

Physiological responses of *Physalis angulata* L. grown under molybdenum rates in hydroponic system

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Abstract

Studies on plant development evaluating the effects of molybdenum (Mo) applications are important for nutritional management in crops, such as *Physalis angulata* L. crops. Therefore, the objective of this work was to evaluate the responses of *Physalis angulata* grown under molybdenum rates in hydroponic system. The experiment was conducted in a completely randomized design, with 12 replications and five treatments (Mo rates: 0, 0.005, 0.01, 0.015, and 0.02 mg dm⁻³ of H₂MoO₄·4H₂O). Chlorophyll content, gas exchange parameters, nitrate reductase activity, total protein content, dry weights of leaves, stems, and roots, and root to shoot ratio were evaluated. Supplying Mo (0.02 mg dm⁻³) provided gains in biomass, which is connected to the total plant weight and total chlorophyll content. The nitrate reductase activity in leaves and roots was stimulated by Mo supplementation; the highest activity was found in the roots. Photosynthetic rate (0.010 mg dm⁻³) and water use efficiency (0.015 mg dm⁻³) were favored by increases in Mo rates. The results showed that application of molybdenum as a fertilizer resulted in positive effects on biomass gain, chlorophyll content, photosynthetic rate, and water use efficiency for *P. angulata* plants grown in hydroponics.

Keywords: Micronutrients, mineral nutrition, nitrate reductase, photosynthesis

Introduction

The maintenance of crop productivity depends on several factors for a good plant development, such as availability of nutrients in the soil. Adequate amounts of nutrients have specific and essential functions for plant metabolism, and each species has specific nutrient requirements to use them efficiently to optimize its production (Hansel & Oliveira, 2016). Studies have shown that the soil depletion and inadequate managements of nutrients and amendments to correct soil acidity are the main causes of imbalances in the availability of macro and micronutrients to plants, resulting in nutritional deficiencies in plants (Hansel & Oliveira, 2016). Micronutrients are required in smaller amounts by plants; however, insufficient availability of micronutrients causes limitation of plant growth and development; on the other hand, high amounts of micronutrients in the soil can cause disorders in plant metabolism and induce the

appearance of nutritional deficiencies by antagonism, directly impacting plant productivity (Hansel & Oliveira, 2016; Moschini et al., 2017).

Molybdenum (Mo) is among the essential micronutrients for plant development. This element is highly important for plant metabolism, as it participates in the composition of at least 5 enzymes—nitrogenase, nitrate reductase, aldehyde oxidase, sulfate oxidase, and xanthine oxidase—that catalyze different reactions and are connected to the transport of electrons during biochemical reactions (Resende et al., 2012). The nitrate reductase (NR) is considered the key protein, with molybdenum as its prosthetic group, responsible for reducing nitrate to nitrite during the process of assimilating soil nitrogen (Santos et al., 2014).

The presence of Mo as an enzymatic co-factor in NR activity benefits the N assimilation process via nitrate and nitrite reductases and, consequently, can increase

the efficiency of the N assimilation process in cultivated species. Thus, Mo can be an alternative for increasing the growth and productivity of species that are still little cultivated or underutilized in tropical regions, such as *Physalis angulata* L. However, few studies on the efficiency of Mo in these processes are found in the literature.

Physalis angulata L. stands out due to its high nutritional characteristics. Extracts from this plant have important phytochemical compounds, such as glucocorticoids, flavonoids, and mainly withanolides (present throughout the plant) and physalins (mainly in the leaves) (Renjifo-Salgado & Vargas-Arana, 2013; Sisley et al., 2017). It has proven biological activities, such as leishmanicidal (Silva et al., 2018), anti-inflammatory (Abdul-Nasir-Deen et al., 2020), and anti-parasitic activities (Sun et al., 2016; Sharma et al., 2015); anti-proliferative (Pinto, 2010) and anticancer properties (Baker & Rady, 2020); and aids in regulating the body's natural defense system.

The management and technical information for the cultivation of the genus *Physalis*, in general, are based on the practices used by Colombian growers for the cultivation of *Physalis peruviana* L. (Melo et al., 2017); However, such cultivation practices require adaptations for the edaphoclimatic conditions in Brazil, specifically for regions with a hot humid climate in northeastern Brazil.

