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The use of amino acids as bioactive substance: a strategy to increase yield in summer arugula (*Eruca sativa L. Mill*) crops

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Abstract

Foliar application of amino acids can assist crops to maintain homeostasis under diurnal temperature variations and excessive solar irradiance. The objectives of this study were to investigate the effects of the organic fraction and mineral nutrients of an amino acid solution on arugula plants and the effects of weekly applications of this solution on their growth and biomass yield. Two experiments were conducted in a completely randomized design, in a greenhouse (Experiment 1) and on open-field benches (Experiment 2), in Iracemápolis, SP, Brazil. Experiment 1 consisted of foliar applications of water (control), a solution containing 59 and 40.9 g ha^{-1} of nitrogen and sulfur, respectively (N+S), and a solution containing 85 g ha^{-1} of amino acids, with seven replications. Experiment 2 consisted of four different number (0, 1, 2, and 3) of weekly foliar applications of 85 g ha^{-1} of amino acids, with six replications. Chlorophyll *a* fluorescence, chlorophyll and carotenoid contents, and fresh and dry biomass weights were evaluated in both experiments. In Experiment 1, shoot fresh weight of plants treated with amino acids increased by 21% and 6% compared to those in the control and N+S treatment, respectively. Amino acid application increased chlorophyll and carotenoid contents, improving photosynthetic performance by increasing effective quantum efficiency of photosystem II and photochemical quenching. In Experiment 2, weekly application of 85 g ha^{-1} of amino acids increased shoot fresh weight by approximately 10% per application. Therefore, foliar application of amino acids can increase fresh biomass of arugula plants grown under both mild and warmer climate conditions.

Keywords: abiotic stress, foliar application, leaf vegetables, nutrients, photosynthetic pigments

Introduction

Leafy vegetables can be grown year-round; however, summer is the peak period for their consumption and highest prices (Hortifruti Brasil, 2023). Arugula (*Eruca sativa* L. Mill) crops currently account for 19% of the 200.000 ha of leafy vegetables cultivated in Brazil. The increased consumption of this vegetable is mainly due to its high contents of health-promoting phytochemicals, as sulfur metabolites (glucosinolates) (Di Gioia et al., 2018). This crop is sensitive to excessive solar radiation and high air temperatures, presenting a better growth under mild environmental conditions (Cabral, 2023).

The growth and yield of plants exposed to challenging environmental conditions are negatively affected due to impairment in homeostasis. Water or light stress induces reductions in leaf water potential and stomatal closure, downregulation of photosynthesis, photooxidation, increased concentrations of reactive oxygen species (ROS), and damage to membranes and proteins (Osakabe *et al*. 2014; Rivero *et al*. 2022). Additionally, diurnal and seasonal variations in air temperature and vapor pressure deficit have been identified as significant drivers of plant metabolism (Grossiord *et al*. 2020). These factors act as plant stressors, affecting plant growth by reducing carbon fixation and increasing leaf damage (Matthews *et al*. 2017). Plants have some strategies to respond to these stresses, as modulation of stomatal opening and membrane depolarization through synthesis of abscisic acid and accumulation of osmolytes, including amino acids (Osakabe *et al*. 2014; Yang et al., 2020); however, these processes consume energy, such as glucose, thus reducing plant growth.

Studies have focused on alternatives to mitigate or eliminate negative effects of stress in plants and the application of amino acids as bioactive substances has

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been extensively investigated (Ali et al., 2019), as they are vital metabolites utilized in the biosynthesis of both protein and non-protein compounds. Furthermore, some amino acids are involved in mitigating drought or heat stress by controlling stomatal conductance, membrane permeability and polarization, and protecting proteins from denaturation (Yang et al., 2020). Thus, the exogenous application of amino acids has increased concentrations of proline and other osmolytes, protecting photosynthetic pigments (Noroozlo *et al*. 2019; Alfosea-Simón *et al*. 2021), improving ROS scavenging, preventing electrolyte leakage and lipid peroxidation (Hammad & Ali 2014; Alfosea-Simón *et al*. 2021), and enhancing photosynthesis and water use efficiency (Peña Calzada *et al*. 2022).

