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DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v28n12e277584 ORIGINAL ARTICLE

Biomass and spilanthol content of hydroponic jambu as a function of nitrogen supply[1]

Biomassa e teor de espilantol de jambu hidropônico em função do fornecimento de nitrogênio

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HIGHLIGHTS:

Nutritional disorder due to low nitrogen supply does not stimulate spilanthol biosynthesis in jambu.
Spilanthol biosynthesis in jambu is stimulated by nitrogen supply.
Higher concentrations of spilanthol were identified in the inflorescences, followed by the shoot (leaf and stem) and root.

ABSTRACT: Jambu (*Acmella oleracea*) is a typical culinary vegetable from Northern Brazil that has gained increased demand and recognition due to its sensory and bioactive properties attributed to the presence of spilanthol. Strategies for plant management have been explored; however, the influence of N supplementation on spilanthol biosynthesis in jambu in a hydroponic system is unknown. Thus, this study aimed to evaluate the effect of N on biomass production, photosynthetic pigments, leaf nitrogen concentration, and spilanthol content in jambu cultivated hydroponically. The plants were subjected to six concentrations of N in the nutrient solution (11, 13, 15, 17, 19, and 21 mmol L^{-1}), using a completely randomized design with four replications, each consisting of one plant. Fresh and dry biomass of inflorescence, shoot (leaves and stems), and root, N and pigment content in the leaves, and spilanthol content in different plant organs were evaluated. The N concentrations positively affected biomass production, photosynthetic pigments, leaf N concentration, and spilanthol content in jambu inflorescence, shoot, and root. The supply of 21 mmol L^{-1} N in the nutrient solution resulted in a higher concentration of N in the leaves, leading to increased production of photosynthetic pigments, shoot biomass, and inflorescence. Conversely, a moderate supply of 17 mmol L^{-1} N resulted in a higher synthesis of spilanthol in the organs of the jambu plant. Therefore, appropriate nitrogen supplies should be considered an indispensable tool for the nutritional management of jambu cultivated in hydroponic systems.

Key words: *Acmella oleracea*, cultivation without soil, secondary metabolite, nutrition

RESUMO: Jambu (*Acmella oleracea*) é uma hortaliça condimentar típica do Norte do Brasil que tem adquirido maior procura e notoriedade devido as suas propriedades sensoriais e bioativas, atribuídas à presença do composto espilantol. Estratégias para o manejo da planta têm sido buscadas, entretanto, a influência da suplementação com N sobre a biossíntese de espilantol no jambu em sistema hidropônico é desconhecida. O objetivo deste estudo foi avaliar o efeito do N na produção de biomassa, de pigmentos fotossintéticos, na concentração de N nas folhas e no teor de espilantol em jambu cultivado hidroponicamente. Estas plantas foram submetidas a seis concentrações de N na solução nutritiva (11, 13, 15, 17, 19 e 21 mmol L^{-1}), utilizando delineamento inteiramente casualizado. Avaliaram-se a produção de biomassa fresca e seca das inflorescências, parte aérea (folhas e caules) e raiz, teor de N e pigmentos nas folhas e teor de espilantol nos diferentes órgãos da planta. As concentrações de N afetaram positivamente a produção de biomassa, de pigmentos fotossintéticos, a concentração de N nas folhas e o teor de espilantol nas inflorescências, parte aérea e raiz do jambu. O suprimento de 21 mmol L^{-1} N na solução nutritiva resultou em maior concentração de N nas folhas, promovendo maior produção de pigmentos fotossintéticos, biomassa da parte aérea e inflorescência. Suprimento médio de 17 mmol L^{-1} N resultou em maior síntese de espilantol nos órgãos do jambu. Portanto, suprimentos adequados de N devem ser considerados indispensáveis para o manejo nutricional do jambu cultivado em sistema hidropônico.

Palavras-chave: *Acmella oleracea*, cultivo sem solo, metabólitos secundários, nutrição

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Introduction

Jambu (*Acmella oleracea* (L.) R. K. Jansen) is a culinary vegetable that holds significant importance in the cuisine and culture of the Brazilian Amazon region (Sampaio et al., 2021). Spilanthol is the major compound in the species, responsible for the momentary anesthetic sensation caused by consuming its vegetative parts as inflorescences, leaves, and stems (Barbosa et al., 2016; Balieiro et al., 2020; Rondanelli et al., 2020).

The cultivation of jambu primarily occurs in soil with low technological input. The limited technological support in cultivation, combined with the incidence of soil-borne diseases such as jambu smut (*Thecaphora spilanthes* Frei. & Van.) and jambu rust (*Puccinia* sp.), contribute to significant losses and/or variations in plant production and quality (Sampaio et al., 2021). In this context, hydroponic systems may provide a viable alternative for cultivating this species, as it is an environmentally friendly and modern technology with significant capacity for managing biotic and abiotic factors (Sambo et al., 2019; Nascimento et al., 2020). Balancing and supplying nutrients adequately in the nutrient solution are fundamental factors for increasing the yield and quality of crops grown in hydroponics (Sambo et al., 2019). Variations in the nutrient solution composition have been effective in increasing biomass production and bioactive compound content in basil and perilla (Argyropoulou et al., 2015; Lu et al., 2017).