Information on the effects of the element Mo on plant metabolism is important as a reference and may allow the use of Mo as a fertilizer for nutritional management of plants, such as *P. angulata* and other species from the family Solanaceae, which are commonly cultivated by small farmers in the semiarid region of the state of Bahia, Brazil.

Thus, the objective of this study was to evaluate the responses of *Physalis angulata* grown under molybdenum rates in hydroponic system.

Material And Methods

The experiment was conducted in a greenhouse at the Horto Florestal Experimental Unit of the State University of Feira de Santana (UEFS), Feira de Santana, Bahia (BA), Brazil (12°14'S, 38°58'W, and an altitude of 258 m), from August 2019 to September 2020.

The seeds used to produce seedlings were from fruits collected at stage three of development, considering the color of the calyx (brownish yellow) (Tanan et al., 2018) from plants that occur naturally in the region of Serra Preta, BA (12°2'S, 39°16'W, and an altitude of 160.22 m). Data of temperature and relative air humidity during the the experimental period (**Figure 1**) were obtained through a digital hygrometer (INCOTERM).

Three seeds were sown at 2 cm depth in 200 mL disposable plastic containers filled with a commercial substrate (Plantamax ®). Daily irrigation was performed twice a day (09:00 a.m. 16:00 p.m.) with the aid of a manual timer turned on for 15 minutes, keeping a 6 mm water depth. The seedlings were thinned 15 days after sowing, keeping those with approximately 4 cm in height and 2 pairs of expanded leaves, which were transplanted into individual containers (6 dm³ capacity) in a floating hydroponic system.

The experiment was conducted using 60 polypropylene containers. The pots were kept on a galvanized iron bench with spacings of 0.8 m between rows and 0.2 m between plants. The nutrient solution was aerated at 15-minute intervals every three hours using a compressor (SCHULZ 250 W) with flow of 100 L min⁻¹.

The nutrient solution of Sarruge (1975) was used, with adaptations for cultivation of *P. angulata* (Leite et al., 2017), consisting of macronutrients (in mg.L⁻¹): N = 210, P = 31, K = 234, Ca = 200, Mg = 48, and S = 64; and micronutrients (in µg.L⁻¹): B = 500, Cu = 39, Cl = 722, Fe = 5000, Mn = 502, and Zn = 98. The treatments consisted of five molybdenum (Mo) rates: 0, 0.005, 0.01, 0.015, and 0.02 mg dm⁻³ of H₂MoO₄·4H₂O (**Figure 2**); the Mo rate of 0.01 mg dm⁻³ is the recommended application rate for species grown in hydroponics (Sarruge, 1975). The pH and electrical conductivity (EC) of the nutrient solution were monitored daily during the experiment, using a portable pH meter (ASKO 50) and a portable conductivity meter (ASKO 90), respectively; pH was maintained at 6.5 and EC at a mean of 2 dS.m⁻¹. The solution was completely replaced whenever the EC decreased by 20% of the initial value (on average every 30 days). The plants remained under these growing conditions for 60 days after transplanting, and the evaluations were conducted at the end of the period.

Chlorophyll content was determined using the method described in Tanan et al. (2017), removing leaf discs from the first pairs of fully expanded leaves collected between 10:00 a.m. and 11:30 a.m. Each sample consisted of five discs (10 mm in diameter) from each plant, which were immediately immersed in 5 mL of 95% alcohol inside sealed tubes (37 mL capacity), without thread, and wrapped in aluminum foil to limit light incidence. The absorbances (664.2 and 648.6 nm) were read in a spectrophotometer (FEMTO 800 XI) after 24 hours. The data were used to calculate the total chlorophyll contents, according to the equations proposed by Lichtenthaler (1987), and the results were expressed as mg.g⁻¹ MF.