A commonly used amino acid source in agriculture is industrial waste. An organic residue of the sugarcane fermentation process for glutamic acid production is a solution rich in free amino acids and chemical compounds such as nitrogen, sulfur, and other nutrients (Sato et al, 2001). Considering this mixture of organic and inorganic substances, should the effects of these amino acid sources be considered bioactive function or only nutritional? Studies attempting to answer this question are still limited. Geshnizjani & Khosh-Khui (2016) compared the application of amino acids and ammonium nitrate as source of N for gerbera plants and found that the mineral fertilizer did not improve growth as effectively as the amino acid solution.

In this context, the objective of the present study was to investigate whether the responses of arugula plants to the foliar application of an amino acid solution are due to its bioactive or nutritional effects and evaluate its potential as a strategy to increase crop yield under warm environmental conditions.

Materials and Methods

The study was conducted at the Innovation Center of the ICL company, in Iracemápolis, São Paulo, Brazil (22°38'38.8"S, 47°30'7.2"W, and altitude of 600 meters). Two experiments were carried out from January to March in a greenhouse (Experiment 1) and on an open-field benches (Experiment 2).

Arugula (*Eruca sativa*) seeds were germinated in 128-cell expanded polystyrene trays for both experiments. Twenty days after sowing, seedlings were transplanted into 8-liter pots filled with a sieved mixture of soil (Typic Hapludox), sand, and a commercial substrate for growing horticultural crops (1:1:1) (Basa Plant Hortaliças). Seedlings from two tray cells were transplanted into each pot. Some plants were thinned five days after transplanting, keeping the five most vigorous plants in each planting hole. The pots were watered daily using an automatic drip irrigation system to ensure no water deficit throughout the crop cycle. Soil fertilizers were applied following recommendations of Trani et al. (2014). A nutrient solution was applied before transplanting the arugula plants, at equivalent rates of 50 kg ha^{-1} of N, 180 kg ha^{-1} of P_2O_5, 80 kg ha^{-1} of K_2O, and 25 kg ha^{-1} of S, and complemented with micronutrients (B, Cu and Zn), followed by an application of a nutrient solution at equivalent rates of 70 kg ha^{-1} of N, 20 kg ha^{-1} of P_2O_5, and 35 kg ha^{-1} of K_2O, two weeks later.

Experiment 1 was designed to test the following hypothesis: i) the effects of foliar application of an amino acid solution as bioactive substances is due to its organic fraction, not its nutrient content, and ii) the foliar application of this bioactive substance improves plant metabolism and growth even when plants are grown without water deficit.

The potted seedlings were divided into three uniform groups and subjected to foliar applications 15 days after transplanting (DAT). The treatments used were: deionized water (control); solution containing 85 g ha^{-1} of free amino acids (AA treatment); and solution containing 59 g ha^{-1} of nitrogen (N) and 40.9 g ha^{-1} of sulfur (S), corresponding to 173.1 g ha^{-1} of ammonium sulfate and 51.4 g ha^{-1} of urea in deionized water (N+S treatment). The amino acid solution used was a byproduct of the bacterial fermentation process of a sugarcane industry; its recommended rate for field crops is 1.5 L ha^{-1}, which corresponds to 85 g ha^{-1} of free amino acids. Nutritional analysis of this amino acid solution showed 7.25% N and 5% S. The plants were kept under greenhouse conditions until harvest.

Experiment 2 was designed to test the hypothesis that weekly foliar applications of the amino acid solution benefits plant growth and it could be a strategy to improve yield of arugula plants grown under warm climate conditions. Potted seedlings were divided into four uniform groups and subjected to 0, 1, 2, or 3 weekly foliar applications of the same amino acid solution at the same rate described in Experiment 1 (85 g ha^{-1}). The seedlings were placed on benches under open-field conditions after the first treatment application until harvest.

All treatments were applied using a CO_2-pressurized backpack sprayer equipped with a spray nozzle set to a flow rate of 0.34 L min^{-1}, pressure of 0.3 MPa, and speed of 2 m s^{-1}. Weather conditions during the experimental periods were monitored using a Vantage Pro2 weather station (Davis Instruments, Hayward, USA).

