



Foliar urea application improves acclimatization of ‘Turiaçu’ pineapple grown *in vitro*

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ABSTRACT: *In vitro* propagation is an efficient technique for producing ‘Turiaçu’ pineapple (*Ananas comosus* var. *comosus*) plantlets, ensuring uniform and pathogen-free plants. However, the acclimatization phase remains challenging, requiring appropriate nutritional strategies, with nitrogen being a key element for plant growth and metabolism. This study evaluated the effects of foliar fertilization with urea as a nitrogen source on the acclimatization of *in vitro*-grown ‘Turiaçu’ pineapple plantlets, using a completely randomized design with five foliar-applied urea concentrations (0%, 0.25%, 0.5%, 1.0%, and 1.5%) and ten replications. After 72 days of acclimatization, growth (leaf area, number of leaves, length of D leaf, rosette diameter, shoot and root dry mass), physiological (chlorophylls, carotenoids, photochemical efficiency), and nutritional (leaf macro- and micronutrients) parameters were analyzed. Foliar application of 1.0% urea promoted increases of 86.3% in leaf area, 38.7% in D leaf length, 30% in rosette diameter, 83% in shoot dry mass, and 13% in root dry mass, without causing phytotoxicity to the plantlets. It is concluded that foliar application of 1.0% urea is recommended to optimize the acclimatization of *in vitro* cultivated ‘Turiaçu’ pineapple plantlets, representing a promising approach for the commercial production of vigorous plantlets and for guiding future studies on foliar nutrition in bromeliads.

Key words: *Ananas comosus* var. *comosus*, foliar fertilization, plant nutrition.

A aplicação foliar de ureia melhora a aclimação do abacaxizeiro ‘Turiaçu’ cultivado *in vitro*

RESUMO: A propagação *in vitro* é uma técnica eficiente para a produção de mudas de abacaxizeiro ‘Turiaçu’ (*Ananas comosus* var. *comosus*), garantindo plantas uniformes e livres de patógenos. No entanto, a fase de aclimação permanece desafiadora, exigindo estratégias nutricionais adequadas, sendo o nitrogênio um elemento-chave para o crescimento e metabolismo vegetal. Este estudo avaliou os efeitos da adubação foliar com ureia como fonte nitrogenada na aclimação de mudas de abacaxizeiro ‘Turiaçu’ produzidas *in vitro*, por meio de um delineamento inteiramente casualizado com cinco concentrações de ureia (0%, 0,25%, 0,5%, 1,0% e 1,5%) aplicadas via foliar e dez repetições. Após 72 dias de aclimação, foram analisados parâmetros de crescimento (área foliar, número de folhas, comprimento da folha D, diâmetro da roseta, massa seca da parte aérea e radicular), fisiológicos (clorofilas, carotenoides, eficiência fotoquímica) e nutricionais (macro e micronutrientes foliares). A aplicação foliar de 1,0% de ureia promoveu aumentos de 86,3% na área foliar, 38,7% no comprimento da folha D, 30% no diâmetro da roseta, 83% na massa seca da parte aérea e 13% na massa seca radicular, sem causar fitotoxicidade às mudas. Conclui-se que a ureia a 1,0% é recomendada para otimizar a aclimação de mudas de abacaxizeiro ‘Turiaçu’ cultivadas *in vitro*, representando uma abordagem promissora para a produção comercial de mudas vigorosas e orientar estudos futuros sobre nutrição foliar em bromeliáceas.

Palavras-chave: *Ananas comosus* var. *comosus*, adubação foliar, nutrição de plantas.

INTRODUCTION

Pineapple [*Ananas comosus* (L.) Merril.] is a perennial plant of the Bromeliaceae family native to tropical and subtropical America, beyond its nutritional value and high vitamin C content, offers notable health benefits, including antioxidant, anti-inflammatory, and digestive properties, largely attributed to the enzyme bromelain (CASAS-RODRÍGUEZ et al., 2024). Brazil ranks among the largest global producers, with

cultivation concentrated in the domestic market (PIRES et al., 2023; SILVA-MORAES et al., 2024). The ‘Turiaçu’ pineapple [*Ananas comosus* var. *comosus* (L.) Merril], native to Maranhão State, has gained national and international attention due to its yellowish pulp, high sugar content, and medium fruit size (ARAUJO et al., 2012). However, production still relies on traditional, labor-intensive methods, highlighting the need for technological improvements to enhance yield and quality (ARAUJO et al., 2012; REIS et al., 2024).

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Despite its economic and nutritional relevance, pineapple propagation remains a challenge, especially for traditional cultivars like ‘Turiaçu’, which often exhibit low uniformity, reduced vigor, and phytosanitary vulnerabilities when propagated conventionally (ALVES et al., 2024). *In vitro* propagation has emerged as a promising strategy to overcome these constraints, enabling the large-scale production of homogeneous and pathogen-free plantlets. Protocols employing temporary immersion bioreactors have demonstrated enhanced morphophysiological performance, including under photoautotrophic and photomixotrophic conditions (ALVES et al., 2024).