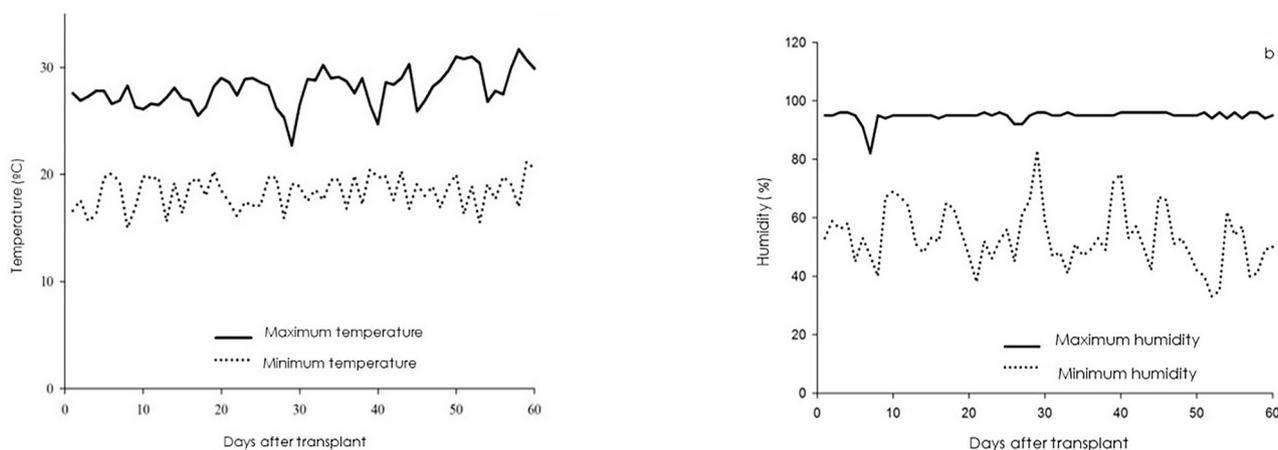


Figure 1 - Air temperature (a) and relative air humidity (b) during the experimental period.

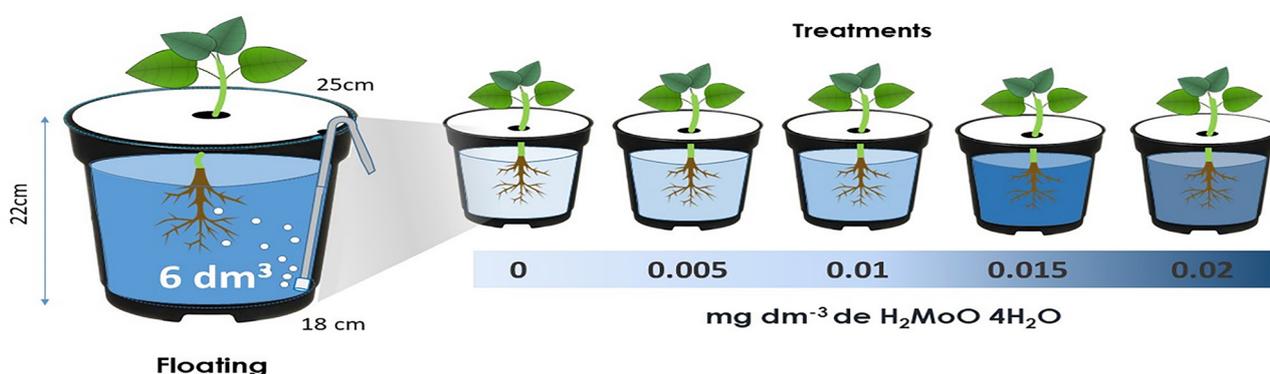


Figure 2 - Treatments consisting of five molybdenum (Mo) rates (0, 0.005, 0.01, 0.015, and 0.02 mg dm^{-3} of $\text{H}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$) in the nutrient solution for hydroponic growing of *Physalis angulata* plants.

Gas exchange measurements were carried out using the Portable Photosynthesis System - IRGA (Infrared Gas Analyzer; PP Systems), with a photon flux of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, CO_2 reference of $400 \mu\text{mol mol}^{-1}$, and constant temperature ($\sim 25 \text{ }^\circ\text{C}$). The following parameters were evaluated: internal carbon concentration (C_i ; $\mu\text{mol mol}^{-1}$), stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), photosynthetic rate (A ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$), transpiration rate (E ; $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), and water use efficiency (WUE; $\text{mmol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$). The measurements were performed using five plants per treatment, on fully developed leaves in the middle third of the plant, recording three measurements for each leaf at 20-second intervals, between 10:00 a.m. and 11:30 a.m.