Chlorophyll *a* fluorescence was assessed one week after the application of the treatments in both experiments. Measurements were taken on healthy, newly expanded leaves under steady-state conditions between 10:00 and 11:00 AM on a sunny day using a Junior PAM pulse-modulated fluorometer (Walz® GmbH, Effeltrich, Germany). The readings were obtained by applying an actinic light source with multi-flash saturating pulse, coupled to a PAR-clip, to determine photosynthetically active radiation. Fluorescence quenching data were used to calculate the effective quantum yield of photosystem II (Φ_{PSII}), maximum photochemical efficiency of *PSII* (Fv'/Fm'), and photochemical quenching based on the lake model (qL) (Kramer et al. 2004; Baker 2008). Two readings were taken per replication.

Pigment contents (chlorophyll and carotenoids) were determined for all treatments in both experiments three weeks after the first treatment application, following the method of Wellburn (1994), using dimethyl sulfoxide (DMSO) as extractant. Absorbances were measured using a UV-Vis spectrophotometer at 649, 665, and 480 nm, and the equations of Wellburn (1994) were used to calculate the contents of carotenoids, chlorophyll a, and chlorophyll b.

Subsequently, shoots and roots of harvested plants were separated and dried at 65 °C for three days to determine their dry weights.

The experiments were conducted in a completely randomized design, with seven replications in Experiment 1 and six replications in Experiment 2. Data were subjected to analysis of variance (ANOVA) using Minitab®, LLC (2020), and significant differences between means were determined using the Fisher's test at a 5% significance level. The experimental data were evaluated for homoscedasticity (Levene and Bonferroni test) and normality (Kolmogorov-Smirnoff test).

Results and Discussion

Experiment 1

The objective of this experiment was to isolate the effects of the organic components from those of the nutritional components in the amino acid solution used. The temperature Inside the greenhouse increased in the first few days after transplanting the seedlings into the pots, reaching more than 40 °C, with radiation intensity of 700 W m^{-2} (approximate PAR of 1400 μmol m^{-2} s^{-1}) (**Figure 1**). Afterwards, the average and maximum temperatures were approximately 25 and below 30 °C, respectively, until harvest. The air temperature range after transplanting was similar to that reported by Costa *et al*. (2011) for a good plant yield and quality. However, optimal temperatures for arugula growth are between 15 and 18 °C, according to Trani et al. (1992).

Plants treated with foliar application of a solution containing amino acids (AAs treatment) presented 14.5% more leaves and 21.1% more shoot fresh weight than those in the control (without any treatment) at harvest, 17 days after application of the treatments (**Figure 2**A and 2B). Total dry weight (shoots + roots) was 10% higher in plants in the AAs treatment compared to those in the control, but with no significant difference among treatments (Figure 2C). Dry weight percentage (%DW) was significantly lower in the AAs treatment than in the control (Figure 2D). Thus, plants subjected to the AAs treatment presented higher hydration level than those in the control or in the treatment with foliar application of mineral fertilizer containing nitrogen (N) and sulfur (S) (N+S treatment). This effect is particularly important for arugula plants, whose marketable part is fresh leaves. The N+S treatment, applied with the same rate of mineral nutrients (N and S) as the amino acid solution used, had lower positive effects on plant growth. Similar results were reported by Geshnizjani & Khosh-Khui (2016), who evaluated gerbera plants grown under no stress conditions with foliar application of a mixture of 19 essential amino acids or ammonium nitrate. These results confirm that the positive effects are due to the organic fraction rather than the mineral nutrients available in amino acid solutions.

The applied treatments significantly affected chlorophyll and carotenoid contents in leaves at harvest. Plants in the AAs treatment had total chlorophyll contents (16.1 μg cm^{-2}) and carotenoid (4.6 μg cm^{-2}) contents 60% higher than control plants (10.1 and 2.8 μg cm^{-2}, respectively) (**Table 1**). Plants in the N+S treatment presented a slightly higher total chlorophyll (11.5 μg cm^{-2}) and carotenoid (3.5 μg cm^{-2}) contents compared to the control plants, respectively. This reinforces that the growth-promoting effect of amino acid solutions is connected to the organic fraction and not to the mineral compounds in their compositions.