During the acclimatization phase, the nutritional status of *in vitro* – derived plantlets play a decisive role in their successful establishment and long-term performance in the field (PIRES et al., 2023). Urea is the most widely used nitrogen fertilizer in agriculture due to its high nitrogen content, affordability, and rapid assimilation by plants (MOTASIM et al., 2024). Its effectiveness lies in supplying nitrogen predominantly as ammonium (NH_4^+) and nitrate (NO_3^-), forms readily absorbed by plants (MOTASIM et al., 2024). Nitrogen, in turn, is one of the most demanded macronutrients by pineapple and is essential for fundamental physiological processes such as photosynthesis, protein synthesis, enzyme activation, and chlorophyll production (KHUONG et al., 2024). Despite its potential, the optimal use of foliar-applied urea during the acclimatization of pineapple plantlets remains poorly understood, particularly in traditional cultivars like ‘Turiaçu’, where this phase often marked by physiological stress, limited growth, and high mortality rates.

Given this scenario, we hypothesized that foliar application of urea during the acclimatization of *in vitro*-grown ‘Turiaçu’ pineapple plantlets enhances morphophysiological development. To test this hypothesis, the general objective of this study was to evaluate the effect of foliar urea fertilization on the acclimatization of ‘Turiaçu’ pineapple plantlets. Specifically, we determined the most effective urea concentration for optimizing growth parameters, assess the impact of foliar urea on physiological traits such as chlorophyll content and photochemical efficiency, and evaluate the leaf nutritional status in response to increasing doses.

MATERIALS AND METHODS

Micropropagation of the ‘Turiaçu’ pineapple plantlets

The experiment was performed at the Tissue Culture Laboratory of the Universidade

Estadual do Maranhão (LCT/UEMA). Previously established *in vitro* shoots of ‘Turiaçu’ pineapple were sub-cultured four times in MS (MURASHIGE & SKOOG, 1962) medium (PhytoTechnology®, Lenexa, KS, USA), supplemented with 100 mg L⁻¹ myo-inositol (Sigma-Aldrich®, St. Louis, MO, USA), 2 µM naphthaleneacetic acid (NAA) (Sigma-Aldrich®, St. Louis, MO, USA), 4 µM benzylaminopurine (BAP) (Sigma-Aldrich®, St. Louis, MO, USA), 2 g L⁻¹ Phytigel (Sigma-Aldrich®, St. Louis, MO, USA), and 30 g L⁻¹ sucrose (Isosfar® Ltda, Duque de Caxias - RJ). The pH was adjusted to 5.7 ± 0.1 and the medium was autoclaved at 121 °C for 15 minutes.

In a laminar flow cabinet, shoots (approximately 2 cm long) were transferred to temporary immersion bioreactors (TIS-RALM®) containing 600 mL of liquid MS medium supplemented with salts and vitamins, 30 g L⁻¹ sucrose, 100 mg L⁻¹ myo-inositol, and 2.7 µM NAA as described by ALVES et al. (2024). The pH was adjusted to 5.7 ± 0.1 and the medium was autoclaved at 121 °C for 15 minutes.

Plantlets were kept in a growth room for 55 days under a 16-hour photoperiod and a light irradiance of 85 µmol m⁻² s⁻¹ provided by four white LED lamps (Tubular T8 LED, 20 W, Elgin, Mogi das Cruzes, SP, Brazil).

Acclimatization and experimental design of ‘Turiaçu’ pineapple plantlets

After 55 days of *in vitro* cultivation, pineapple plantlets measuring 5 cm in length were removed from the bioreactors, washed in running water, and transferred to polystyrene pots with a capacity of 380 mL containing Carolina Soil® substrate. Plantlets were then placed in the greenhouse of the Graduate Program in Agricultural Sciences for acclimatization.

The experiment was conducted in a completely randomized design, with five concentrations of foliar urea: 0%; 0.25%; 0.5%; 1.0%, and 1.5%, expressed in grams per liter (g L⁻¹), with ten replications each. Each experimental unit consisted of one plant per polystyrene pot. The experimental period comprised 72 days of acclimatization (or *ex vitro* cultivation), during which plants were subjected to the respective foliar urea treatments.

Determination of growth parameters

After 72 days of acclimatization, the following growth parameters were assessed: root length (cm); leaf D length (cm); rosette diameter (cm); leaf area (cm²), determined using ImageJ® software; shoot dry mass (g); and root dry mass (g).

Plant tissues were separated into shoots and roots; and subsequently, oven-dried at 60 °C for 72 h in a forced-air circulation oven (Solidsteel®) to obtain the dry mass. The sample size (n) was 10 for all evaluated variables.

