments. The application of 15 mL of ANE impaired seedling growth during and after the drought period. *I. edulis* seedlings cultivated with 45 mL ANE had better nutritional status and physiological efficiency, resulting in greater growth in the post-stress, indicating potential for ecological resilience due to phenotypic plasticity.

Abbreviations

ANE – *Ascomyllum nodosum* extract, Ca – Calcium, DQI – Dickson quality index, F_0 – Initial fluorescence, F_v/F_m – The quantum efficiency of photosystem II, K – Potassium, LA – Leaf area, LAR – Leaf area ratio, Mg – Magnesium, Nitrogen, P – Phosphorus, PPI – Phenotypic plasticity index, REC – Recovery, RL – Root length, SLM – Specific leaf mass, SRR – Shoot/root ratio, SWD – Symptoms of water deficit, WR – Water restriction, WRC – Water retention capacity

Declarations

Competing interests: The authors declare no competing interests.

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Figures

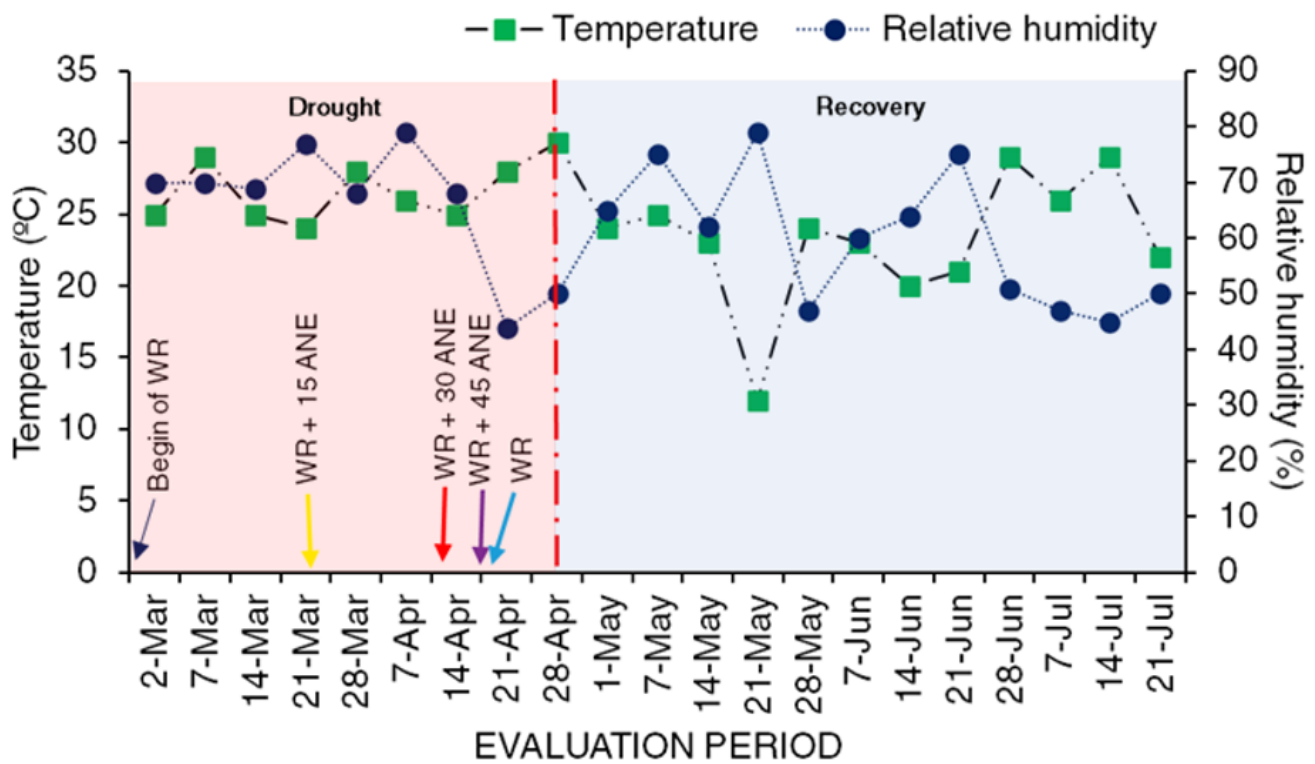


Figure 1

Temperature and relative humidity of the air inside the environment during all the evaluation period. Yellow, red, purple, and blue arrows indicate the days of *Inga edulis* Mart. seedlings of each treatment that showed symptoms of water deficit (SWD) and recovery (REC, post-stress).