The applied treatments significatively affected some chlorophyll *a* fluorescence parameters. Plants treated with AAs showed higher photochemical quenching *(qL)* and effective quantum yield of photosystem II (Φ_{PSII}) (**Figure 3**A and 3B), presenting a *qL* 5.8% higher than control plants, indicating an improved state of PSII reaction centers (increased fraction of oxidized Q_A), accordingly to the lake model (Kramer et al. 2004). This denotes a greater capacity to absorb energy after foliar application of AAs compared to plants int the control and N+S treatment. The Φ_{PSII} parameter

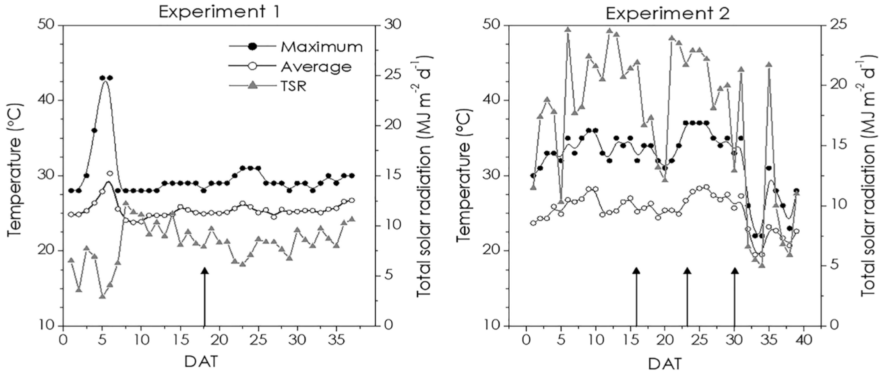

Figure 1: Variations in air temperature (maximum and average) and total daily solar radiation (MJ m^{-2} day^{-1}) from the transplanting to harvest of arugula seedlings during Experiments 1 and 2 (DAT: days after transplanting). ICL Innovation Center, Iracemápolis, SP, Brazil.

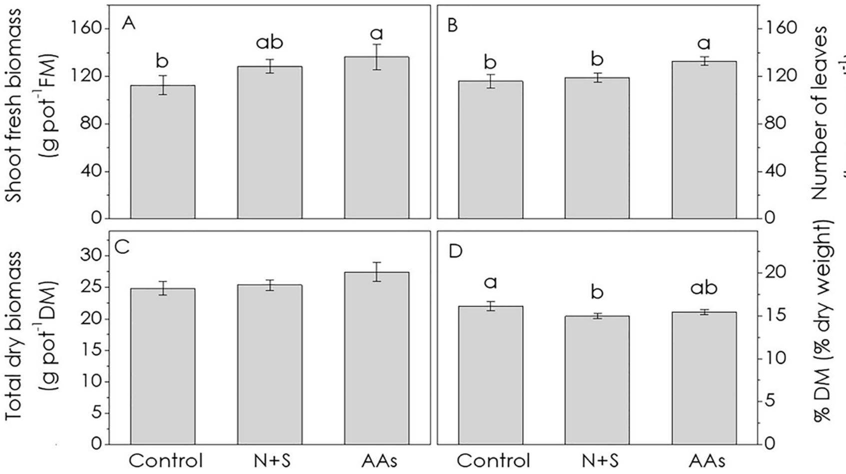

Figure 2: Shoot fresh weight (A), number of leaves per pot (B), total dry weight (shoots + roots) (C), dry weight percentage (%DW) (D) at harvest of arugula (*Eruca sativa*) plants subjected to foliar application of an amino acid solution (AAs) or mineral fertilizer with nitrogen and sulfur (N+S) in Experiment 1. ICL Innovation Center, Iracemápolis, SP, Brazil. Bars (mean of seven replications) with the same letter are not significantly different from each other by the Fisher's test at a 5% significance level. Vertical lines represent the standard deviation.