Quantification of photosynthetic pigment concentration

Photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, and the total chlorophyll-to-carotenoid ratio, were quantified using five leaf discs (5 mm diameter) collected from leaf D. The discs were immersed in 5 mL of dimethyl sulfoxide (DMSO) and kept in the dark for 48 h. Absorbance readings were performed using a UV-Vis spectrophotometer (BEL Engineering, UV-M51 UV/Vis Spectrophotometer) at wavelengths of 480, 649, and 665 nm, following the methodology described by WELLBURN (1994). The results were expressed as $\mu\text{g cm}^{-2}$. The sample size (n) was 5 for all evaluated variables.

*Measurement of gas exchange and chlorophyll *a* fluorescence*

Gas exchange and chlorophyll *a* fluorescence were evaluated on the D leaf using a portable infrared gas analyzer (LI-6400XT, Li-Cor, Lincoln, NE, USA) and a portable fluorimeter (Pocket-PEA, Hansatech, Norfolk, UK), respectively. Gas exchange parameters included net photosynthetic rate (A ; $\mu\text{mol m}^{-2} \text{s}^{-1}$), measured under an external CO_2 concentration of 400 $\mu\text{mol mol}^{-1}$, a photosynthetic photon flux density of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and an average temperature of 25 °C. Subsequently, chlorophyll *a* fluorescence parameter, including energy absorbed per active reaction center (RC/ABS) and the performance index (PI), were assessed after dark adaptation of the leaves for 30 min using specific leaf clips, according to BOLHAR-NORDENKAMPF et al. (1989). The sample size (n) was 5 for all evaluated variables.

Tissue nutrient analysis

Dried and ground leaf samples were used for nutrient analysis. The concentrations of K, Zn, Fe, and B were determined following nitric-perchloric acid digestion (4:1, v/v), while N content was determined by sulfuric acid digestion using the Kjeldahl method (SARRUGE & HAAG, 1974). Potassium (K) was quantified by flame photometry (Corning 400, Corning Inc., NY, USA); iron (Fe) and zinc (Zn) by atomic absorption spectrophotometry (Varian, Mulgrave, Victoria, Australia); and boron

(B) by colorimetric analysis using the azomethine-H method, as described by MALAVOLTA et al. (1997). The results were expressed as mg Kg^{-1} dry mass. The sample size (n) was 3 for all nutrients evaluated.

Statistical analysis

The data were subjected to regression analysis using the Sisvar statistical software (FERREIRA, 2011). Linear and quadratic regression models were tested and selected based on the significance of the F-test from the analysis of variance (ANOVA) and the highest coefficient of determination (R^2).