Nitrogen (N) is the most abundant nutrient in plants, and its supply promotes an increase in metabolic activity by influencing the biosynthesis of biomolecules such as proteins, amino acids, chlorophylls, nucleic acids, and secondary metabolites. It is one of the main factors regulating plant growth (Ueda et al., 2017; Saloner & Bernstein, 2020). In this regard, this study aimed to evaluate the effect of N on biomass production, photosynthetic pigments, leaf N concentration, and spilanthol content in jambu grown hydroponically.

Material and Methods

The experiment was conducted in a greenhouse located at the Universidade Federal Rural da Amazônia, Belém, Pará, Brazil (1° 27' 11.08" S, 48° 26' 34.21" W, and an altitude of 9 m), between September and December 2018. The climate of the region is Af-type according to the Köppen classification (Alvares et al., 2013). During the experimental period, the air temperature and relative air humidity were monitored daily using a digital thermo-hygrometer (Plastic HTC Instruments 288-ATH, Taiwan, China) installed in the greenhouse at a height of 2 m above the ground. The average maximum and minimum air temperatures were 35.8 and 28.1 °C, and relative air humidity was 83.9 and 43.1%, respectively.

The experimental design used was completely randomized with four replications. The experimental unit consisted of one jambu plant. The treatments comprised six nitrogen concentrations in a hydroponic nutrient solution (11, 13, 15, 17, 19, and 21 mmol L^{-1}).

The variety of jambu used in the study was the most common: the yellow-flowered variety. The sowing was conducted in polystyrene trays with 128 cells filled with a coconut powder-based substrate (Golden Mix Misto, AMAFIBRA, São Paulo, Brazil). Three days after emergence, the trays were transferred to wooden benches. The trays were irrigated every two days with a 25% strength Hoagland nutrient solution (Hoagland & Arnon, 1950). Seven days after sowing, thinning was performed, leaving one seedling per cell. Transplanting of the seedlings occurred 21 days after sowing.

The plants were cultivated using a semi-hydroponic system. Sterilized ground silica was used as the substrate, with pots having a capacity of 2 L. The pots were perforated near their base, and a rubber tubular mechanism was attached to control the drainage of the solution. Additionally, the pots were covered with aluminum foil to prevent heating of the substrate and nutrient solution. Furthermore, the nutrient solution collectors were coated with aluminum metallic paint and adapted through a support system at the neck of the jars to facilitate solution drainage. After transplanting, the jambu plants underwent a seven-day acclimatization period in the hydroponic system, receiving a 50% strength Hoagland nutrient solution (Hoagland & Arnon, 1950). After this period, they were subjected to their respective treatments.

During the experiment, the nutrient solutions were oxygenated by draining the solution in the late afternoon and replenishing it in the early morning daily. The solutions were renewed weekly, and the water lost through evapotranspiration was replaced to maintain a daily volume of 0.4 L per pot. The pH of the solutions was monitored daily using a pH meter GroLine HI98118 (HANNA Instruments Inc., Woonsocket, Rhode Island, USA) and kept within the range between 5.5 and 6.5 by using 1 M NaOH or 1 M citric acid when necessary.

The plants were harvested at 56 days after transplanting. The plants were separated into root, shoot (stem and leaves), and inflorescence for analysis of the following characteristics: root fresh mass (RFM, in g per plant), shoot fresh mass (SFM, in g per plant), and inflorescence fresh mass (IFM, in g per plant). For dry mass determination, fresh material was placed in paper bags and dried at 65 °C for 72 hours in a ventilated oven until a constant mass was reached. Root dry mass (RDM, in g per plant), shoot dry mass (SDM, in g per plant), and inflorescence dry mass (IDM, in g per plant) were obtained. The masses were determined using a semi-analytical balance AD3300 (Marte Científica, São Paulo, Brazil).

The shoot of jambu was freeze-dried for 24 hours in a lyophilizer L101 Liotop (LIOBRAS, São Carlos, São Paulo, Brazil). Posteriorly, the material was crushed with a Willye-type knife mill (STAR FT-50-Fortinox) and sieved through a 10 mm mesh to prepare the crude extract. Two hundred and twenty-five g of SDM were used to produce a shoot crude extract in a glass flask with a screw cap using a 1:9 v/v ratio with 95% ethanol at 55 °C for 1 hour in a water bath. After extraction, the supernatant was separated by filtration through an 80 µm mesh and concentrated using a rotary evaporator in a CentriVap acid-resistant concentrator system (Labconco Corporation, Kansas, MO, USA) under vacuum to a concentration of 183.26 mg SMD mL^{-1} of ethanol.