The *in vivo* activity of the enzyme nitrate reductase (NR) was evaluated using fully expanded young leaves (Figure 4) and root samples, according to the methodology proposed by Jaworski (1971) and adapted for *P. angulata*; the n-propanol concentration used was 1% (Tanan, 2019). Samples of 200 mg of fresh leaf tissue (approximately 20 discs) and 300 mg of root tissue were used, totaling three plant samples per treatment. The absorbance of the samples was determined using a dual beam spectrophotometer (FEMTO 800XI), adjusted

for wavelength = 540 nm. The amount of μmol of nitrite released by 1 g of fresh tissue per hour of incubation ($\mu\text{mol g}^{-1} \text{h}^{-1} \text{NO}_2^-$) was determined to calculate the NR activity using a linear equation obtained from a nitrite standard curve that was prepared beforehand.

Contents of soluble proteins and total free amino acids were determined using one leaf collected from the middle third of the plant. A crude extract was obtained by macerating 1 g of leaf tissue, per treatment, in 10 mL of 0.1M phosphate buffer at pH 7. The homogenate formed was centrifuged at $12000 \times g$ for 15 minutes and the supernatant was withdrawn for analysis. The soluble protein content was determined by the method of Bradford (1976), with bovine serum albumin (BSA) as standard protein. The total free amino acid content was determined by the ninhydrin method, using pure $0.1 \mu\text{mol mL}^{-1}$ Glycine as standard (Yemm & Cocking, 1955). The absorbance of the samples was determined using a dual beam spectrophotometer (FEMTO 800XI) adjusted for absorbance of $\lambda = 595 \text{ nm}$ for proteins and $\lambda = 570 \text{ nm}$ for amino acids; the results were expressed as $\text{mg g}^{-1} \text{MF}$.

Organs (leaves, stems, and roots) of four plants per treatment were separated from the plant at the end of the experiment, placed in paper bags, and left in a

forced air circulation oven at 60 °C until constant weight for determining shoot (leaves and stems), root, and total dry weights (g). Then, root to shoot ratio was calculated.

The experiment was conducted in a completely randomized design, with 12 replications per treatment, considering each pot with a plant an experimental unit, totaling 60 plants.

The data found were subjected to analysis of variance using the F test and to regression analysis at a 5% significance level, adjusting the equations of the evaluated parameters. The statistical analyses were carried out using the software Sisvar (Ferreira, 2011).

Results and discussion

Physalis angulata plants grown in hydroponic system responded to increasing molybdenum (Mo) rates (Figure 3).

A direct correlation between Mo rate and positive linear response ($p < 0.5$) was found for leaf dry weight (LDW) (Figure 3a), stem dry weight (StDW) (Figure 3b), root dry weight (RDW) (Figure 3c), and total dry weight (TDW) (Figure 3d). These variables increased 34.7% (LDW), 60.4% (StDW), 29.7% (RDW), and 48.1% (TDW) compared to those found in plants grown under absence of Mo in the nutrient solution (Figure 4).