Table 1: Chlorophyll *a* (Chl *a*) (µg cm^{-2}), Chlorophyll *b* (Chl *b*) (µg cm^{-2}) and total chlorophyll (Total Chlor) contents, chlorophyll *a* to chlorophyll *b* ratio (Chl a: Chl b), and carotenoid contents at harvest in arugula (*Eruca sativa*) plants subjected to foliar application of an amino acid solution (AAs) and mineral fertilizer (N+S) in Experiment 1 (E1) and to different number (0, 1, 2, and 3) of weekly foliar application of AAs in Experiment 2 (E2). ICL Innovation Center, Iracemápolis, SP, Brazil

	Chl *a*		Chl *b*		Total Chl		Chl a: Chl b		Carotenoids	
	------ µg cm^{-2} ------								-- µg cm^{-2} --	
Treatment (E1)										
Control	7.57	b	2.57	b	10.13	b	3.37		2.80	c
N+S	8.78	b	2.68	b	11.46	b	3.27		3.43	b
AAs	12.61	a	3.53	a	16.15	a	3.57		4.57	a
Mean	9.65		2.93		12.58		3.40		3.60	
CV%	19.32		23.23		18.80		7.43		10.69	
F-value	12.05*		4.27*		10.87*		2.19		30.02*	
No. of weekly AAs application (E2)										
0	13.96		3.72		17.67		3.79	b	2.43	a
1	12.70		3.47		16.17		3.69	b	2.02	b
2	13.70		3.28		16.97		4.35	a	2.50	a
3	12.82		2.94		15.76		4.42	a	2.46	a
Mean	13.29		3.35		16.64		4.06		2.35	
CV%	14.54		22.3		15.88		9.53		10.11	
F-value	0.63		1.14		0.62		4.38*		4.93*	

CV = coefficient of variation. *Statistical difference at a 5% significance level. Means followed by the same lowercase letter in the columns are not significantly different from each other by the Fisher's test (p > 0.05).