Figure 2

Visual aspect of *Inga edulis* Mart. seedlings grown under water regime control (daily irrigation), drought by water restriction (WR), WR + 15 mL L⁻¹ de ANE, WR + 30 mL L⁻¹ ANE and WR + 45 mL L⁻¹ ANE, showing symptoms of water deficit (SWD) at 0, 48, 21, 45 and 48 days after WR, respectively. Source: Authors

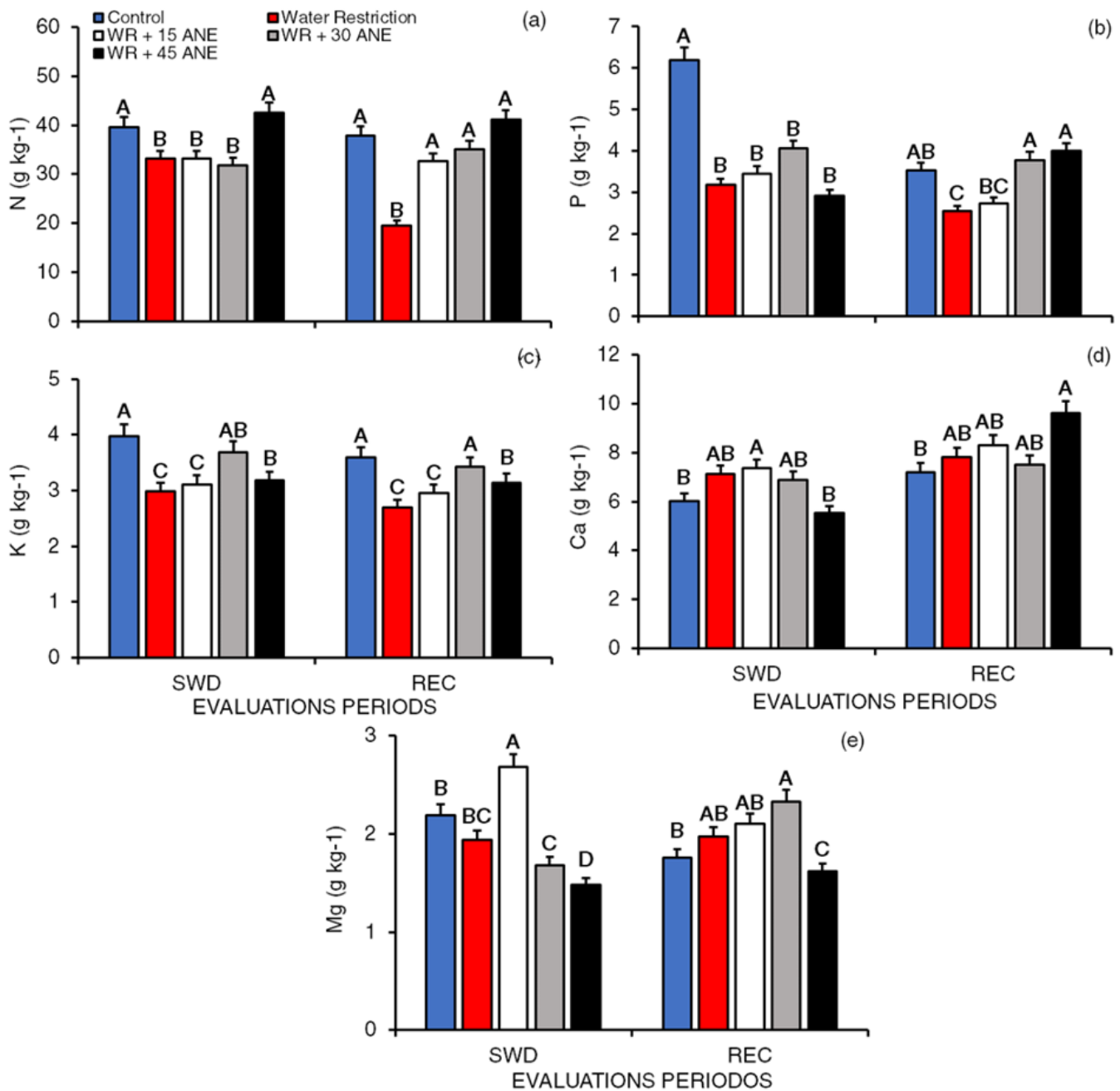


Figure 3

Total content of nitrogen – N (a), phosphorus – P (b), potassium – K (c), calcium – Ca (d), and magnesium – Mg (e) in *Inga edulis* Mart. seedlings under water regimes – control (daily irrigation) and drought by water restriction (WR) – with or without the application of *Ascophyllum nodosum* L. seaweed extract (ANE) doses in both evaluation periods (SWD and REC). Different letters compare the effect of treatments in each evaluation period $\pm$ SE (Tukey test, $p \leq 0.05$).