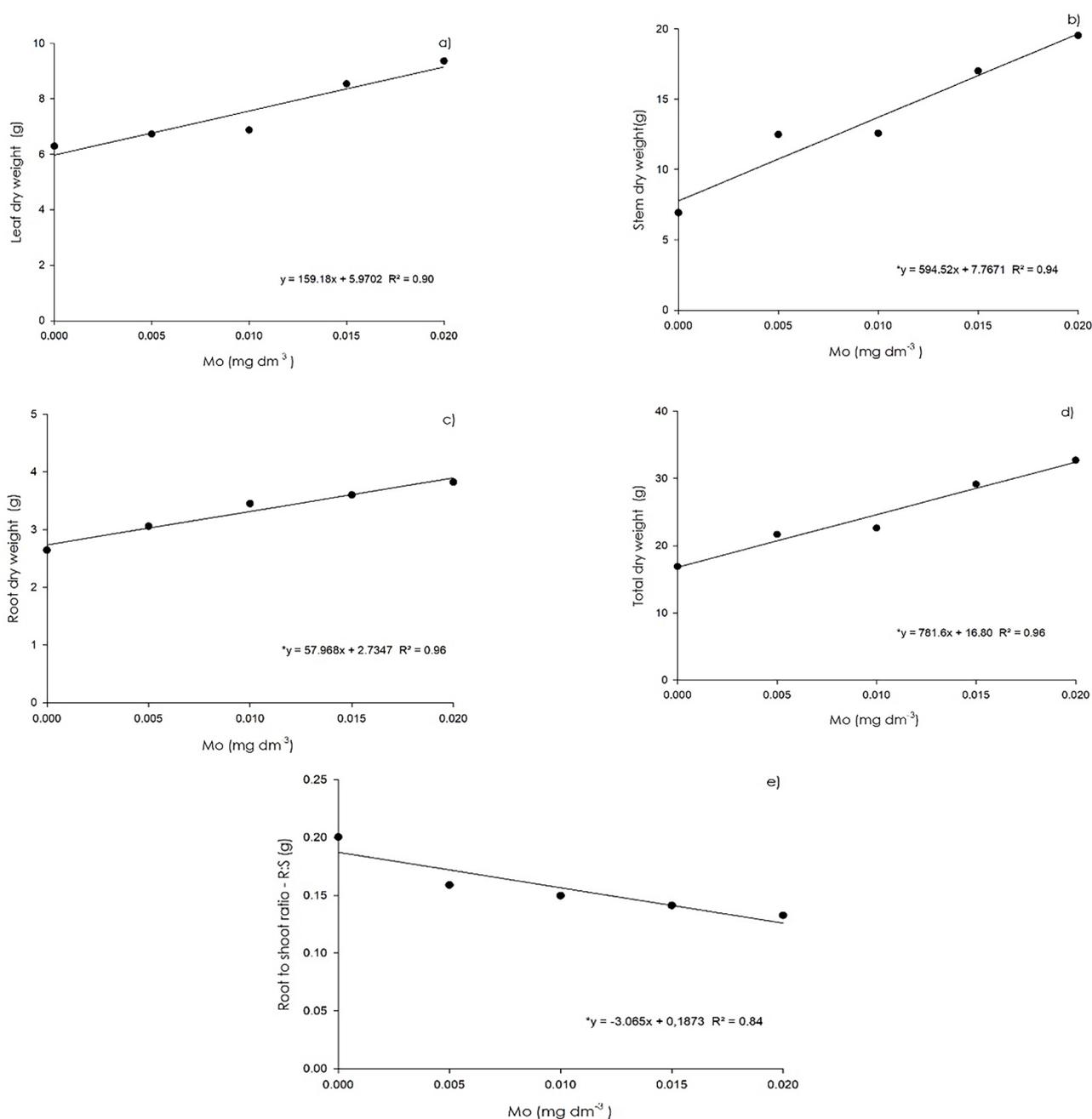


Figure 3 - Biomass accumulation in *Physalis angulata* plants subjected to molybdenum (Mo) rates: leaf dry weight (a), stem dry weight (b), root dry weight (c), total dry weight (d) and root to shoot ratio - R:S (e). *Significant at 5% level.



Figure 4 - Graphic summary of the physiological responses of *Physalis angulata*. L. to molybdenum rates in the nutrient solution.

Skarpa et al. (2013) evaluated sunflower (*Helianthus annuus* L.) plants and found that foliar fertilizer application using Mo rates increased leaf and stem dry weights by 21.4% and 32.6%, respectively.

A decreasing linear response was found for root to shoot ratio (R:S) (Figure 3e), which presented a decrease of 33% compared to that found without addition of Mo (0 mg dm^{-3}) in the nutrient solution. As the Mo rates were increased (0.0015 and 0.020 mg dm^{-3}), a trend towards greater allocation of photoassimilates to the shoots (leaves and stems) was observed, denoting a negative impact on the root system. The results showed that, under conditions of greater micronutrient availability, the biomass production was directed to the aerial part of the plants (shoots). In addition, the plants were at the reproductive phenophase with the presence of flowers, which may have contributed to the allocation of biomass production to the shoots.