An extraction was performed considering the relative influence of Na^+ and Cl^- ions on chlorophyll stability

(Lichtenthaler & Buschamann, 2001). In the first step, 15.4 mL of concentrated crude extract (in 9.2% ethanol) was added to a 0.16 mol L^{-1} NaCl solution (91:9 v/v), followed by homogenization (30 s at 2800 rpm) using an agitator (Basic K40-2810-Kasvi) and centrifugation (CT15RE-Eppendorf) at 14,000 rpm for 20 min at 25 °C. The supernatant was collected, and 14 mL of ethanol was added to the pellet, followed by homogenization (30 s at 2800 rpm), addition of 1.1 mL of distilled water, and a 0.16 M NaCl solution (1:9 v/v). This mixture was centrifuged under the same conditions as the previous step, and the supernatant was collected—finally, the same procedure as the second step was applied to the pellet again.

Four grams of Amberlite XAD 02 resin (Supelco) were thoroughly washed with ultrapure water, dried at 70 °C for 24 hours in a circulating air oven, and cooled in a desiccator for 2 hours. After this period, 3.4 g of resin was weighed and added to absolute ethanol in a ratio of 2:17 m/v and left to stand for 12 hours (Silva et al., 2007). Subsequently, two washes with XAD 02 were performed in a ratio of 2:17 m/v using ultrapure water at 40 °C under agitation in a shaker incubator for 1 hour. After each wash, all liquid was removed by vacuum filtration using a qualitative filter paper with a Büchner funnel.

The combined supernatant from the chlorophyll removal steps was adsorbed onto XAD 02 resin (2:17 m/v) and agitated on a shaker at 25 °C and 183 rpm for 1 hour. Then, two washes with ultrapure water (2:17 v/v) and three washes with 30% ethanol (2:17 m/v) were conducted to remove impurities (30 min, 25 °C, and 183 rpm). After each wash, all liquid was removed by vacuum filtration using a qualitative filter paper with a Büchner funnel. Finally, three desorptions with 96% ethanol were conducted (2:17 v/v, 25 °C for 1 hour at 183 rpm) to recover the purified spilanthol fraction. The ethanoic fractions containing the compound of interest were combined and stored at -22 °C until spilanthol was isolated in preparative chromatography.

The unified less polar fraction from the purification process was concentrated in a rotary evaporator under vacuum at 60 °C for 4 hours to an extract concentration of 77.85 mg mL^{-1}. This extract was diluted twice with water and injected into a preparative PLC (preparative liquid chromatography) 2020 chromatograph (Gilson) at a flow rate of 3 mL min^{-1} for 16 min, with an injection volume of 1 mL and isocratic elution using a mobile phase of 60:40 ethanol:water (v/v) on a Gemini 5 µm C18 250 × 100 mm column (Phenomenex). All collected injections (between 12.5 and 15.3 min of elution) were combined, concentrated in a rotary evaporator under vacuum at 60 °C, and lyophilized (L101 Liotop Lyophilizer, Liobras, São Carlos, SP, Brazil) to determine the amount of dry mass obtained from isolated spilanthol.

The identification of spilanthol in the chlorophyll removal fractions was performed using a Thermo Scientific Ultimate 3000 UHPLC system equipped with a UV-VIS detector with a scanning range between 200 - 400 nm, with 229 nm being the compound's maximum absorption wavelength. The extracts were filtered using a 0.22 µm syringe filter (PVDF), and then 5 µL were injected into a Kinetex EVO C18 100 Å, 1.7 µm 100 × 2.1 mm column (Phenomenex) at 25 ºC. The mobile phase consisted of ultrapure water (Solution A) and acetonitrile (Solution B), both filtered through a 0.22 µm membrane (Nylon). Elution was performed in isocratic mode, with a constant 30% Solution B for 30 min at 0.3 mL min^{-1} (Bae et al., 2010).

Thirty fractions were collected in the interval between 12.5 and 15.35 min elution. The fractions collected after isolation in PLC were pooled, concentrated under vacuum, and lyophilized, obtaining 49.28 mg of isolated compound. By analyzing the diode array detection (DAD) absorption spectrum in the UHPLC chromatograph, it was observed the presence of a chromatographic peak with maximum absorption at 229 nm referring to spilanthol. The chromatographic purity of the isolated spilanthol was observed by analyzing the normalized areas and subtracting peaks present in the analytical blank, which resulted in a compound of 95.1% chromatographic purity. The DAD absorption spectrum was also compared with the literature (Bae et al., 2010), showing the absorption at 229 nm characteristic of spilanthol.

A 5 mg lyophilized mass of the isolated compound after PLC was solubilized in 5 mL of 80% ethanol in a volumetric flask. The chromatographic purity of the isolated spilanthol was determined from the analysis of normalized areas and subtraction of peaks present in the analytical blank and by the DAD absorption spectrum. After solubilization of the isolated compound and injection into an Ultimate 3000 UHPLC, an analytical calibration curve with six concentration points in triplicate was constructed from dilutions of the stock solution (Bae et al., 2010), obtaining LD and LQ values of 1.22 and 3.69 µg mL^{-1}, respectively and $R^2 = 0.996$.

