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Algal extract and proline promote physiological changes in mango trees during shoot maturation

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Abstract - Water stress should be adequately managed during shoot maturation to improve the floral induction of mango grown in semi-arid regions. In this scenario, proline association with algal extracts has a potential stress-mitigating effect. However, even though this practice has been reported separately for some crops, its effects on mango are still unknown. From this perspective, this study investigated the role of proline and algal extract in mitigation of water deficit effects during shoot maturation of the mango variety 'Tommy Atkins' grown in the tropical semi-arid region. The study consisted of five consecutive individual experiments (five foliar application treatments) in a randomized block design and a 4 x 2 factorial corresponding to i) four proline concentrations (Pro): 0.0% (without proline), 0.287, 0.575, and 1.150%, and ii) the presence of algal extract (AE) (presence and absence). The following parameters were evaluated: photosynthetic pigments, soluble carbohydrates, photochemical and non-photochemical efficiency, and flowering and production uniformity. Carotenoids were interactively affected by the factors evaluated. On the other hand, as additive effects, proline provided the highest values of gas exchange and total soluble carbohydrates, with a higher internal CO_2 concentration and greater flowering uniformity. The application of proline and the algal extract of *A. nodosum* promoted different responses in mango physiology and can be used during shoot maturation.

Index terms: *Ascophyllum nodosum*; total soluble carbohydrates; photoprotective pigments; flowering uniformity.

Extrato de algas e prolina promovem alterações fisiológicas em plantas de mangueira durante a maturação de ramos

Resumo - Para um melhor gerenciamento da indução floral na mangueira cultivada na região semiárida, é necessário que a imposição do estresse hídrico durante a fase de maturação de ramos seja manejada de forma apropriada. Nesse sentido, prolina associada ao extrato de algas são moléculas com potencial efeito mitigador do estresse, e mesmo que para algumas culturas esta prática tenha sido relatada separadamente, na mangueira, os resultados ainda são desconhecidos. Investigou-se o papel da prolina e do extrato de algas na mitigação dos efeitos ocasionados pela imposição do déficit hídrico durante a fase de maturação de ramos na cultura da mangueira variedade 'Tommy Atkins' cultivada na região semiárida tropical. O estudo realizou cinco experimentos individuais consecutivos (cinco aplicações foliares de tratamentos e amostragem), em delineamento experimental de blocos casualizados, em esquema fatorial 4 x 2, correspondendo a i) concentrações de prolina (Pro): 0,0% (sem prolina), 0,287; 0,575; e 1,150%; e ii) extrato de algas (EA) (presença e ausência), avaliando pigmentos fotossintéticos, carboidratos solúveis, trocas gasosas, parâmetros de eficiência fotoquímica e não fotoquímica, uniformidade de floração e produção. Os carotenoides sofreram efeitos interativos dos fatores avaliados. Como efeitos aditivos, a prolina proporcionou as maiores taxas de troca gasosa e de carboidratos solúveis totais, sendo que, na ausência de extrato de algas, maior concentração interna de CO_2 pôde ser observada, contrapondo-se à maior uniformidade de floração. O fornecimento de prolina e extrato de algas de *A. nodosum* promove diferentes respostas na fisiologia das plantas de mangueira, podendo ser inserido no manejo empregado na etapa de maturação de ramos.

Termos para indexação: *Ascophyllum nodosum*; Carboidratos solúveis totais; Pigmentos fotoprotetores; Uniformidade de floração.

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Introduction

Mango production in the São Francisco Valley, Brazil, stands out both nationally and internationally due to the technologies employed to manage floral induction, a stage with important metabolic changes that trigger the development of reproductive buds (CLONAN et al., 2021). Under semi-arid conditions, floral induction comprises steps such as pruning and the application of plant growth regulators (paclobutrazol), induced water stress, and products with physiological effects (SILVA et al., 2021) to promote shoot maturation (lignification).

Although employed in commercial mango orchards in the semi-arid region, shoot maturation is not a simple task due to physiological changes during water stress induction. However, in spite of negative effects on plants, it is considered a 'necessary evil' in the region. The primary purpose of this technique is to potentialize shoot maturation through ethylene production and make the apical meristem more sensitive to low levels of floral stimuli (ALBUQUERQUE et al., 2002; RAMÍREZ; DAVENPORT, 2016).

Carbohydrate deposition on the tissues during shoot development is another key factor for plants to withstand the 'reproductive effort' or form reproductive structures using photoassimilates (DAS et al., 2019; SILVA et al., 2020) while simultaneously tolerating the water stress induced by osmotic adjustment. However, this accumulation can be compromised depending on the intensity and duration of stress, and energy reserves can be drastically reduced as a survival attempt (FALCHI et al., 2020).

On the other hand, plants have developed other defense mechanisms associated with carbohydrate consumption, e.g., proline accumulation in plant cells, an acclimation mechanism to withstand water stress conditions by maintaining a low water potential without significantly damaging the tissues. In addition to the primary purpose of osmotic adjustment and the consequent protection of macromolecules against denaturation, proline also eliminates free radicals and acts as an energy sink to regulate redox potentials. Furthermore, it is also a nitrogen source for rapid post-stress growth (ZHENG et al., 2015; NAKHAIE et al., 2020; LOPEZ-DELACALLE et al., 2020).

As previously mentioned, proline has multiple benefits. However, water stress induction in mango can last up to 60 days and, when associated with high temperatures and intense solar radiation, proline accumulation alone may not be enough and thus require foliar supplementation, as reported in the literature (SOROORI et al., 2021; HANIF et al., 2021; SEMIDA et al., 2021; CUNHA et al., 2022).