Furthermore, the pattern of biomass allocation varies according to plant species and growth environment (Poorter et al., 2012). The plants evaluated in the present work were maintained under the same crop conditions, varying only the Mo rate, which was the factor that promoted increased biomass in the stems when compared to the leaves. Increases in shoot biomass production in plants with medicinal potential, such as *P. angulata*, can contribute to increases in contents of secondary compounds that occur mainly in the shoots of these plants (Cruz et al., 2015).

P. angulata stands out due to the synthesis of simple withanolides derivatives, called physalins, which are synthesized in the leaves along with the other green parts and roots of the plant (Tomassini et al., 2000).

The increase found for phytomass production in *P. angulata* plants denotes a beneficial effect of the evaluated treatments. The limited supply of mineral elements (macro and micronutrients) can cause direct effects, mainly those connected to decrease in biomass

production. Thus, even indirectly, reduced Mo rates can have a negative impact on both the biomass production and the production of compounds derived from withanolides.

Therefore, the accumulation of dry biomass as a response of *P. angulata* plants to increasing Mo rates is a result that reinforces the importance of this micronutrient for physiological processes, such as N metabolism. The Mo ion acts as an enzymatic cofactor of nitrate reductase (NR) activity, which is affected by environmental factors, such as ambient temperature and availability of water and nutrients to plants (Mazid et al., 2012). The activity of this enzyme is essential for capturing and incorporating inorganic N into important organic molecules, such as proteins, amino acids, and chlorophylls.

The results for NR activity showed that using Mo as a fertilizer provided gains in the enzyme activity in leaves and roots of *P. angulata* (Figure 5). The data of NR activity in leaves fitted to a linear equation, since the enzyme activity increased as the Mo rate was increased. The highest amount of NO_2^- ($0.357 \text{ } \mu\text{moles of NO}_2^-$) released in the incubation solution was found for plants subjected to the highest Mo rate tested, resulting in an increase of 98% compared to plants grown in nutrient solution without addition of the micronutrient. These results are significant in terms of micronutrients as growth stimulants for Solanaceae species, particularly when considering chemical fertilization with macronutrients alone. Moreover, few studies have been conducted to evaluate the response of NR activity to crop fertilization using Mo.

The results found for the evaluated *P. angulata* plants reinforce that Mo supplementation has an important function as an enzymatic cofactor in nitrate reductase synthesis, which is further intensified by the presence of nitrate as a substrate (Almeida et al., 2013); once available to plants as inorganic N, nitrate reductase can be incorporated into organic molecules, such as

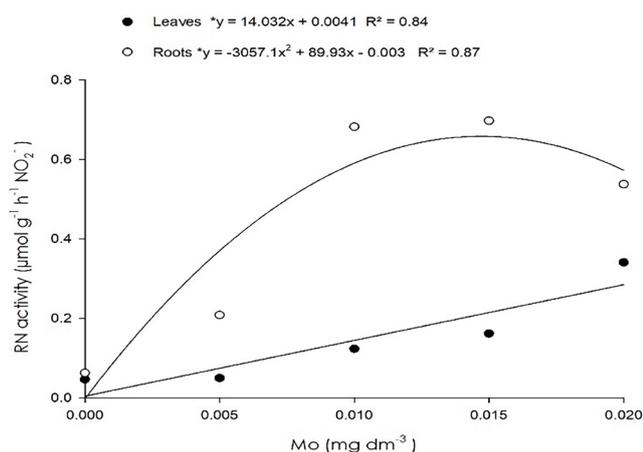


Figure 5 - Nitrate reductase activity in leaves and roots of *Physalis angulata* plants subjected to different molybdenum (Mo) rates in the nutrient solution in a hydroponic system. *Significant at 5% level.

amino acids, proteins, and chlorophylls, resulting in increases in plant dry weight (Figure 3).

A greater NR activity in roots of *P. angulata* plants was found in response to Mo rates; the highest estimated NR activity was $0.68 \mu\text{mol}$ of NO_2^- for the Mo rate of 0.014 mg dm^{-3} , but it decreased to a similar level to that found for leaves when using the Mo rate of 0.02 mg dm^{-3} (Figure 5).