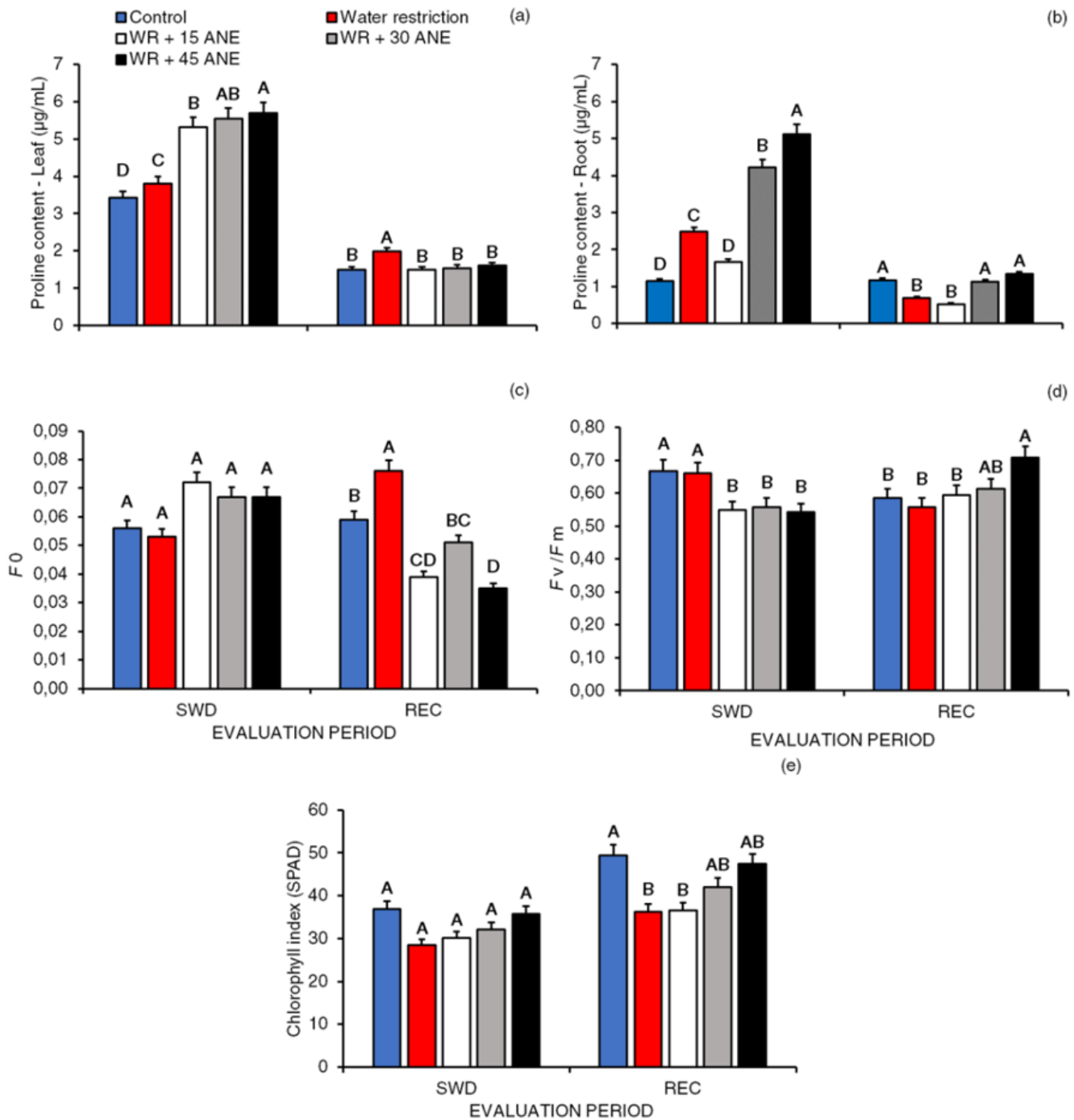


Figure 4

Proline content in leaves (a), proline content in roots (b), initial fluorescence – F_0 (c), the quantum efficiency of photosystem II – F_v/F_m (d) and chlorophyll index SPAD (e) in *Inga edulis* Mart. seedlings under water regimes – control (daily irrigation) and drought by water restriction (WR) – with or without the application of *Ascophyllum nodosum* L. seaweed extract (ANE) doses, in both evaluation periods (SWD and REC). Different letters compare the effect of treatments in each evaluation period $\pm$ SE (Tukey test, $p \leq 0.05$).