Jambu plants were washed under running water and then with distilled water. They were then separated into shoot (leaves and stem), root, and inflorescence, then frozen and freeze-dried. After freeze-drying, the material was crushed in a Willye-type knife mill equipped with a 10-mesh sieve opening. Each extraction was performed in a 10 mL glass flask with 95% ethanol (1:9, m/m) at 55 °C for 1 hour, closed with a screw cap (Bae et al., 2010). The supernatant post centrifugation (4,500 rpm, 15 min at 4 °C) was removed and subjected to chlorophyll removal at a ratio of 1:9 v/v with 0.16 M NaCl solution under stirring for 30 s and centrifugation (14,000 rpm, 20 min at 25 °C) followed by two repetitions of the process. The resulting supernatants were pooled, saturated with N_2 and stored at -22° C until spilanthol quantification by UHPLC (Bae et al., 2010).

Fresh leaves from the second pair of leaves from the apex of the main stem were harvested from the plants. Subsequently, 0.1 g of the leaves was crushed in a mortar with 5 mL of 80% acetone and 0.05 g of $CaCO_3$ while protected from sunlight. The obtained extracts were filtered through qualitative filter paper into 25 mL volumetric flasks and made up to volume with 80% acetone (Lichtenthaler, 1987). An aliquot of the extracts was analyzed at wavelengths of 470, 646, and 663 nm, and the absorbance was recorded to quantify carotenoids (Car), chlorophyll a (Chl a), and chlorophyll b (Chl b), employing equations 1, 2, and 3, respectively. The results for each pigment were expressed on a fresh weight basis (mg g^{-1} FW).

The dried leaf material was crushed, samples of jambu were ground using a Willye-type mill, sieved through a 20-mesh

screen, and stored in paper bags. The nitrogen (N) content was determined using the Kjeldahl method with a conversion factor of 6.25 (Silva, 2009).

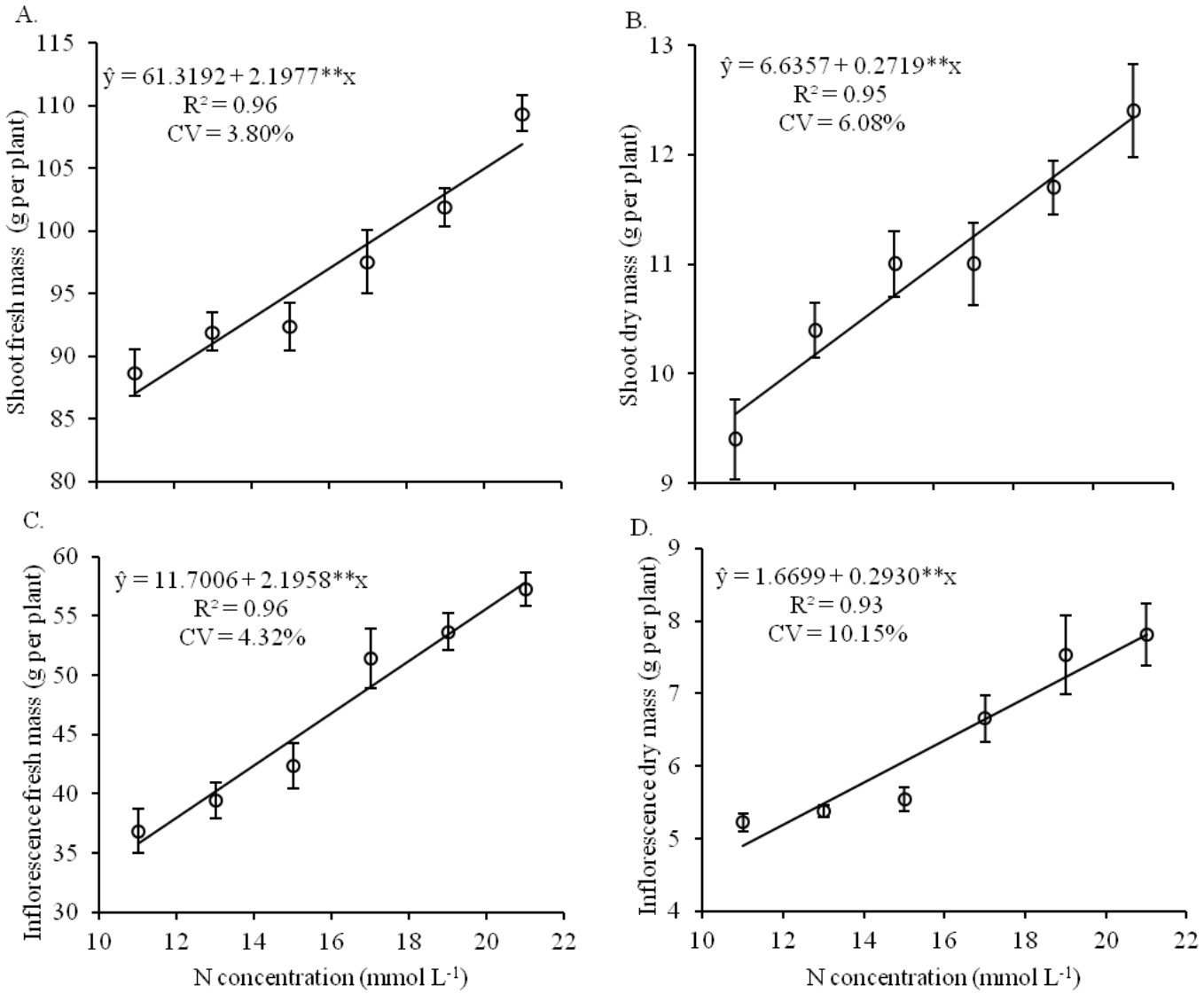
The data were subjected to analysis of variance and the F test ($p \leq 0.01$), and when a significant effect was detected, regression models were fitted for N concentrations in the nutrient solution. Pearson correlation and principal component analysis (PCA) were applied using the FactoMineR and Hmisc packages, respectively, to examine the relationship between nitrogen availability, biomass accumulation, and spilanthol concentration in jambu organs. The R software version 3.4.3 (R Core Team, 2017) was used for data analysis.