The reduction of NO_3^- can vary depending on the plant organ (Tanan, 2019). The absorbed NO_3^- can be reduced in roots or transported through the xylem to the leaves for further reduction (Taiz et al., 2017). However, the assimilation of NO_3^- in leaves is generally more efficient, whereas the reduction of NO_3^- and assimilation of NH_4^+ in roots or other non-green tissues depend on the chemical energy from the photoassimilates provided by the leaves' metabolism.

The reduction of NO_3^- in roots (Figure 5) increased as the Mo rate was increased, denoting that the assimilation routes can be competitive. This competitiveness between NO_3^- reduction sites may be related to environmental conditions, as the NR activity in *P. angulata* roots is affected by high solar incidence and temperature. Accordingly, Tanan et al. (2019) evaluated variations of NR activity in roots and leaves of *P. angulata* plants and found that the plants showed peaks of NR activity in the period of highest solar incidence and temperature: the highest activities of NR in leaves were found at 10:00 a.m. and 2:00 p.m., and NR activity in the leaves increased after 4 hours of light incidence.

In addition, several works have been developed evaluating the performance of the enzyme nitrate reductase due to its regulatory function and its effect on plant growth and development under different

experimental conditions; they are based on the hypothesis that plants with high NR enzyme activity may have greater potential to assimilate NO_3^- and respond better to nitrogen fertilization (Beevers & Hageman, 1969).

Viana & Kieh (2010) evaluated the effects of combinations of nitrogen rates on the enzymatic activity of nitrate reductase in leaves of wheat plants and found the highest enzymatic activity for the N rate of 158 mg dm^{-3} , but from this rate onwards, the NR activity decreased. They attributed this result to the period when the plants were collected (79 days after nitrogen supply), when nitrogen was no longer available in the soil solution to maintain the NR activity, which may be further limited by the absence of other nutrients, such as molybdenum.

These studies contribute to the understanding of the effects of external factors on the plant responses found in the present work. An adequate Mo supply to plants can increase NR activity and, consequently, N assimilation as it is available to the plant. This mechanism may be favorable for the synthesis and high maintenance of amino acids and proteins in leaf and root tissues.

Regarding the soluble protein contents in *P. angulata* plants, the highest estimated content was 11.72 mg g^{-1} MF for the Mo of rate 0.011 mg dm^{-3} (Figure 6a). Oliveira et al. (2017) found that the results of protein contents in soybean seeds fitted to an increasing linear equation as the Mo rate was increased. They attributed this result to the fact that Mo is part of the nitrogenase molecule and the enzyme nitrate reductase, which are directly connected to N metabolism in soybean plants, which can contribute to increases in N incorporation and, consequently, in protein contents.

Increasing the Mo rates from 0.015 mg dm^{-3} onwards caused a quadratic response with decreasing protein contents in the leaves. These decreases were significant and can be attributed to the plant development stage at the period of evaluations. Despite the absence of physical signs of senescence, the evaluations were conducted at the early stage of the reproductive phase, which is characterized by the continuous emergence of flower buds and small fruits. Silva (2017) evaluated tomato crops to determine the nutritional crop status and found that some of the photoassimilates produced are used to maintain the physiological activity of the leaves during the reproductive stage. On the other hand, the maximum daily absorption of nutrients occurs during the beginning of the fruiting stage, when a mobilizing force of nutrients and assimilates is established, driven by an increase in metabolic activity connected to hormonal activity, as well as cellular division and growth.