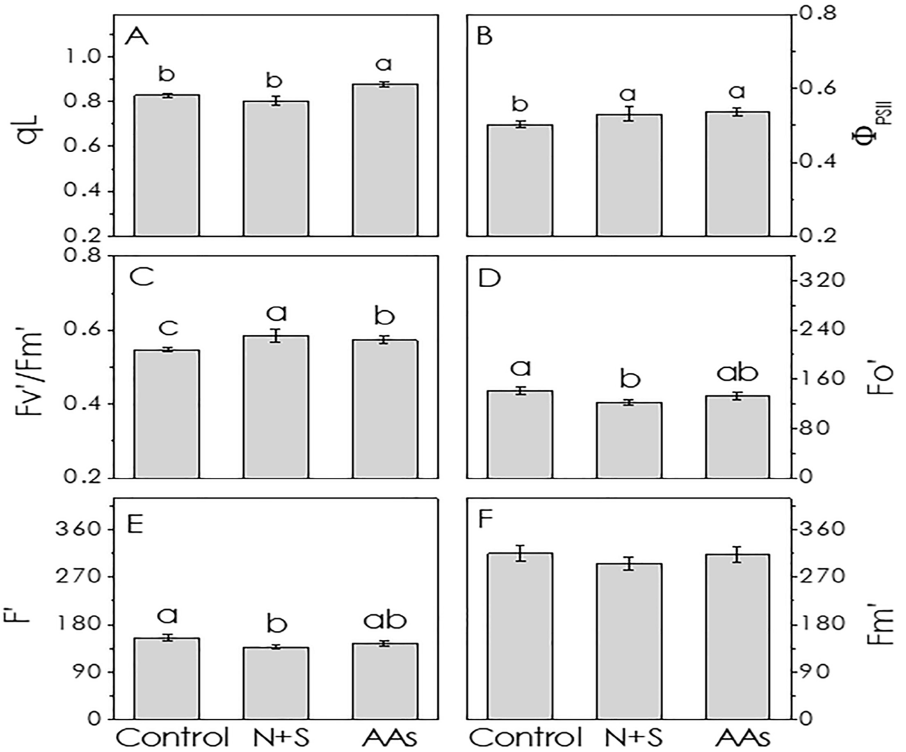

Figure 3: Photochemical quenching based on the lake model (qL) (A), effective quantum yield of PSII (Φ_{PSII}) (B), maximum photochemical efficiency of PSII (F'/Fm') (C), light-adapted minimum fluorescence (F_o') (D), fluorescence emission from leaves adapted to actinic light (F') (E), and maximum fluorescence of light-adapted leaves (Fm') (F) in arugula (*Eruca sativa*) plants subjected to foliar application of an amino acid solution (AAs) or mineral fertilizer (N+S) in Experiment 1 six days after treatment application. ICL Innovation Center, Iracemápolis, SP, Brazil. Bars (mean of seven replications) with the same letter are not significantly different from each other by the Fisher's test at a 5% significance level. Vertical lines represent the standard deviation.

increased by 6.5% compared to that in the control plants; this parameter is related to oxygen evolution and linear transport flow. In the absence of photorespiration, Φ_{PSII} is linearly correlated with CO_2 assimilation (Baker 2008). The higher qL and Φ_{PSII} found for plants treated with AAs were due to a lower light-adapted minimum fluorescence (F_o') and steady-state fluorescence yield (F') (Figure 3D and 3E). Increases in F_o' are connected to the inactivation of reaction centers and increased rate of thermal energy dissipation (Hong *et al.* 1999). Additionally, this could be attributed to net loss or transient inactivation of D1 protein or reduction in plastoquinone pool, leading to increased F_o' (Hong et al. 1999). The higher F_o' and F' values found for control plants may be connected to the reaction center inactivation caused by daily light excess and high air temperatures. The higher carotenoid contents in AAs-treated plants may have protected the D1 protein in the reaction centers, allowing for the maintenance of qL and Φ_{PSII} under daily stressful environmental conditions (Baker 2008).

Experiment 2

Considering that the organic fraction of the amino acid solution (AAs) used was responsible for the improvement in growth and yield of arugula plants, this second experiment was conducted to test the additional benefits of one to three weekly foliar applications of this solution to the growth of arugula plants. Potted arugula seedlings were placed on open-field benches to assess the effects of challenging environmental conditions (Figure 1). Total solar radiation was twice as high (20-25 MJ m^{-2} d^{-1}) as that under greenhouse conditions (10 MJ m^{-2} d^{-1}), reaching radiation intensity of 1000 to 1200 W m^{-2} (PAR of 2000 to 2400 μmol m^{-2} s^{-1}), with maximum air temperatures ranging from 30 to 36 °C throughout the crop cycle. The field environmental conditions were far from ideal for satisfactory growth and quality of arugula plants (Trani et al., 1992). The shoot dry and fresh weights at harvest under these more challenging environmental conditions were approximately 32% lower than those in Experiment 1, which was conducted under near-optimal environmental conditions. Shoot biomass production increased linearly as the number of weekly foliar applications of AAs increased from one to three. Plants treated with AAs once a week had a 9% higher shoot dry weight than untreated plants, whereas those subjected to three weekly foliar applications showed a 45% higher mean (**Figure 4**). Plants grown under high-stress conditions and subjected to three weekly foliar applications of the amino acid solution (Experiment 2) produced as much fresh biomass as control plants grown under mild environmental conditions (Experiment 1). This denotes that applying an amino acid solution can mitigate yield losses in horticultural crops grown during summer and under high-temperature environmental conditions.

The total chlorophyll contents did not increase as the number of weekly foliar applications of AAs was increased; however, a significant increase in chlorophyll *a* to *b* ratio was found when AAs was applied two or three times a week due to a decrease in chlorophyll *b* contents (Table 1). Chlorophyll *a* fluorescence parameters were not affected by weekly applications of amino acids (**Table 2**). Fv'/Fm', $\Phi_{PSII,}$ and qL were 12% to 26% lower than those in Experiment 1 due to higher air temperatures and light intensity during Experiment 2.

Improvements in crop performance due to the application of amino acid solution.