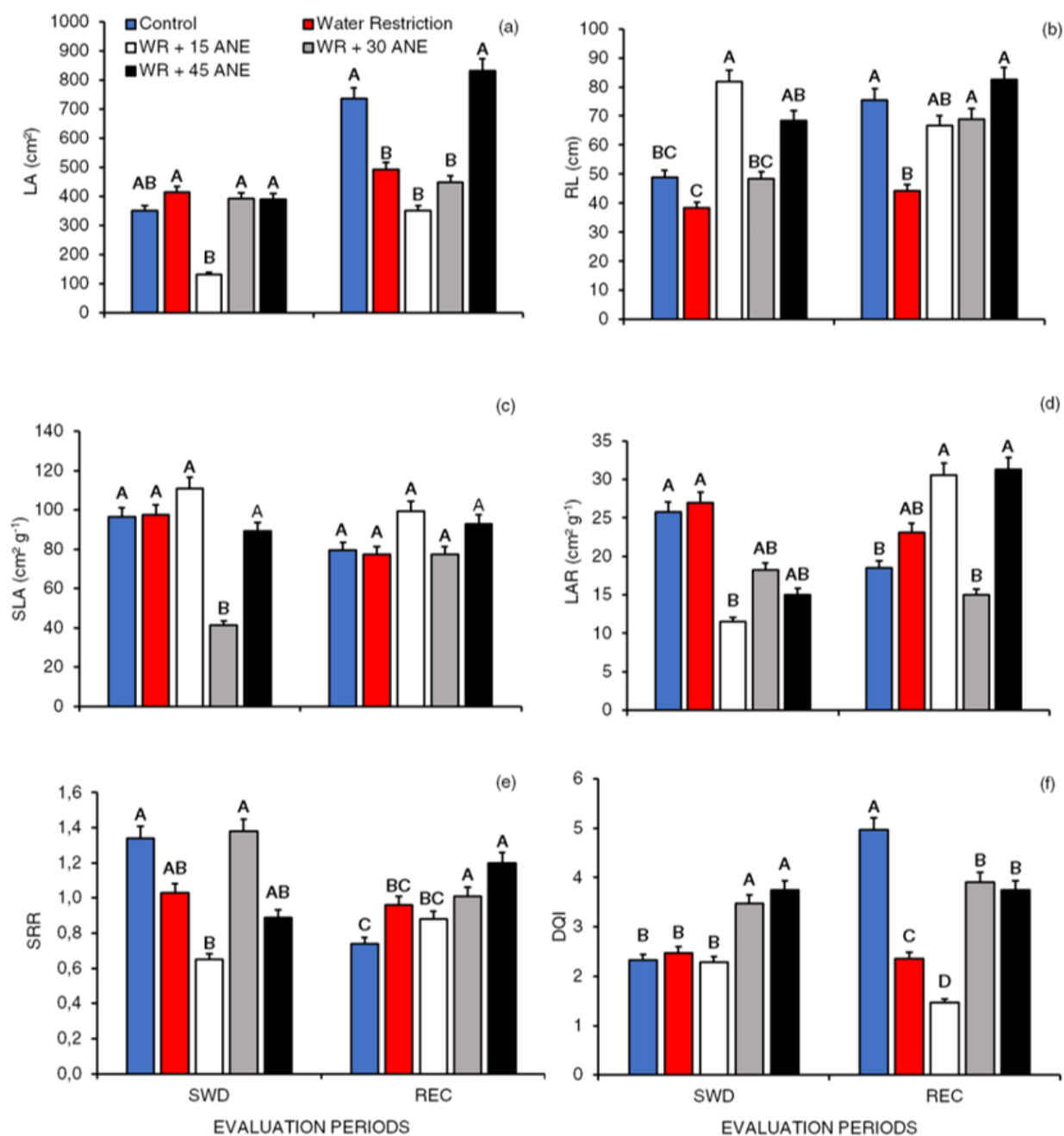


Figure 5

Leaf area – LA (a), root length – RL (b), specific leaf area – SLA (c), Leaf area ratio – LAR (d), shoot/root ratio – SRR (e), and Dickson quality index – DQI (f) in *Inga edulis* Mart. seedlings under water regimes – control (daily irrigation) and drought by water restriction (WR) – with or without the application of *Ascophyllum nodosum* L. seaweed extract (ANE) doses, in both evaluation periods (SWD and REC). Different letters compare the effect of treatments in each evaluation period $\pm$ SE (Tukey test, $p \leq 0.05$).