From this perspective, the use of marine algae, especially *Ascophyllum nodosum*, has outstanding potential in reducing stress in mango trees, as observed by Cavalcante et al. (2018) for the mango cv. 'Palmer' in another study conducted in the São Francisco Valley in which the application of *A. nodosum* increased the carbohydrate contents and yield. Moreover, increases were also observed in the chlorophyll levels of the mango varieties 'Palmer' and 'Parvin' (MORALES-PAYAN, 2013) and in the flowering and yield of the mango varieties Tommy Atkins (SILVA et al., 2020) and Arka Neelachal Kesri (DASH et al., 2021).

The scenario exposed is positive and reinforces the idea that the combined application of proline and algal extract could effectively ensure the maintenance of photosynthetic pigments, gas exchange, and, consequently, carbohydrate production before floral induction. We believe that this association changes the physiology of mango plants and provides significant information on the action of these molecules, especially during water reduction, when the plant is more vulnerable to the adverse effects of abiotic stresses. On the other hand, although the panorama is promising, we must always consider that the combination of proline and algae extract may not influence mango physiology.

From this perspective, the present study aimed to evaluate whether proline and the algal extract act jointly to mitigate water deficit during shoot maturation in the mango variety 'Tommy Atkins' grown in the tropical semi-arid region.

Materials and Methods

Study area description

Five consecutive and individual experiments were carried out in a commercial 'Tommy Atkins' mango orchard located at the FRUTAVI/ARGO BRASIL Farm, in the Irrigation Perimeter "Senador Nilo Coelho", Petrolina, Pernambuco, Brazil (9°18'19.2" S, 40°33'55.9" W, at an elevation of 365.5 m above sea level), from October 2017 to April 2018.

According to the classification by Alvares et al. (2013), the climate of the region is classified as BSh, corresponding to a semi-arid region with a mean annual temperature of 26.0 °C and a mean annual rainfall of 481.7 mm. The meteorological data on rainfall, temperature (minimum, maximum, and mean), relative air humidity, and global solar radiation during the experiments were recorded by an automatic weather station at the Agricultural Sciences Campus (CCA) of the Federal University of Vale do São Francisco (UNIVASF) (Figure 1).
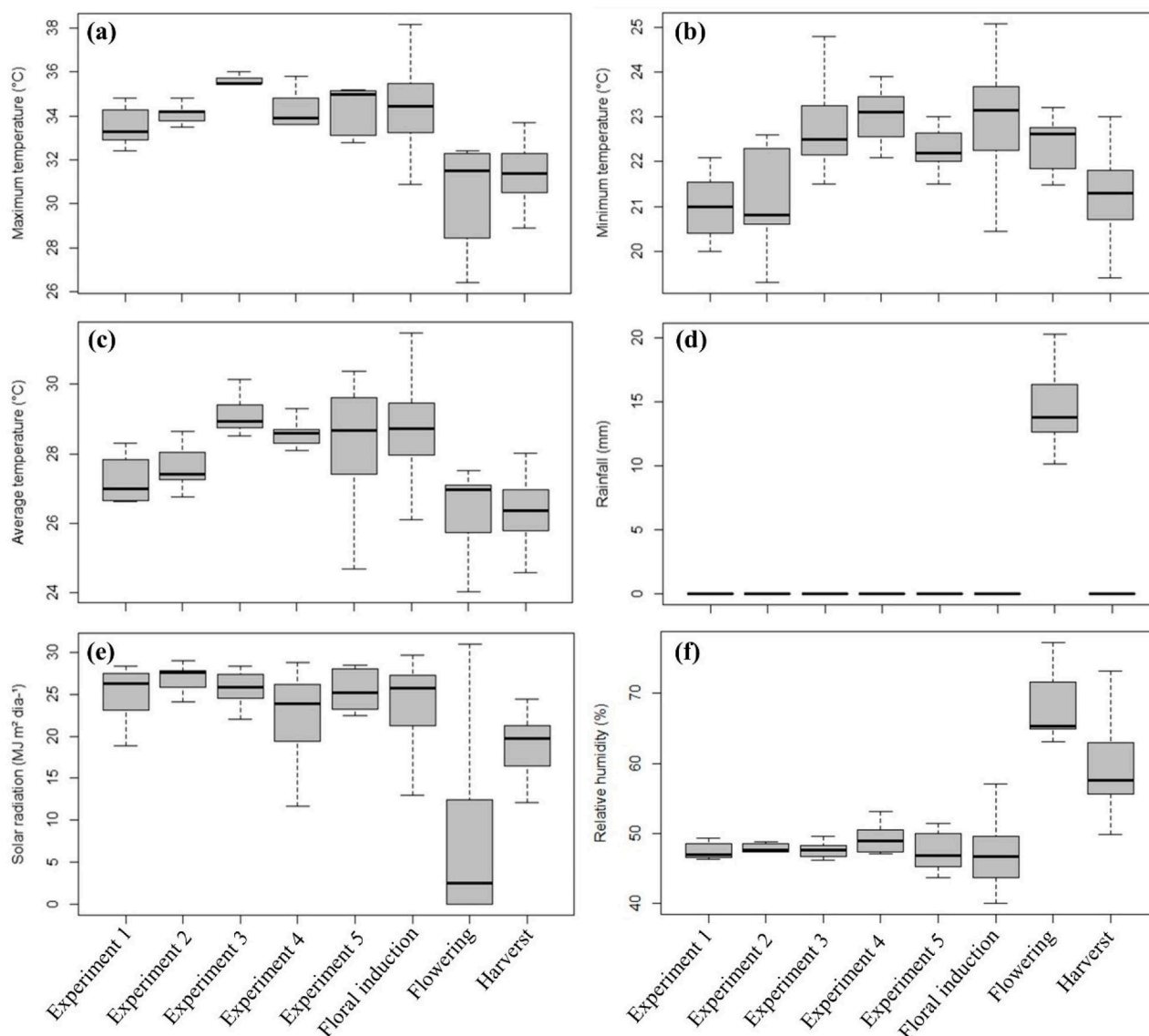
Figure 1. Maximum (a), minimum (b), and mean (c) air temperature, rainfall (d), global solar radiation (e), and relative air humidity (f) recorded during the experiments, floral induction, flowering, and harvest. Petrolina –PE.