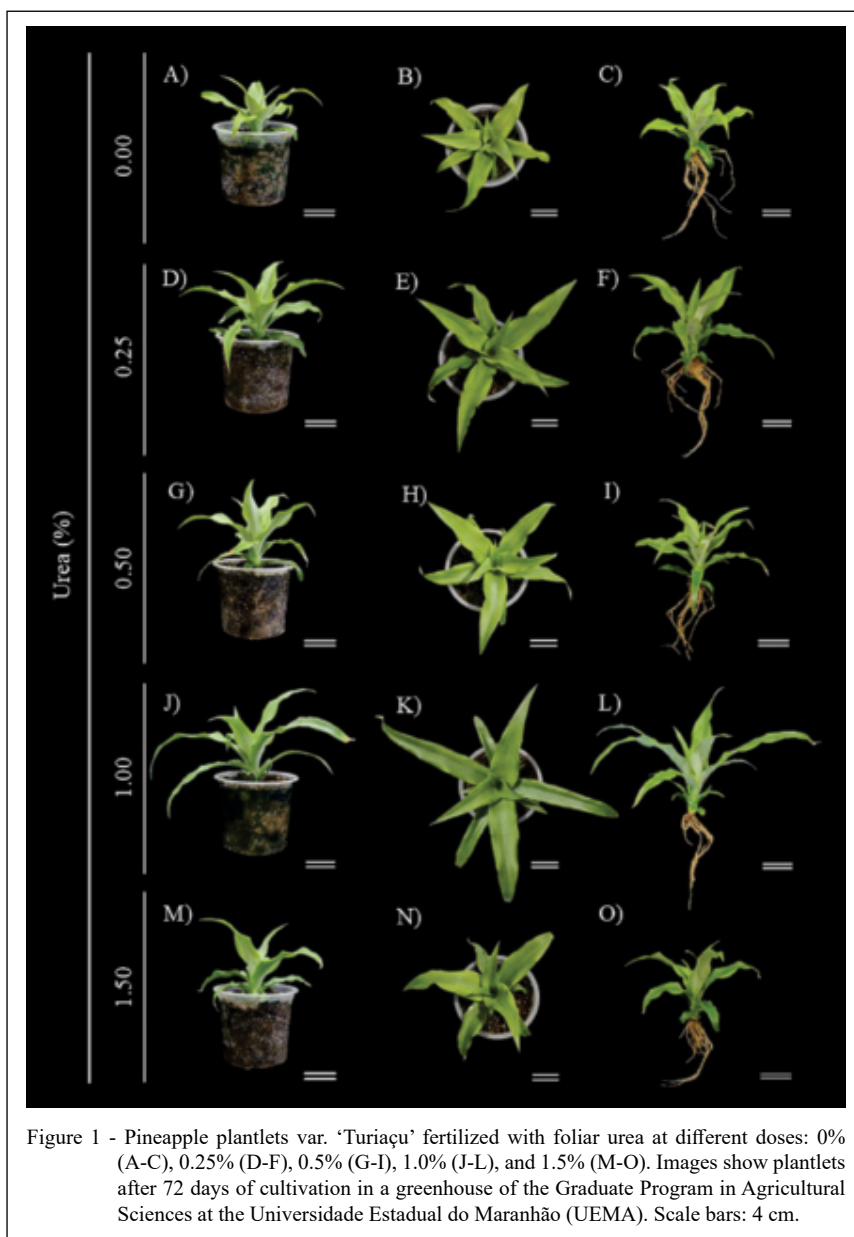
RESULTS AND DISCUSSION

Different concentrations of foliar urea significantly influenced the morphological, physiological, and nutritional parameters of 'Turiacu' pineapple plantlets (Figures 1 to 5). Plantlet growth responded positively to increasing urea doses up to 1.0%, resulting in enhanced vigor and development (Figure 1J-L). Regression models revealed distinct dose-dependent responses.

Root length showed a quadratic response ($R^2 = 0.98$), ranging from 13.35 cm at 1.5% urea to a maximum of 17.48 cm at 0.5% (Figure 2A), suggesting that moderate nitrogen availability favors root development, while higher doses may impair it due to nutritional imbalance (DÍAZ-ÁLVAREZ et al., 2015). Similarly, leaf D length increased quadratically ($R^2 = 0.93$), from 12.11 cm (control) to a peak of 18.05 cm at 1.0%, followed by a slight reduction at 1.5% (Figure 2B), underscoring the role of nitrogen in promoting leaf expansion and light capture.

Rosette diameter increased linearly ($R^2 = 0.61$), from 17.96 cm (control) to 23.35 cm at 1.5% urea (Figure 2C). Leaf area followed a quadratic pattern ($R^2 = 0.86$), reaching a maximum of 240.20 cm^2 at 1.0% urea, compared to 128.93 cm^2 in the control, before declining to 202.73 cm^2 at 1.5% (Figure 2D). Shoot dry mass rose from 0.61 g (control) to 1.12 g at 1.0% urea ($R^2 = 0.85$), while root dry mass ranged from 0.18 g to 0.21 g at the same concentration ($R^2 = 0.81$), with decreases observed at higher doses (Figures 2E-F, respectively). These findings confirmed that nitrogen excess may reduce biomass accumulation due to phytotoxic effects (SALES & BERILLI, 2018).

These morphological gains were accompanied by physiological adjustments, especially related to pigment accumulation and photosynthetic capacity, reflecting the integrated role of nitrogen in