The foliar application of the amino acid solution showed potential to mitigate environmental stress and improve the production of arugula plants under warm environmental conditions. Increases in fresh biomass production were found in both experiments when the amino acid solution was applied. Several studies have shown that foliar application of amino acids promote growth and biomass production in plants under stress or not. Increases of approximately 40% in biomass or marketable plant parts were found for tomato (Alfosea-Simón et al. 2021) and lettuce (Noroozlo et al. 2019) plants grown under well-watered conditions. Wheat plants grown under drought stress or field conditions showed increases of 34% to 60% in biomass depending on the water deficit level (Hammad & Ali 2014). The plant growth promotion was attributed to effects of the foliar application of amino acids on chlorophyll protection from photooxidation, which increased stomatal opening, improving photosynthesis. Foliar application

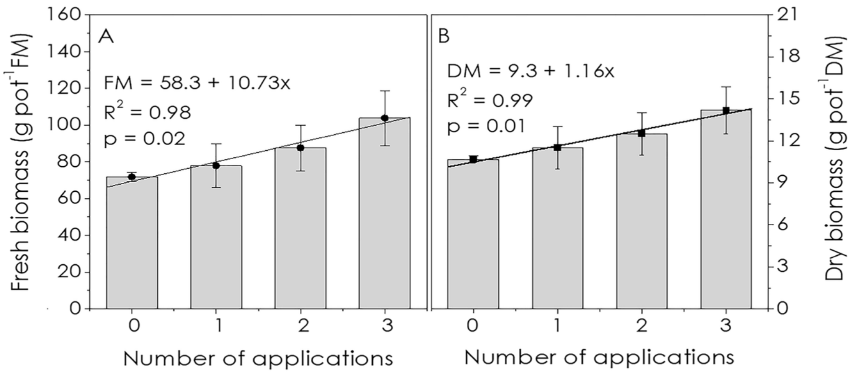

Figure 4: Shoot fresh weight (A) and shoot dry weight (B) at the harvest of arugula (*Eruca sativa*) plants subjected to different number (0, 1, 2, and 3) of weekly foliar applications of an amino acid solution during the crop cycle in Experiment 2. ICL Innovation Center, Iracemápolis, SP, Brazil. Bars represent the mean of seven replications; vertical lines represent the standard deviation.

Table 2: Air temperature (°C), photosynthetic active radiation (PAR), maximum photochemical efficiency of PSII (Fv'/Fm'), effective quantum yield of PSII (Φ_{PSII}), and photochemical quenching (qL) in arugula (*Eruca sativa*) plants subjected to foliar applications of an amino acid solution (AAs) and mineral fertilizer (N+S) in Experiment 1 and to different number (0, 1, 2, and 3) of weekly foliar application of AAs (0, 1, 2, and 3 applications) in Experiment 2. ICL Innovation Center, Iracemápolis, SP, Brazil

	Experiment 1				Experiment 2				F-value
Variable	Mean	±	SE	CV%	Mean	±	SE	CV%	
Temperature (°C)	30.9	±	0.166	3.5	36.9	±	0.278	5.5	302.5*
PAR	888	±	18.6	13,6	1407	±	78.4	41.0	32.1*
Fv'/Fm'	0.5728	±	0.0061	6,8	0.4677	±	0.0064	10.0	138.6*
Φ_{PSII}	0.5268	±	0.0070	8,5	0.3916	±	0.0072	13.5	174.1*
qL	0.8408	±	0.0099	7,5	0.7391	±	0.0157	15.6	24.9*

SD = standard error. CV = coefficient of variation. *Statistical difference at a 5% significance level between experiments.

of amino acids to cabbage plants resulted in increased fresh biomass, number of leaves, and contents of phenolics, anthocyanins, flavonoids, and glucosinolates, consequently enhancing the commercial quality of the product (Haghighi et al., 2022).

Chlorophyll and carotenoid contents in leaves were positively affected by foliar application of amino acids. Amino acids act as osmolytes that can regulate the intracellular pH and increase the concentration of proline, a powerful osmotic regulator (Noroozlo et al. 2019; Peña Calzada et al., 2022). This helps to prevent cell membrane degradation and maintains chlorophyll contents, as found for plants grown under both stress (Ali et al., 2019) and non-stress conditions (Haghighi et al., 2022). Furthermore, glutamic acid is a biosynthetic precursor of glutathione and arginine, which are two amino acids involved in ROS scavenging (Alfosea-Simón et al. 2021). More than 65% of the amino acids in the solution tested in the present study were glutamic acid, which could explain the significant positive effect on chlorophyll protection.