Results and Discussion

The supply of nitrogen in jambu plants positively affected the accumulation of shoot fresh and dry mass (Figures 1A and B, respectively) and inflorescence fresh and dry biomass (Figures 1C and D, respectively). However, root fresh mass (RFM) and root dry mass (RDM) did not significantly respond to increasing nitrogen supply. Nitrogen supply stimulated linear increments in shoot fresh mass (SFM) and shoot dry mass (SDM), with increases of approximately 25.7 and 28.2%, respectively, in plants subjected to a concentration of 21 mmol L^{-1} of nitrogen compared to the lowest nitrogen concentration assessed (11 mmol L^{-1}) (Figures 1A and B). Similarly, inflorescence fresh mass (IFM) and inflorescence dry mass (IDM) increased proportionally with the increase in nitrogen in the nutrient solution, showing increments of 61.3 and 59.9% in the concentration of 21 mmol L^{-1} of nitrogen compared to the lowest nitrogen concentration in the nutrient solution (11 mmol L^{-1}), respectively (Figures 1C and D).

The productive responses observed in this study are consistent with previous studies on jambu grown in soil (Rodrigues et al., 2014; Costa et al., 2020), where additional nitrogen supply stimulated the accumulation of vegetative and reproductive biomass in the plant and significant losses when nitrogen was reduced.

Nitrogen is considered the most limiting nutrient for plant growth and development, as it is a substrate for synthesizing compounds such as chlorophylls, proteins, and nucleic acids (Ueda et al., 2017). Thus, nitrogen deficiency in plant tissues



** Significant at $p < 0.01$ by the F-test. CV- Coefficient of variation; The bar represents the mean ± standard error (n = 4)

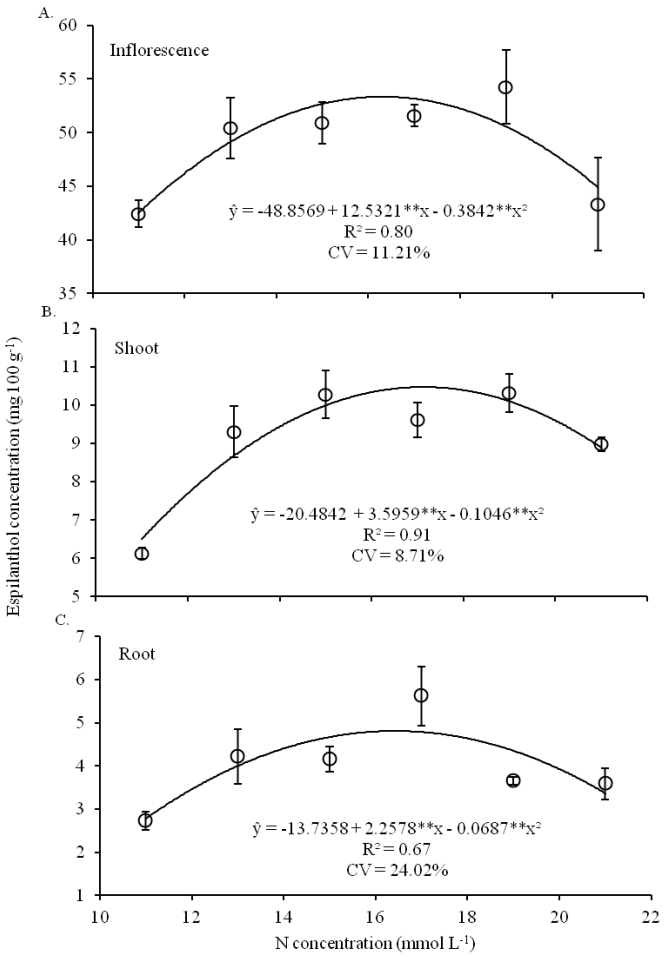
Figure 1. Shoot fresh mass (A), shoot dry mass (B), inflorescence fresh mass (C) and inflorescence dry mass (D) of jambu plants as a function of nitrogen concentration in the nutrient solutions

reduces the production of these key metabolites, reducing vital metabolic processes for plants (Saloner & Bernstein, 2020). Therefore, such functions justify that changes in nitrogen supply significantly affected the vegetative and reproductive growth of jambu in this study.