The boxplots referring to the meteorological data were constructed by considering seven days for each of the five experiments (experimental design), in addition to the flowering period. Floral induction and harvest lasted 57 and 19 days, respectively. Soil and leaf samples were collected to characterize the area by analyzing the fertility, plant nutrient status, levels of chlorophyll *a*, chlorophyll *b*, total carotenoids, and total soluble carbohydrates, as shown in Tables 1 and 2.

Table 1. Chemical analysis of soil (0.0 to 0.40 m depth) and leaves in the experimental area cultivated with 'Tommy Atkins' mango trees before the treatments.

					Soil					
pH	M.O	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	$H+Al^{3+}$	SB	V
H_2O	g kg^{-1}	mg dm^{-3}				cmol$_c$/dm^3				%
6.6	8.9	114	0.24	0.05	4.9	1.7	0.0	1.12	6.89	86

					Leaves					
N	P	K^+	Ca^{2+}	Mg^{2+}	B	Cu	Fe^{2+}	Mn	Zn	Na
		g kg^{-1}					mg kg^{-1}			
16.8	1.03	7.50	26.0	3.3	198	198	183	520	113	130

Soil and leaf macro and micronutrient contents were determined according to the methodology of Teixeira et al. (2017). Extraction methods – soil: M.O: volumetric method; P, K, Na, Cu, Fe, Mn, and Zn: Mehlich (Hcl + H_2SO_4); Ca, Mg, and Al: KCl 1 mol L^{-1}.
Extraction methods - leaves: N: Kjeldahl; P: Spectrometry with vanadate yellow; K: flame photometry; Mg^{2+}, Ca^{2+}, Fe^{2+}, Zn, and Mn: atomic absorption spectrophotometry; B: Spectrophotometry with azomethine-H.

Table 2. Leaf contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, and total soluble carbohydrates (TSC) before the treatments.

Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	TSC
——————————————mg g^{-1} FM——————————				µmol g^{-1} FM
1.06	0.97	2.03	0.69	127.21

FM: Fresh mass

The 'Tommy Atkins' mango plants used in this study had 22 years of productive age and were arranged in an 8 x 8 m spacing. Production pruning was performed on April 24, 2017, and irrigation was performed daily using a micro-sprinkler system with one emitter per plant and a water flow of 50 L h^{-1} in a 1.5 m radius. On August 10, 2017, Paclobutrazol was applied at an average level of 25 ml Cultar 250 SC® per plant, corresponding to 6.25 g of the active ingredient applied via soil under the canopy projection.

Pruning, weeding, and pest and disease control followed the integrated production techniques proposed by Lopes et al. (2003). Nutritional management was performed by fertigation after analyzing the soil, leaves, and crop requirements (SILVA et al., 2002).

Experimental design and application of treatments
Experiments 1, 2, 3, 4, and 5 corresponded to the first, second, third, fourth, and fifth sampling to investigate the physiological parameters mentioned before. The experiment was set up a randomized block design with four replications and three plants per plot. Treatments were distributed according to a 4 x 2 factorial corresponding to: 1) four proline concentrations: 0.0 (no proline), 0.287, 0.575, and 1.150%, and 2) the presence of 1.5% algal extract of *A. nodosum* (presence and ausence).

Treatments were defined by considering the crop requirements and physiological changes during shoot maturation, including a reduction in water availability for 60 days preceding floral induction using nitrate (calcium or potassium), according to the management practices recommended by Genú and Pinto (2002). However, the shoot maturation management used in this study consisted exclusively of applying proline and algal extract (*A. nodosum*) through five different combinations.

Treatments were applied via foliar application, and the concentrations of proline and algal extract were determined according to Abdelhamid et al. (2013) and Carvalho and Castro (2014). The mix volume was standardized at 20 L per plot (6.66 L per plant) to allow sufficient foliar coverage, and application was performed using a Jacto Arbus® airblast sprayer.

On October 6, 2017, the irrigation volume was reduced to 75 L per plant, corresponding to 37.5 % of the total volume provided to each plant in the farm (200 L per plant). Treatments 1, 2, 3, 4, and 5 were applied on October 13, October 20, October 27, November 3, and November 10, 2017, respectively, corresponding to seven, 14, 21, 28, and 35 days after reducing the water volume supplied. Each experiment was evaluated seven days after their application. Finally, the irrigation volume was readjusted to 100 L per plant 27 days after the initial reduction following the management practices used in the farm.

Variables evaluated
Leaf samples were collected before each application. The first collection was performed before applying the treatments and served as a control. Four fresh 12-leaf samples of the last vegetative flush were collected from the four quadrants of the plants (TEDESCO et al., 1995) at mid-canopy height. Then, the samples were put in plastic bags and stored in coolers containing ice.

Photosynthetic pigments and total soluble carbohydrates
The contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were quantified according to Lichtenthaler and Buschmann (2001) by adding 10 mL of methanol (100 %) to 100 mg of each fresh leaf sample in threaded test tubes covered with aluminum foil. The readings of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were performed by spectrophotometry at the wavelengths of 665.2, 652.4, and 470.0 nm. The sulfuric acid-phenol method was used to quantify soluble carbohydrates according to Dubois et al. (1956). D-glucose was used as a standard, and the absorbance was read in a spectrophotometer at the wavelength of 490 nm.

Gas exchange
The variables of net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), maximum photochemical efficiency of PSII (Fv/Fm), photochemical quenching (qP), non-photochemical inhibition coefficient (NPQ), and instantaneous carboxylation efficiency (Eic) were determined during experiment 5 (the fifth sampling used to investigate the physiological parameters). The Eic was determined by the net photosynthesis/internal CO_2 concentration ratio using an infrared gas analyzer (IRGA) (Mod. Li-COR® 6400 XT) coupled to a portable frequency-modulated light fluorometer at 1500 µmol photons m^{-2} s^{-1} (artificial light source). The measurements were performed in mature leaves of the last vegetative flush collected at mid-canopy height between 9:00 a.m. and 11:00 a.m.