Carotenoids are non-enzymatic antioxidants that are oxidized in the presence of ROS, thus preventing photodamage to the D1-D2 protein in PSII. Moreover, they participate in xanthophyll cycles, dissipating excess energy as heat (Demmig-Adams et al. 2013). In both situations, they prevent chlorophyll degradation. Furthermore, changes in chlorophyll *a* to *b* ratio were found (Table 1), which may be connected to structural alterations in the light-harvesting complex and number of reaction centers, as well as to the PSI to PSII ratio. Considering that chlorophyll *a* is more susceptible to degradation, reductions in its concentration may be attributed to increased degradation. Additionally, an increased chlorophyll *b* content improves light absorption under lower light intensity (Beneragama & Goto 2011), explaining the lower chlorophyll *a* to *b* ratio found for greenhouse-grown plants (Experiment 1).

The arugula plants grown in open field were exposed to double the total solar radiation than those in the greenhouse covered with plastic, and to higher amounts of UV-A and UV-B radiation. Plants under these conditions had 38% more chlorophyll contents and 35% lower carotenoid contents. Increases in chlorophyll contents may be connected to reductions in leaf expansion, as it is expressed as concentration per leaf area. Higher light intensity causes decreases in specific leaf area. Moreover, irradiance was 700 W m^{-2} or higher (up to approximately 1400 µmol m^{-2} s^{-1}) on several days during the crop growth cycle (Figure 1). Approximately 1000 µmol m^{-2} s^{-1} of PAR are sufficient to saturate the photon acceptor system in leafy vegetables. Plants under higher irradiance conditions may benefit from shading or other mechanisms to dissipate excess excitation energy (Cometti et al., 2020).

Chlorophyll and carotenoids are essential for capturing energy from solar radiation, therefore, the connection between these pigments and chlorophyll fluorescence and photosynthesis is clear. Environmental conditions were less challenging in Experiment 1, making this connection evident (Tables 1 and 2; Figure 4). Similarly, the application of amino acid solution to tomato and *Crataegus* sp. plants resulted in higher chlorophyll contents and subsequent increases of 5% to 7% in Fv'/Fm' and 30% increase in CO_2 assimilation (Alfosea-Simón et al. 2021). However, the environmental conditions were extremely challenging in Experiment 2 and, consequently, foliar application of the amino acid solution did not mitigate the negative effects of high air temperatures and excess solar radiation on chlorophyll *a* fluorescence (Table 2); therefore, the growth-promoting effect of amino acids was not connected to the PSII function. Therefore, foliar spraying of an amino acid solution could improve plant metabolism by saving glucose for structure repair or incorporating amino acids into plant tissues. This storage of precursors for protein synthesis prepares the plant metabolism for a rapid recovery after stress (Batista-Silva et al., 2019).

Although arugula plants were not subjected to water deficit, they experienced thermal stress (temperatures above 25 °C) and excess light at midday. These stress conditions cause stomatal closure, increased leaf temperature and cell membrane permeability, and reduced biochemical activity (Moore et al., 2021; Peña Calzada et al., 2022), leading to photoinhibition and reduced photosynthesis. Therefore, the application of an amino acid solution promoted protection of cellular components from degradation, improving photosynthesis and plant growth under these stressful conditions.

Conclusions

The results found for all variables analyzed in this study indicate that the effects of foliar application of an amino acid solution on plant metabolism cannot be explained solely attributed to the mineral content in the solution; instead, these effects may be connected to its organic fraction. Moreover, weekly foliar application of the amino acid solution improved the growth of arugula plants grown under warm environmental conditions. Thus, the use of this type of amino acid solution can contribute to improvements in growth and yield of arugula crops grown under adverse environmental conditions. The plant response was primarily connected to the organic fraction of the applied amino acid solution. The results showed the beneficial effects of weekly foliar application of 85 g ha^{-1} of free amino acid solution on arugula plants. Therefore, comparing free amino acid rates of products is essential to take advantage of the benefits of this technology and assist in decision-making for crop management.

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