The concentrations of spilanthol analyzed in the different organs of jambu plants showed variations under N supplementation, fitting a quadratic effect (Figure 2). The highest values obtained for IFM (53.3 mg 100 g^{-1} of fresh mass), SFM (10.4 mg 100 g^{-1} of fresh mass), and RFM (4.8 mg 100 g^{-1} of fresh mass) were achieved at concentrations of 16.3, 17.2, and 16.4 mmol L^{-1} N, respectively (Figures 2A, B, and C), with increases of 25.4, 62.5, and 71.4% compared to the results obtained under the lowest N supply in the nutrient solution (11 mmol L^{-1}), respectively.

The plants had sensitivity to high concentrations of N, as N supply above 17 mmol L^{-1} inhibited the biosynthesis of spilanthol in all evaluated organs, as observed by the quadratic behaviors (Figure 2). Regarding the distribution of spilanthol content in different organs of the jambu plant, it was found that the values decreased in the following order: inflorescences > shoot (leaves and stems) > root.

Previous studies have also shown that the inflorescences of jambu have a higher concentration of spilanthol than the leaf and stem (Dias et al., 2012; Barbosa et al., 2016; Balieiro et al., 2020). In all organs of jambu plants, N concentrations in the deficient, optimal, and excessive nutrient solution regarding the biosynthesis of spilanthol were observed in this study. Thus, N concentrations above and below the range of 17 mmol L^{-1} led to lower concentrations of spilanthol in the tissues, suggesting that depending on the concentration of N in the nutrient solution, there is a change in the allocation of primary metabolites and energy inputs for the growth and development of jambu to the detriment of the production of secondary metabolites (Figure 2). Radušienė et al. (2019) demonstrated that higher N supplies promote higher biomass production in *Hypericum perforatum*; however, a consequent reduction in bioactive compounds occurs.



** Significant at p < 0.01 by the F-test. CV- Coefficient of variation; The bar represents the standard error (n = 4)

Figure 2. Spilanthol concentration in inflorescence dry mass (A), shoot dry mass (B), and root dry mass (C) of jambu plants as a function of nitrogen concentration in the nutrient solution

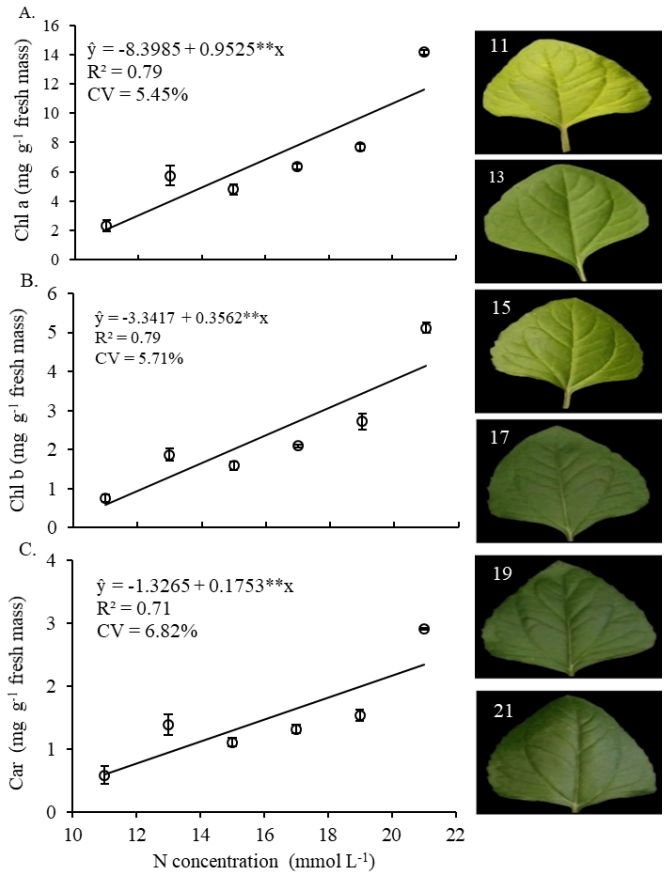
Changes in the biosynthesis of primary and secondary compounds were observed in *Labisia pumila*, where increased N supply promoted higher soluble protein content at the higher N concentrations supplied, as well as a negative correlation between increased protein and the production of total phenols and flavonoids, indicating the occurrence of an upregulation of secondary metabolite synthesis in plants when protein content is reduced (Ibrahim et al., 2011).