Phytotechnical variables

After the five experiments, at full flowering, the ratio of the total number of panicles to the number of branches per plant was used to determine the flowering percentage and analyze the effect of the treatments on the phytotechnical variables. The number of fruits per panicle was determined one week before harvest, which occurred on April 12, April 19, and April 30, 2018, when fruits were in ripening stage 2 and had a cream-yellow pulp color (FILGUEIRAS et al., 2000). Subsequently, the fruits were weighed to determine the production (kg plant^{-1}) and the estimated yield (t ha^{-1}).

Statistical analysis

The data were tested for normality of residuals and homogeneity of variances. Subsequently, analysis of variance was performed with the F-test and, according to its significance, the treatments with *A. nodosum* were compared. The proline concentrations were subjected to regression analysis. All statistical analyses were performed according to Banzatto and Kronka (1995) using the statistical softwares R, version 3.5.2 (R Core Team, 2018), and Sigma Plot, version 10.0 (10.0, Systat Software, San Jose, CA, USA).

Results

Table 3 shows the additive effect of the algal extract (AE) on the chlorophyll *a* levels of experiment 5 and the total soluble carbohydrates (TSC) of experiment 1. In experiment 1, the TSC content was also positively affected by the interaction between the algal extract and proline (AE x Pro). The analysis of experiment 1 revealed that the algal extract and proline had no significant effects on the levels of chlorophyll *a* and chlorophyll *b*, whose behavior was opposite (Table 3), as observed during the initial characterization of the study area (Table 2).

With regard to carotenoids, the analysis of the AE x Pro combination showed variable mean values and fit a quadratic model, with the maximum estimated response of 0.71 mg g^{-1} MF at the proline concentration of 0.484% (in the presence of AE) and 0.72 mg g^{-1} MF at 0.629% (in the absence of AE) (Figure 2). Both models showed high coefficients of determination (R^2).

The analysis of the predictive equations showed very similar responses as the proline concentration required was low even in the presence of algal extract. The mean value of leaf carotenoids in the plants treated exclusively with the algal extract was 41.86% higher than in the absolute control (Figure 2).

The algal extract significantly influenced the TSC levels, with higher mean values for this parameter compared to the plants treated exclusively with proline (Table 3). The influence shown in experiment 1 is even more visible when evaluating the AE x Pro combination (Table 3). However, the TSC values did not fit a regression model in the presence of algal extract. On the other hand, the proline concentrations of 0.287% and 1.150% resulted in values ranging from 115.36 to 138.47 µmol g^{-1} MF. Moreover, the TSC values increased along with the proline concentrations in the absence of algal extract. At the proline concentration of 1.150%, the TSC showed a 114% difference compared to the control. Furthermore, as shown in Figure 3, the lower TSC levels obtained with the control were 42.35% lower than the levels observed in the initial characterization of the area (Table 2).
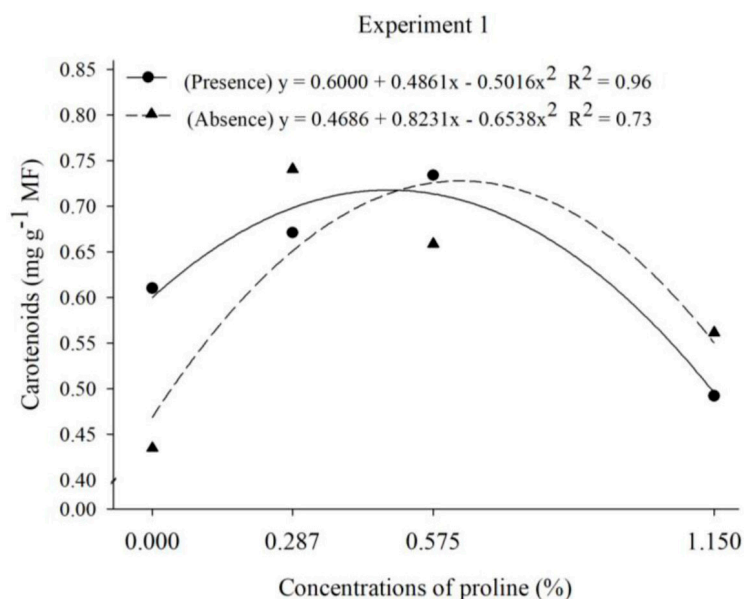

Figure 2. Leaf carotenoids in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

Table 3. Summary of the analysis of the variance for the leaf concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, and total soluble carbohydrates in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