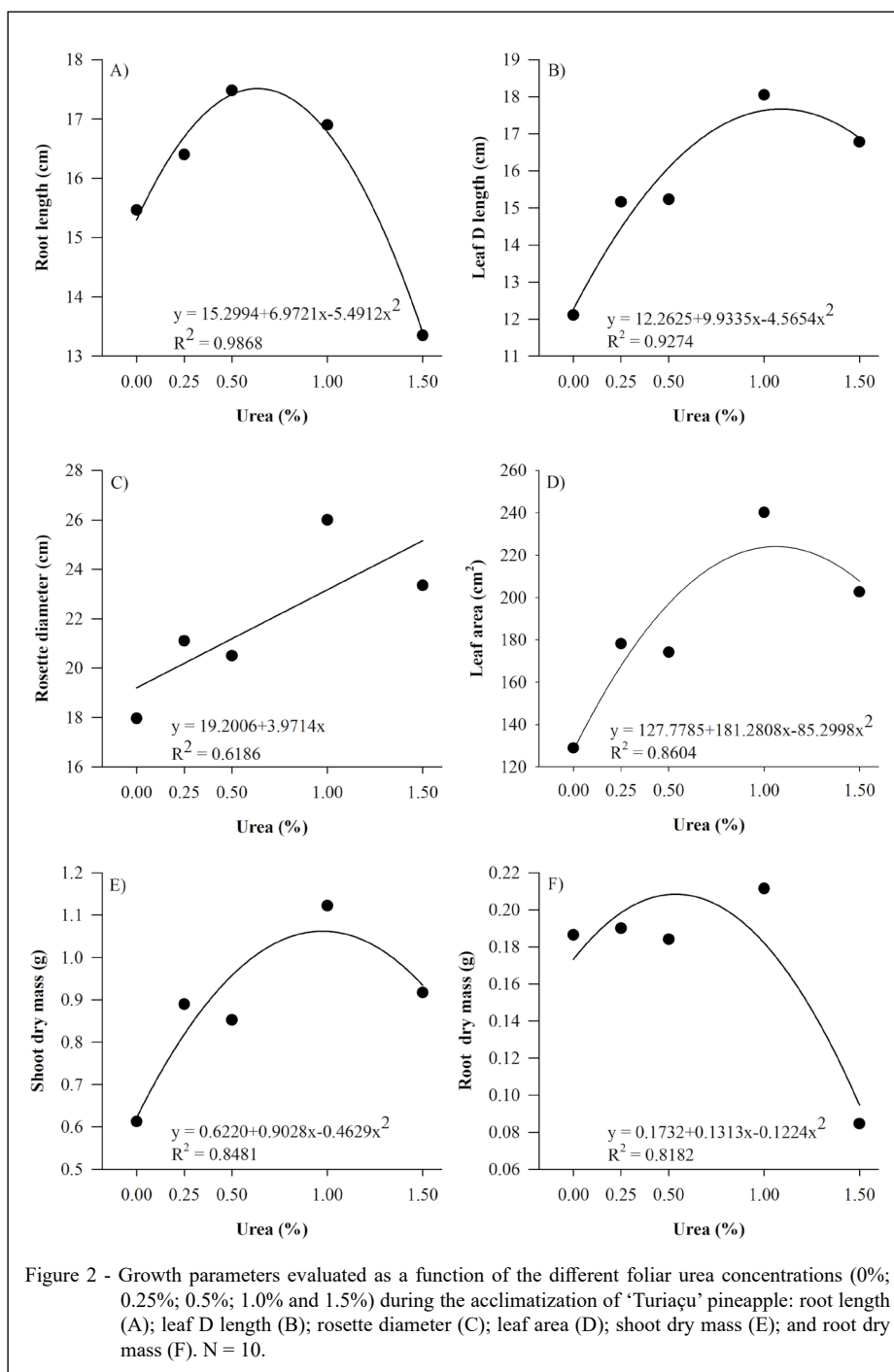


supporting plant metabolism during acclimatization. The observed improvements at 1.0% urea highlighted the pivotal role of nitrogen in coordinating growth, metabolism, and structural development.

Nitrogen is a key component of amino acids, proteins, nucleic acids, and chlorophyll molecules, and is fundamental for cell division, tissue expansion, and overall structural integrity (WANG et al., 2024). Additionally, sufficient nitrogen availability stimulates enzymatic activity and metabolic pathways related to protein synthesis, energy production, and hormonal balance, thereby enhancing the coordination

between source (photosynthate production) and sink (growth demand) tissues (BHATLA & LAL, 2024).

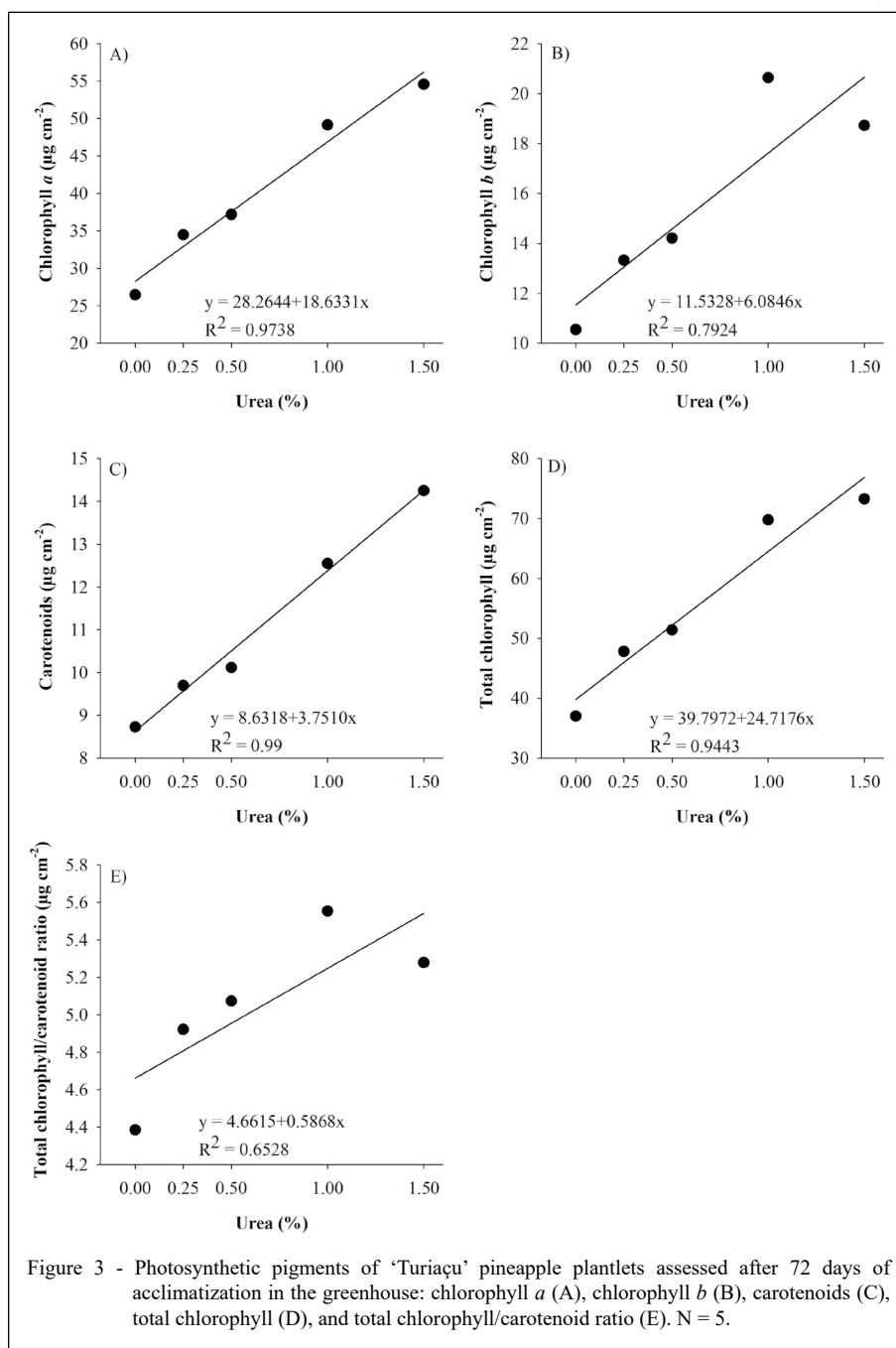
Foliar urea application led to a significant linear increase in all photosynthetic pigments of 'Turiacu' plantlets (Figure 3). Chlorophyll *a* increased from 26.5 to 54.6 $\mu\text{g cm}^{-2}$ ($R^2 = 0.97$), chlorophyll *b* from 10.5 to 18.7 $\mu\text{g cm}^{-2}$ ($R^2 = 0.79$), and carotenoids from 8.7 to 14.2 $\mu\text{g cm}^{-2}$ ($R^2 = 0.99$) (Figure 3A-C, respectively). Total chlorophyll rose from 37.0 to 73.3 $\mu\text{g cm}^{-2}$ ($R^2 = 0.94$), while the chlorophyll-to-carotenoid ratio increased from 4.4 to 5.6 ($R^2 = 0.65$) (Figure 3D-E, respectively). These trends align