Primary metabolism is an important source of precursors for secondary metabolite synthesis, which is positively stimulated by nutrient supply (Becker et al., 2015; Lu et al., 2017; Saloner & Bernstein, 2020). Considering that plant growth is strongly dependent on key nutrients such as N for the synthesis of structural and enzymatic proteins (photosynthetic, biosynthetic, and regulatory), the pronounced biosynthesis of secondary metabolites is dependent on the pronounced development of the primary metabolism, given the need of both metabolisms for common nutrients (Radušienė et al., 2019), a relationship was observed in the biosynthesis of N-alkylamines such as spilanthol and fresh matter production in all jambu organs in this study.

The content of Chl a (Figure 3A), Chl b (Figure 3B), and Car (Figure 3C) increased linearly with the elevation of N supply in the nutrient solution, showing increments of 458.2, 617.9, and 291.3%, respectively, in the leaves of jambu plants cultivated under the concentration of 21 mmol L^{-1} compared to the lowest N concentration tested (11 mmol L^{-1}). Another aspect affected by the photosynthetic pigments concentrations, due to the N levels in the nutrient solution, was the color of the jambu leaves. The chlorotic yellowish-green leaves supplied with the lowest levels of N in the nutrient solution reflect the low levels of chlorophyll, while the dark green leaves in the range of 17-21 mmol L^{-1} indicate an adequate level of pigments.

The synthesis of chlorophylls and carotenoids depends on mineral nutrition (Ueda et al., 2017). The N availability stands out as it is vital in cell division and formation of photosynthetically active pigments, protein synthesis in the stroma and thylakoid in leaves, and chloroplast formation during leaf growth (Saloner & Bernstein, 2020).

Increased N supply stimulated the production of photosynthetic pigments in jambu (Figure 3), similar to what has been shown for several plant species (Mampholo et al., 2018; Saloner & Bernstein, 2020). Thus, the elevation of photosynthetic pigments with higher N supplies in the nutrient



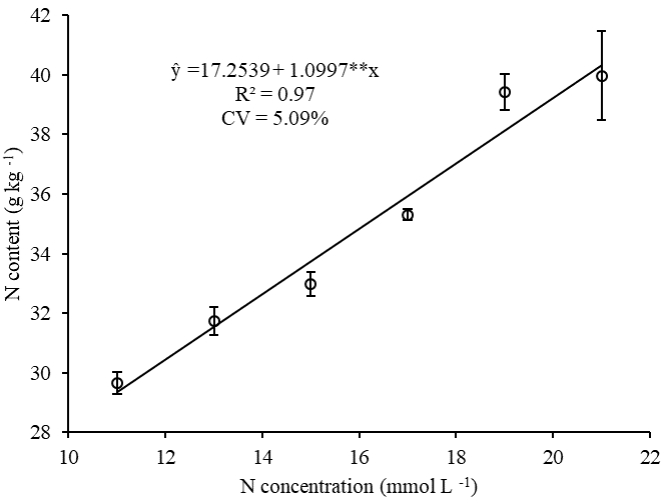
** Significant ($p < 0.01$) by the F-test. CV- Coefficient of variation; The bar represents the standard error (n = 4); Leaf images are of the second pair of fully developed leaves taken from the apex of the main stem of the plant, the no. inserted in the leaf refer to N concentration (mmol L^{-1}) in the nutrient solution

Figure 3. Chlorophyll a (Chl a - A), chlorophyll b (Chl b - B), and carotenoids (Car - C) of jambu leaves grown in hydroponics as a function of nitrogen concentration in the nutrient solution

solution acted positively in increasing the photosynthetic capacity of jambu plants, resulting in higher accumulation of shoot (Figures 1A and 1B) and inflorescence (Figures 1C and D) biomass observed in this study. Similar results were observed by Saloner & Bernstein (2020), who demonstrated a positive correlation between pigment contents and increased photosynthetic capacity of *Cannabis sativa* plants, promoting greater carbon fixation and, consequently, biomass accumulation.

Increasing the N supply in the nutrient solution resulted in changes in the uptake and translocation of total N content in jambu leaves, with higher contents being observed as the nutrient concentration was increased. As a result, plants grown at high N concentrations (21 mmol L^{-1}) have total N content in leaves of 40.3 g kg^{-1} and plants under low concentrations (11 mmol L^{-1}) of 29.4 g kg^{-1}, representing a 37.1% increase in leaf N concentration (Figure 4).