Sources of Variation	'F' value				
	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	Total soluble carbohydrates
	---------------------------mg g^{-1} MF---------------------------				μmol g^{-1} MF
Experiment 1					
A. Extract (AE)	1.05^{ns}	0.31^{ns}	0.20^{ns}	0.04^{ns}	5.68^*
Absence	1.41	0.51	1.92	0.59	134.14 a
Presence	1.28	0.56	1.84	0.60	127.69 b
Proline (Pro)	2.12^{ns}	1.93^{ns}	2.92^{ns}	4.20^*	154.99^{**}
AE x Pro	2.22^{ns}	0.97^{ns}	2.25^{ns}	3.10^*	88.20^{**}
CV (%)	26.91	48.77	26.28	20.42	5.85
Experiment 2					
A. Extract (AE)	2.82^{ns}	1.58^{ns}	3.84^{ns}	3.63^{ns}	1.12^{ns}
Absence	1.41	0.49	1.90	0.59	133.59
Presence	1.61	0.54	2.16	0.66	121.36
Proline (Pro)	1.12^{ns}	1.09^{ns}	0.63^{ns}	0.60^{ns}	0.16^{ns}
AE x Pro	0.18^{ns}	4.60^{ns}	0.69^{ns}	0.89^{ns}	0.26^{ns}
CV (%)	22.02	23.79	17.99	0.0702	25.62
Experiment 3					
A. Extract (AE)	0.06^{ns}	0.31^{ns}	0.20^{ns}	1.45^{ns}	2.44^{ns}
Absence	1.61	0.61	2.12	0.61	159.69
Presence	1.62	0.57	2.20	0.66	134.59
Proline (Pro)	1.08^{ns}	0.75^{ns}	1.65^{ns}	2.28^{ns}	1.65^{ns}
AE x Pro	1.69^{ns}	0.53^{ns}	1.60^{ns}	2.25^{ns}	0.85^{ns}
CV (%)	18.49	30.44	24.49	18.17	30.88
Experiment 4					
A. Extract (AE)	3.06^{ns}	3.33^{ns}	0.27^{ns}	0.33^{ns}	0.16^{ns}
Absence	1.15	1.01	2.16	0.60	166.50
Presence	1.46	0.57	2.04	0.63	160.08
Proline (Pro)	0.46^{ns}	0.78^{ns}	0.22^{ns}	0.68^{ns}	0.69^{ns}
AE x Pro	0.50^{ns}	0.83^{ns}	0.51^{ns}	0.66^{ns}	2.01^{ns}
CV (%)	38.61	84.69	31.43	27.64	27.27
Experiment 5					
A. Extract (AE)	7.03^*	0.68^{ns}	0.27^{ns}	2.20^{ns}	3.85^*
Absence	1.43 b	0.61	2.04	0.47	211.91 a
Presence	1.74 a	0.69	2.43	0.55	185.02 b
Proline (Pro)	1.24^{ns}	0.19^{ns}	0.22^{ns}	0.46^{ns}	1.70^{ns}
AE x Pro	0.61^{ns}	1.49^{ns}	0.51^{ns}	0.50^{ns}	0.33^{ns}
CV (%)	20.68	41.41	31.43	29.58	19.52

CV: Coefficient of variation; ns = non-significant; ** = significant at 1% probability ($p < 0.01$); * = significant at 5% probability ($p < 0.05$).
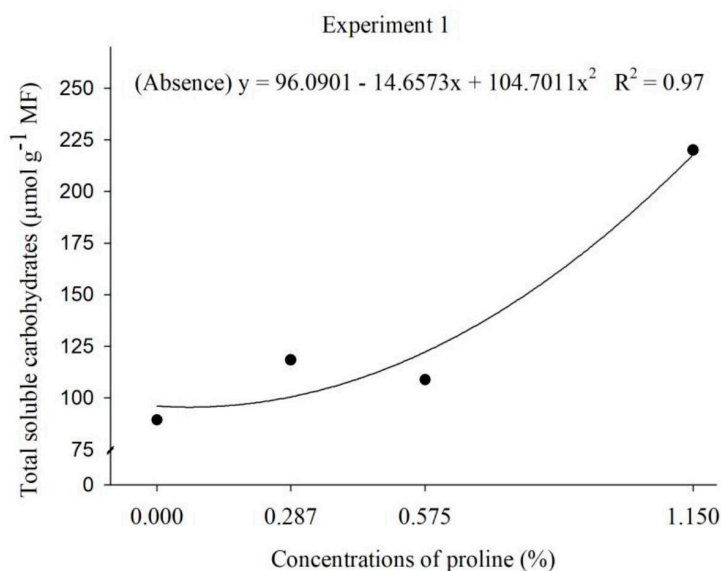
Figure 3. Leaf contents of total soluble carbohydrates in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

With regard to the algal extract, the TSC showed a slight reduction during experiments 1 and 2. However, this parameter increased gradually as stress increased and was significantly affected in experiment 5 (Table 3). Moreover, there was a positive influence of exogenous proline application 42 days after water reduction (experiment 5), inducing the highest TSC values one week before the onset of floral induction.

Table 4 shows significant interactions between AE and Pro and the variables of net photosynthesis (A), stomatal conductance (gs), transpiration (E), and the non-photochemical inhibition coefficient (qN). In the absence of AE, the variables of net photosynthesis, stomatal conductance, and transpiration adjusted to the "peak" regression model, with a directly proportional data distribution pattern (Figure 4). However, in the presence of AE, the data did not fit any regression model, ranging from 2.32 to 4.54 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$, 0.01 to 0.04 $mol\ H_2O\ m^{-2}\ s^{-1}$, and 0.95 to 2.51 $mmol\ H_2O\ m^{-2}\ s^{-1}$ for net photosynthesis, stomatal conductance, and transpiration, respectively.

Table 4. Summary of the analysis of the variance for net photosynthesis (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci), instantaneous carboxylation efficiency (Eic), maximum photochemical efficiency of PSII (*Fv/Fm*), photochemical quenching (qP), and non-photochemical inhibition coefficient (NPQ) in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

Sources of Variation	Value 'F'							
	A	gs	E	Ci	Eic	*Fv/Fm*	qP	NPQ
A. Extract (AE)	0.18[ns]	0.18[ns]	0.04[ns]	4.57*	4.74[ns]	0.084[ns]	0.004[ns]	1.43[ns]
Absence	3.46	0.02	1.63	170.64 a	0.027	0.46	0.22	1.85
Presence	3.62	0.02	1.65	132.33 b	0.020	0.44	0.22	1.75
Proline (Pro)	3.76*	1.55[ns]	4.39*	1.42[ns]	3.49[ns]	1.552[ns]	0.29[ns]	1.28[ns]
AE x Pro	16.51**	5.94**	15.04**	0.97[ns]	1.90[ns]	3.107*	0.51[ns]	4.92*
CV %	29.82	47.52	29.46	33.45	47.47	25.57	33.44	12.46