with nitrogen's central role in pigment biosynthesis and chloroplast development (WANG et al., 2024). However, these results should be interpreted as evidence of improved photosynthetic potential and photoprotective capacity rather than direct increases in photosynthetic efficiency. Pigment accumulation may reflect nitrogen-stimulated biosynthetic activity

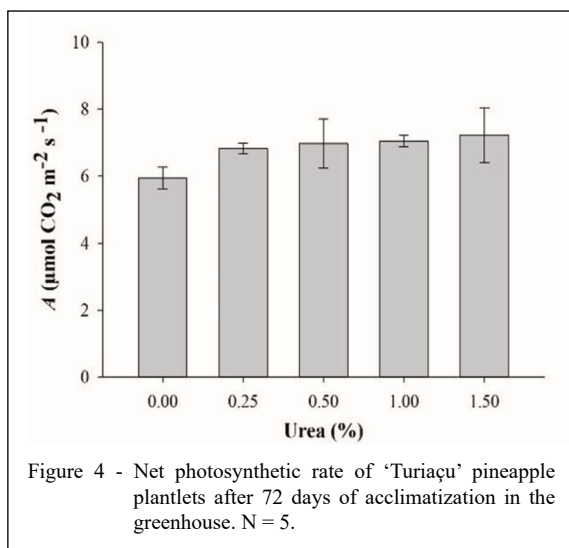
or acclimatory responses to elevated light exposure during the *ex vitro* transition (OLESZKIEWICZ et al., 2025).

Gas exchange data further support this interpretation, as no significant differences in net photosynthetic rate were observed among treatments (Figure 4). Hence, higher pigment levels



indicated a more robust photosynthetic apparatus but do not imply greater carbon assimilation under the given conditions. This decoupling between pigment accumulation and carbon fixation suggests that nitrogen primarily enhanced structural and photochemical components of the photosynthetic machinery rather than the biochemical capacity for carbon assimilation.