In order to establish a relationship between N supplies and the variables evaluated in this study, a Principal Component Analysis (PCA) was conducted, demonstrating a variability of 72.9% in the first two principal components (PC1 and PC2). Chl a, Chl b, Car, N, SFM, SDM, IFM, and IDM showed a higher and positive relationship with the higher nitrogen concentration in the nutrient solution (21 mmol L^{-1}). In contrast,

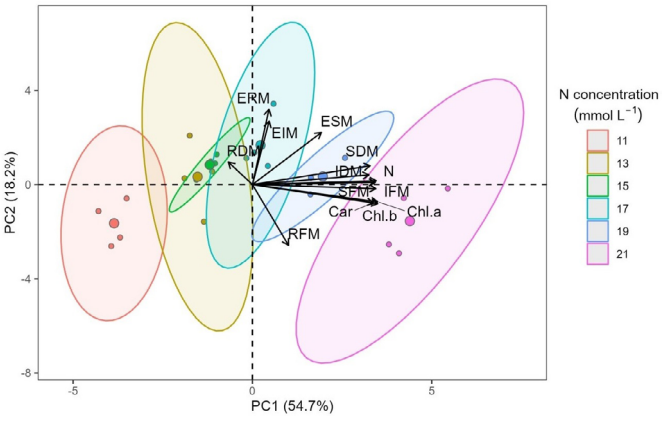


** Significant ($p < 0.01$) by t test. R^2 - Coefficient of determination; CV- Coefficient of variation; The bar represents the standard error (n = 4)

Figure 4. N content in jambu leaves as a function of nitrogen concentration in the nutrient solution

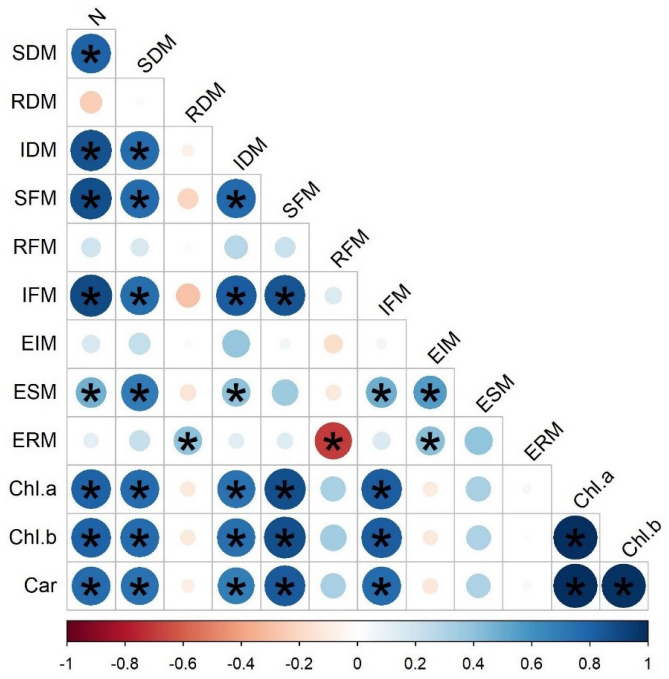
spilanthol concentration in root, shoot, and inflorescence had a greater relationship with the concentration of 17 mmol L^{-1} (Figure 5).

The correlation analysis (Figure 6) revealed that the higher total N content in jambu leaves correlated positively and significantly with the synthesis of photosynthetic pigments, production of fresh and dry biomass of inflorescence and shoot, and spilanthol concentration in the shoot. These correlations can be explained by the fact that the products of primary metabolism are important precursors of secondary metabolites and, given this, these can be affected by nutritional status, although there is not necessarily a direct effect between these and the nutritional supply (Mampholo et al., 2018; Radušienė et al. 2019). Therefore, it demonstrates that alterations in N supply in the growth medium modulate differentiated responses



Scatter plots with different colors indicate the treatments (11, 13, 15, 17, 19 and 21 mmol L^{-1} N in nutrient solution)

Figure 5. Principal component analysis (PCA) demonstrating the impact of nitrogen (N) concentrations in nutrient solution on biomass accumulation in shoot fresh mass – SFM, shoot dry mass – SDM, root fresh mass– RFM, root dry mass – RDM, inflorescence fresh mass – IFM and inflorescence dry mass – IDM; in pigments chlorophyll a - Chl a, chlorophyll b – Chl b and carotenoids – Car, total N concentration in leaf (N) and spilanthol concentration in inflorescence (EIM), aboveground (ESM), and root (ERM) of jambu plants



Shoot fresh mass – SFM, shoot dry mass – SDM, root fresh mass– RFM, root dry mass – RDM, inflorescence fresh mass – IFM and inflorescence dry mass – IDM; in pigments chlorophyll a - Chl a, chlorophyll b – Chl b and carotenoids – Car, total N concentration in leaf (N) and spilanthol concentration in inflorescence (EIM), aboveground (ESM) and root (ERM) of jambu plants. * Significant ($p < 0.05$) by t test. (n = 4)

Figure 6. Pearson correlation between productive variables, pigment concentration, leaf N content, and spilantol concentration in different jambu organs

regarding spilanthol biosynthesis, pigment concentration, and biomass accumulation in jambu.

Conclusions

1. The concentration of 21 mmol L^{-1} of N in the nutrient solution promotes an increase in fresh and dry biomass of the shoot and inflorescences and the content of N and photosynthetic pigments in jambu leaves.

2. The concentration of 17 mmol L^{-1} of N in the nutrient solution stimulates the greater synthesis of spilanthol in the different organs of the jambu..

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