CV: Coefficient of variation; ns = non-significant; ** = significant at 1% probability (p < 0.01); * = significant at 5% probability (p < 0.05). A – $\mu mol\ CO_2\ m^{-2}\ s^{-1}$; gs - $mol\ H_2O\ m^{-2}\ s^{-1}$; E - $mmol\ H_2O\ m^{-2}\ s^{-1}$. Ci - $\mu mol\ m^{-2}\ s^{-1}$; Eic $[(\mu mol\ m^{-2}\ s^{-1})\ (\mu mol\ mol^1)]^{-1}$

The highest mean values of the gas exchange variables were achieved at the proline concentration of 0.287% (Figure 4). However, the proline concentrations of 0.575% and 1.150% caused the opposite effect. Table 4 shows that the plants treated exclusively with exogenous proline (absence) were significantly affected and showed a higher internal CO_2 concentration. In these plants, amino acids preserve cell turgidity and facilitate CO_2 assimilation due to stomatal conductance. However, the algal extract and proline did not affect the instantaneous carboxylation efficiency (Eic) despite its close relationship with the internal CO_2 concentration (Table 4).

The performace of the Fv/Fm ratio was statistically similar to the gas exchange variables (Figure 5a). Moreover, photochemical quenching (qP) was not affected by the two variables, and the mean values of this parameter were the same (0.22) for both AE levels (Table 4). However, unlike qP, the interaction between AE and Pro significantly affected the NPQ (non-photochemical quenching) as the presence of AE resulted in a quadratic behavior with a maximum response of 1.96 at the proline concentration of 0.604%. In the absence of AE, the data showed a linear behavior and increased by 0.34 per unit increase in the proline concentration (Figure 5b).
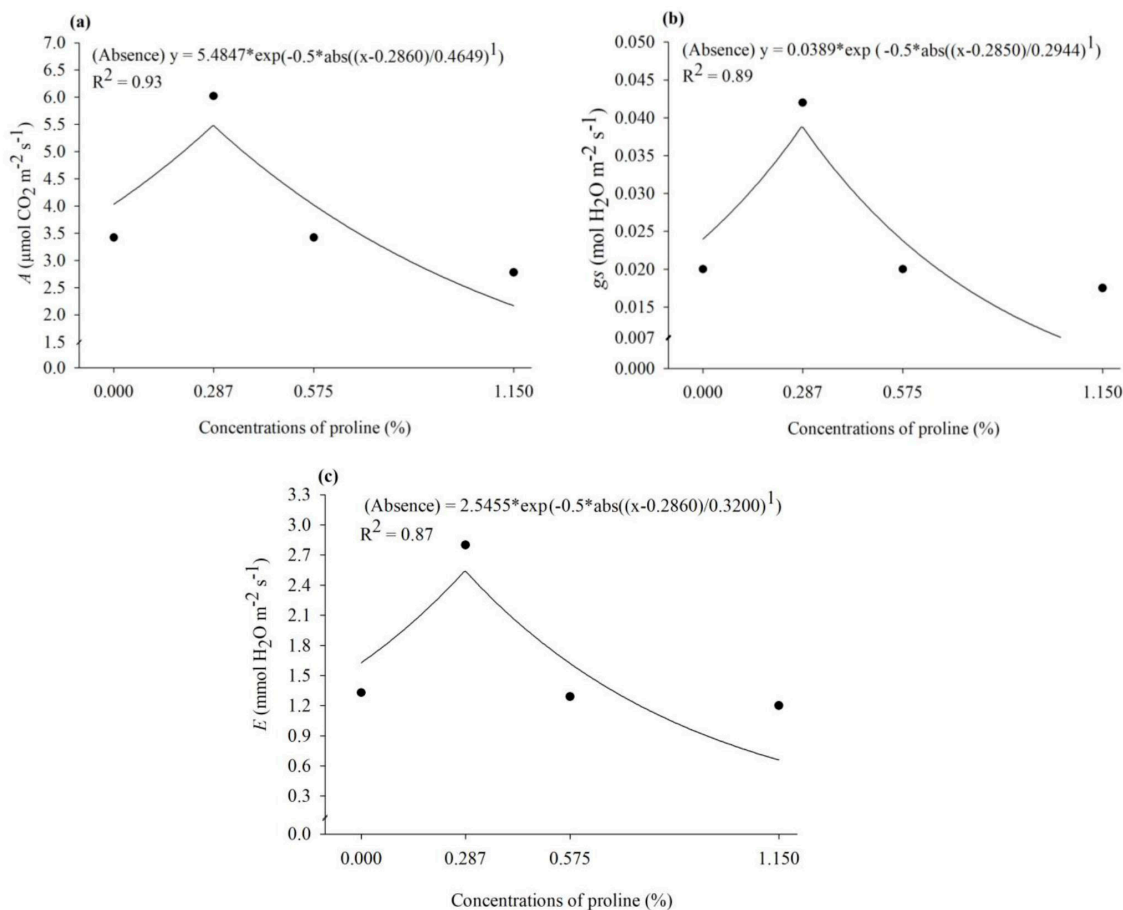