Nitrogen supply often promotes pigment biosynthesis, chloroplast biogenesis, and energy absorption, while carbon fixation may remain constrained by the biochemical limitations of the Calvin cycle, including Ribulose-1,5-bisphosphate (RuBP) activity and regeneration (BHATLA & LAL, 2023; WANG et al., 2024). This phenomenon is common during acclimatization, when energy-



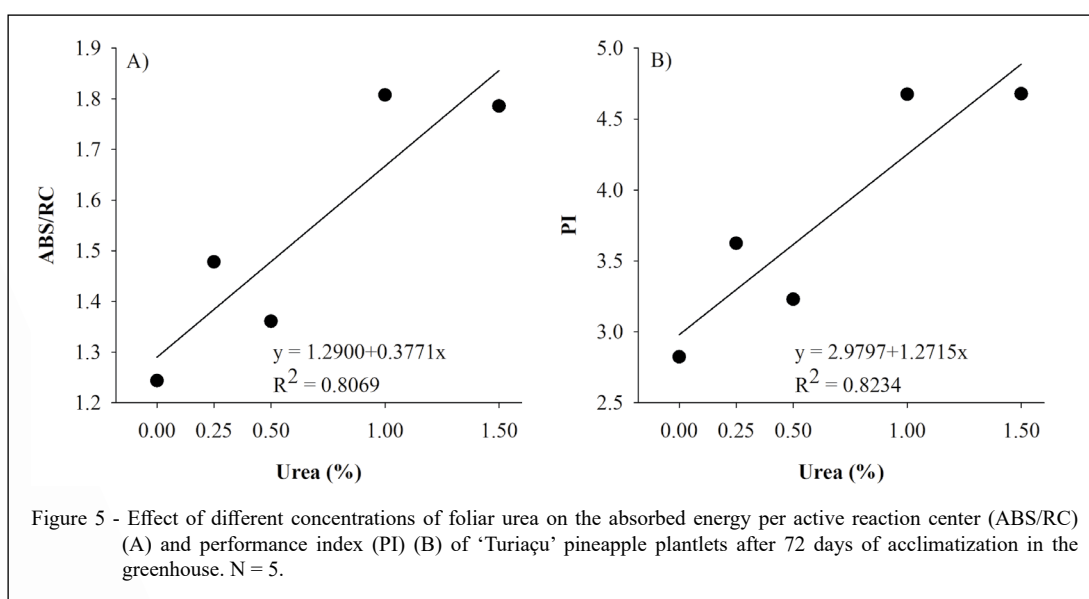
harvesting and conversion components are upregulated independently of carboxylation processes or metabolic sinks (OLESZKIEWICZ et al., 2025).

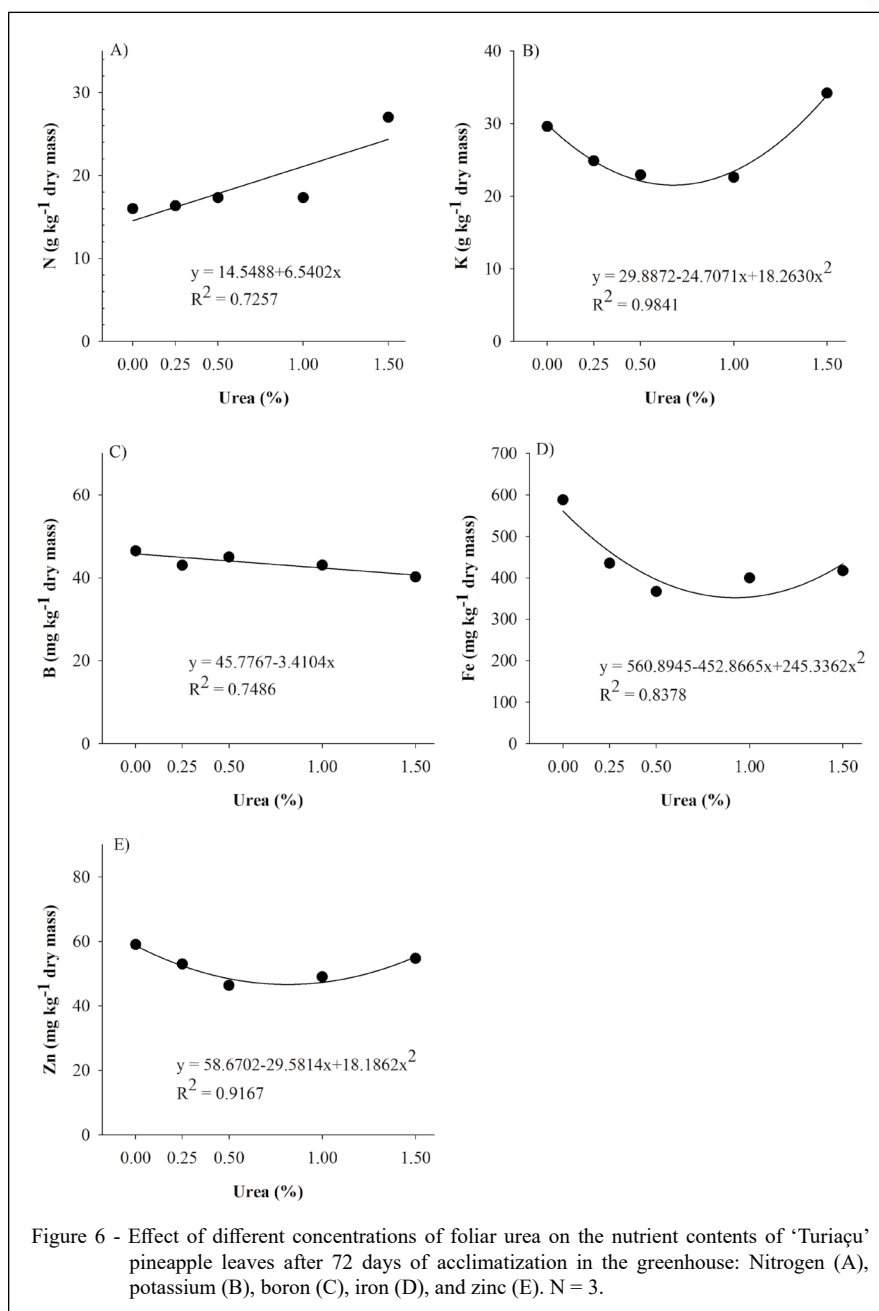
Photochemical performance was also affected by urea. ABS/RC increased from 1.29 (control) to 1.85 at 1.5% urea ($R^2 = 0.81$) (Figure 5A), which may signal a reduction in the number of active reaction centers, increasing energy load per unit. While this could indicate photoinhibition or excitation imbalance, such stress was not corroborated by the performance index (PI), which rose from 2.98 to 4.88 ($R^2 = 0.82$), particularly at 1.0% urea (Figure 5B).