Figure 4. Net photosynthesis-*A* (a), stomatal conductance-*gs* (b), and transpiration-*E* (c) in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.
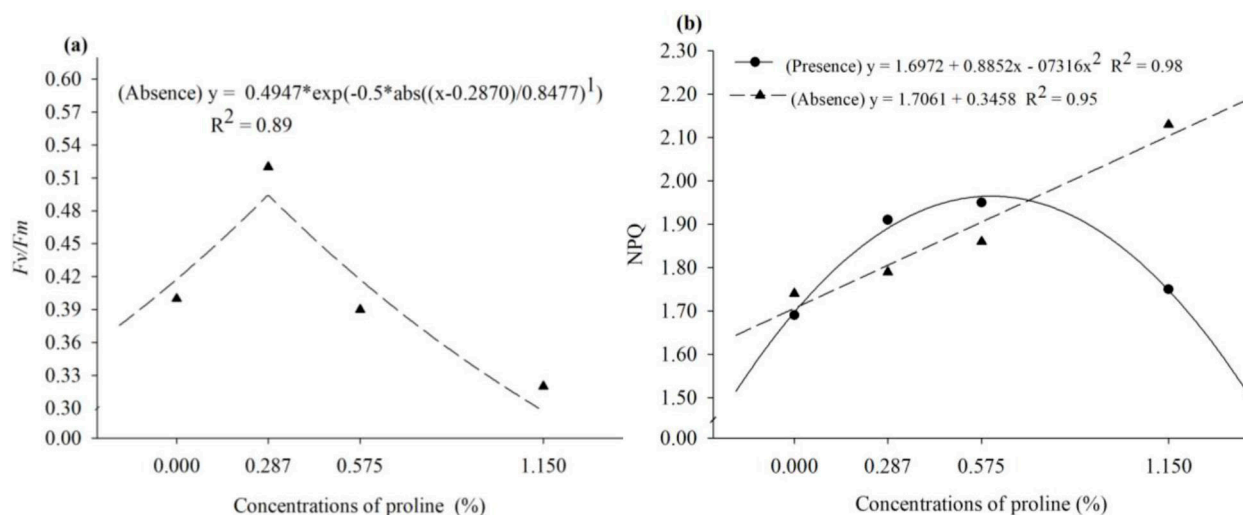

Figure 5. Maximum photochemical efficiency of PSII-Fv/Fm (a) and non-photochemical quenching-NPQ (b) in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

Table 5. Summary of the analysis of the variance for flowering uniformity, fruits per panicle, yield and productivity in 'Tommy Atkins' mango trees after proline and algal extract application during shoot maturation.

Sources of Variation	Flowering uniformity	Fruits per panicle	Yield	Productivity
	'F' value			
	%	-	Kg planta^{-1}	t ha^{-1}
A. Extract (AE)	58.57**	3.39^{ns}	1.89^{ns}	1.89^{ns}
Absence	26.67 b	1.56	65.15	10.16
Presence	48.53 a	1.91	78.96	12.31
Proline (Pro)	1.98^{ns}	0.65^{ns}	0.39^{ns}	0.39^{ns}
AE x Pro	2.72^{ns}	0.29^{ns}	0.52^{ns}	0.52^{ns}
CV (%)	21.54	29.53	39.43	39.43

CV: Coefficient of variation; ns = non-significant; ** = significant at 1% probability ($p < 0.01$); * = significant at 5% probability ($p < 0.05$).

The summary of the analysis of the variance of the phytotechnical variables (Table 5) reveals that the algal extract only significantly affected flowering uniformity. The plants that received the algal extract of A. nodosum showed an 81.96% higher flowering uniformity. The remaining variables were not affected by any of the studied factors.

Discussion

Water stress (drought) is one of the most critical aspects of mango farming in semi-arid regions as a harmful phenomenon characterized by water reduction during shoot maturation, demanding cultivation strategies capable of mitigating such effects. From this perspective, according to the answers obtained in our experiment, we discarded the null hypothesis since the application of algal extract and proline significantly interfered with the physiological development of mango plants during the shoot maturation phase.

This is a pioneering study on the combined application of proline and algal extract in mango trees grown in the semi-arid region of Brazil since the physiological responses of mango derived from using these molecules are still incipient. Compared to the initial characterization, the data showed that the relationship between chlorophyll *a* and *b* and the algal extract increased and decreased, respectively (Table 2), since the plants grew under the same conditions.

More specifically, the algal extract significantly increased the chlorophyll *a* content, probably because it contains secondary metabolites that positively influence the synthesis and protection of chlorophyll when provided to plants (CARVALHO; CASTRO, 2014).

Unlike chlorophyll, carotenoids responded positively to proline and algal extract in experiment 1 (Figure 2). *A. nodosum* has anti-stress properties and induces the synthesis of proline (CARVALHO et al., 2018; JITHESH et al., 2019). From this perspective, high carotenoid levels could be obtained by using low proline concentrations via foliar applications since the amount of proline provided was complementary to its endogenous levels already present in plants.

Even fitting the quadratic model, the use of proline alone or its association with the algal extract always increased the carotenoid content. The benefits include the protection of the plant photosynthetic apparatus against the adverse effects of excessive solar radiation exposure, energy transfer from the triplet state of chlorophyll to carotenoids, and the extinction of reactive oxygen species (ROS), especially singlet oxygen ($^1O_2$) (REHMAN et al., 2021), which is associated with different types of cell damage.

The accumulation of soluble sugars helps eliminate ROS by inducing the synthesis of proline (OZDENIZ, 2019). Therefore, the present study showed higher TSC levels in the plants treated only with proline in experiments 1 and 5. These results were mainly due to the protection of cell structures and chloroplasts provided by proline (MOUSTAKAS et al., 2011), and, consequently, the maintenance of photosynthetic pigments such as carotenoids during stress.

Figure 3 shows that the plants that received no stress-reducing treatment showed low TSC levels in crucial moments, unlike the plants treated with proline. Low TSC levels are one of the adverse effects caused by water stress as plants tend to reduce the production of photoassimilates and consume those that have already been stored (ALMEIDA et al., 2015). These results corroborate those obtained by Helaly et al. (2017) with the mango cultivars Ewaise, Hindy, Misk Ewaise, and Fagri Kalan under water stress.

This observation highlights the relevance of supplying proline in the early stage of water reduction, aiming to influence the TSC. Although proline contributes substantially to osmotic adjustment under stress situations, the sum of these compatible solutes accumulated in the cells can probably provide greater protection to the cell structure than any of them used alone (VERSLUES; SHARMA, 2010). Nevertheless, few reports available in the literature have addressed the influence of TSC levels on osmotic adjustment after adding proline to mango plants under water stress conditions.