These findings suggested that foliar urea did not induce photodamage and may have enhanced overall energy conversion efficiency. Meanwhile, CO₂ assimilation remained similar among treatments, ranging from 5.94 to 7.22 μmol m⁻² s⁻¹, indicating that although, structural and photochemical components improved, photosynthetic metabolism remained largely unchanged.

Foliar urea application also altered the leaf nutrient composition of 'Turiaçu' plantlets (Figure 6). Nitrogen content increased linearly from 15.4 to 24.3 g kg⁻¹ ($R^2 = 0.72$), confirming the effectiveness of urea as a nitrogen source (Figure 6A). Potassium exhibited a quadratic pattern, with higher concentrations at the control and 1.5% doses, possibly reflecting ion competition or translocation dynamics (Figure 6B). Boron content decreased with rising urea concentrations ($R^2 = 0.75$), likely due to a dilution effect associated with biomass expansion (Figure 6C). Nevertheless, nitrogen may also influence boron uptake by altering transpiration and membrane transport activity (MARSCHNER, 2012).

Iron and zinc showed quadratic responses ($R^2 = 0.83$ and 0.92 , respectively), with reduced concentrations at intermediate urea doses and recovery at the highest level (Figure 6D-E, respectively). These trends suggest complex interactions between nitrogen availability and micronutrient uptake, transport, or utilization. Nitrogen can modulate rhizosphere and phylloplane pH, root exudate composition, and nutrient transporter expression, thereby influencing micronutrient acquisition





(CHEN & BARAK, 1982). Additionally, foliar urea may transiently affect the surface pH and ion exchange properties of leaf tissues, altering nutrient solubility and permeability. These findings suggest that the effects of foliar nitrogen extend beyond nitrogen accumulation, influencing the homeostasis and functionality of other essential nutrients during acclimatization.

Collectively, the improvements in growth, pigment levels, photochemical behavior, and nutrient

status under 1.0% foliar urea application underscore its physiological efficacy in promoting successful acclimatization of 'Turiçu' pineapple plantlets, while avoiding the phytotoxic risks observed at higher concentrations.

It is important to note that growth parameters were assessed using ten replicates, while physiological and nutritional traits were evaluated with five and three replicates, respectively. This limitation may affect the statistical robustness of

highly variable parameters such as photosynthetic pigments and micronutrient concentrations. These results should, therefore, be interpreted cautiously. Future studies are encouraged to increase the number of replicates to strengthen data reliability. Furthermore, the absence of phytotoxicity was based solely on visual observations, including the lack of necrosis, chlorosis, or visible growth suppression. However, no physiological or biochemical stress markers were evaluated. We therefore recommended that future studies incorporate more sensitive indicators, such as stomatal conductance, membrane integrity, ammonia accumulation, and malondialdehyde content, to better assess potential phytotoxic effects of foliar nitrogen application.

CONCLUSION

Foliar application of urea is recommended at a concentration of 1.0% to improve the acclimatization of *in vitro*-cultivated 'Turiaçu' pineapple plantlets. This practice enhances growth parameters (leaf area, shoot and root dry mass, and rosette diameter), increases photosynthetic pigment levels, and improves photochemical efficiency. In addition, it optimizes the leaf nutritional status by increasing the accumulation of essential macronutrients (nitrogen and potassium) and micronutrients (iron and zinc). Although this study focused on the acclimatization phase, future studies should investigate the long-term agronomic effects of foliar urea application, including its impact on fruit development, nutrient use efficiency, and commercial productivity in different pineapple cultivars.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

All the original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

We declare that this work did not use any artificial intelligence resources for the conception and writing of the manuscript.

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