Freitas et al. (2019) studied sorghum under salt stress and observed that the proline concentration of 30 mM increased the TSC levels compared to the control treatment. This result was associated with the function of proline as a carbon skeleton, favoring the synthesis of other osmotically active molecules and nitrogen compounds, accumulating TSC, and mitigating the effects of stress exposure.

The imposition of water restriction, the high temperatures of the semi-arid region, and the use of PBZ as an inhibitor of gibberellin biosynthesis promoted vegetative flushes and uniform shoot maturation, consequently increasing carbohydrate accumulation (CAVALCANTE et al., 2018). Our results suggest that, along with the practices described above, proline could be an alternative to promote higher stability and TSC concentrations.

The TSC, A, gs, and E rates fit a significant regression model when treated only with proline. This result highlights the efficiency of this amino acid in stabilizing the electron transport chain in the chloroplast, increasing the activity of enzymes such as Rubisco (ALTUNTAŞ et al., 2020) and increasing the photosynthetic attributes.

Thakur et al. (1988) reported that proline destabilizes the bond of abscisic acid with specific proteins in guard cell membranes. Therefore, in ideal amounts, proline favors potassium accumulation in the membranes and helps maintain the stomata open, increasing the A, gs, and E rates. On the other hand, the adverse effects caused by high proline concentrations (0.575 and 1.150%), depending on the species, could disrupt the chloroplast membranes and affect gas exchange (HARE et al., 2002). From this perspective, Silva et al. (2018) stated that the application of high proline concentrations could cause effects different from those caused by the endogenous levels of this amino acid.

Although reducing the photosynthetic rates at some concentrations, the internal CO_2 concentration was responsive to proline in the absence of the algal extract (Table 4). Since mango shows a C3 respiratory metabolism, requiring constant CO_2 uptake by the leaf mesophyll through the action of Rubisco, as seen in the plants treated exclusively with proline, the results suggest that this amino acid, especially at lower concentrations (0.287%), stimulates stomatal opening and the subsequent increase in the internal CO_2 concentration, which was positively influenced in the absence of AE.

Moreover, as mentioned before, the proline concentration of 0.287% resulted in higher stomatal conductance compared to other concentrations, corroborating Alyemeni et al. (2014), who maintained a constant CO_2 supply by delivering ten mM of proline to chickpea, thus obtained increased stomatal conductance, photosynthesis, and plant efficiency.

In the present study, the Fv/Fm ratio was directly related to gas exchange. However, the values obtained with 0.287% proline can effectively favor the capture of light energy by the PSII (NAKHAIE et al., 2020). Threfore, although the qP was not affected (Table 4) to the point of distinguishing a concentration in which the linear dissipation of electrons occurs, inter-systems could be occurring without damage to the PSII.

On the other hand, the 0.575% and 1.150% concentrations significantly reduced the Fv/Fm ratio and the A, gs, and E values in the absence of AE, suggesting that the dissipation of excess energy in plants treated with these proline concentrations occurred through photon re-emission at longer wavelengths or in the form of heat (BUCHANAN et al., 2015). Therefore, gas exchange was lower at higher proline concentrations since energy was not sent to the qP but rather to the NPQ, showing a linear behavior with the use of proline alone (Figure 5b).

This study revealed that the PSII reaction centers were open in the same proportion (qP) regardless of the AE, without a significant difference. Energy dissipation for NAP$^+$ reduction resulted from the electron transport (extinction) caused by the photochemical process (MAXWELL; JOHNSON, 2000).

The non-photochemical inhibition coefficient (qN) showed a different behavior due to the interaction between factors (Pro x AE). The plants treated exclusively with proline (no algal extract) showed higher energy dissipation due to non-photochemical processes (qN), with increasing proline concentrations. The proline concentration of 1.150% resulted in a mean value of 2.1, or 26% higher than the control. Cao et al. (2018) described that high qN values favor the release of extra energy and strengthen the resistance of thylakoid membranes to injury, e.g., those caused by water restriction, also resulting in higher chlorophyll protection through thermal dissipation.

With regard to the phytotechnical variables, only flowering uniformity responded to the algal extract, which could be attributed to the strict relationship between algal extract hormones (auxins, gibberellins, cytokinins, and ethylene) and their effects on floral induction in mango (AVILAN; ALVAREZ, 1990). According to Mouco (2008), these hormones integrate environmental and intrinsic plant factors, e.g., photoassimilates, nutrients, and water availability by inhibiting or stimulating signs that influence flowering on a quantitative basis.

Rainfall accumulation during flowering is another significant element to be considered (Figure 1d) since it probably reduced the proline levels, favoring the more effective joint action of algal extract components (as mentioned before).

The greater flowering uniformity provided by the algal extract did not result in a significant yield increase, which could be attributed to the same growing treatments applied. We emphasize the need for water and nutritional management proportional to flowering. In addition, the responses of mango trees subjected to water deficit should be monitored even after flowering to consider further applications of stress-reducing molecules.

This study showed that the application of appropriate proline concentrations and algal extract based on *A. nodosum* promoted satisfactory responses by increasing the flowering of mango grown in the semi-arid region of Brazil.

Conclusion

The extract of marine algae (*Ascophyllum nodosum*) and proline improved the carotenoids and photosynthetic components during water restriction, resulting in higher carbohydrate accumulation and favoring shoot maturation. These physiological changes resulted in higher flowering yield and probably increase the tolerance of mango to the deleterious effects of water restriction in semi-arid regions.

Nevertheless, new studies that allow devising strategies capable of mitigating the effects of abiotic stress in mango trees are increasingly necessary, having as a central point proteomic, metabolomic, and genomic responses and aiming to elucidate physiological, biochemical, and molecular cascades, especially during the shoot maturation phase.

Acknowledgements

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