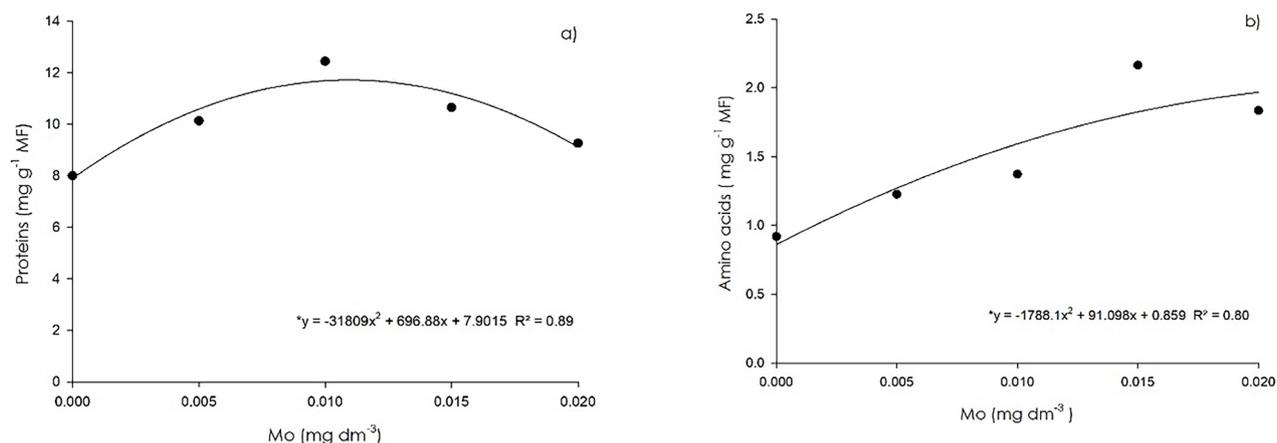


Figure 6 - Contents of soluble proteins (a) and total free amino acids (b) in leaves of *Physalis angulata* plants subjected to different molybdenum (Mo) rates in the nutrient solution in a hydroponic system. *Significant at 5% level.

Concomitantly with the decrease found for total soluble proteins in *P. angulata* plants, an increase in free amino acid contents was found (**Figure 6 b**), which presented a positive exponential correlation with the increase in Mo rates; the highest estimated content of free amino acids in the plants was 0.0025 mg dm⁻³.

Increases in protein and amino acid contents were found up to the Mo rate of 0.01 mg dm⁻³ (**Figure 6**). The plants were evaluated when they were in the reproductive phase and the NR activity was higher in roots than in leaves up to the Mo rate of 0.014 mg dm⁻³ (Figure 5); therefore, nitrogen assimilation may have been more efficient in the roots, resulting in formation of nitrogenous organic compounds, which may have been subsequently transferred to both the leaves and the reproductive parts.

The nitrate assimilation rate in the roots decreased as it increased in the leaves from the Mo rate of 0.014 mg dm⁻³ (Figure 5). This indicates that a portion of the nitrate was transported to the leaves, where it was assimilated and used to form amino acids, which could have been subsequently transported to other sink organs. Amino acid contents in leaves increased while protein contents decreased from the Mo rate of 0.014 mg dm⁻³. This denotes that the amino acids that were being used for protein synthesis in the leaves may have been directed to other sink organs, such as the flowers, which are stronger sinks than vegetative organs (leaves); the reduction in NR activity in the roots is also an important factor to consider.

Nogueira et al., (2015) evaluated the effects of combining the nutrients boron, zinc, and molybdenum on contents of amino acids and proteins in pea grains 72 days after emergence. The authors found that a single Mo rate did not affect significantly the amino acid and protein contents in pea grains. They attributed this result to the possible presence of sufficient amounts of

these micronutrients in the reserves of seeds used for sowing, which may have supported the growth and establishment of seedlings without the need for additional Mo supplementation.

Total Chlorophyll contents increased significantly ($p < 0.05$) as the Mo rate was increased (**Figure 7**). The mean total chlorophyll content found was 0.44 mg g⁻¹ MF. Liu et al. (2010) studied the effects of applications of Mo and zinc on grain yield of winter wheat (*Triticum aestivum* L.) plants and found significant increases in total chlorophyll contents following applications of single Mo rates, whereas chlorophyll synthesis reduced in plants grown without applications of Mo rates.

The increases found for total chlorophyll contents due to increases in Mo rates denote that the mechanisms of absorption of inorganic N (an essential nutrient for chlorophylls) and NR activity can be favored by the increase in the Mo supplementation, thus establishing a positive correlation with levels of photosynthetic pigments.

Increases in chlorophyll contents can increase light absorption and electron transmission during the

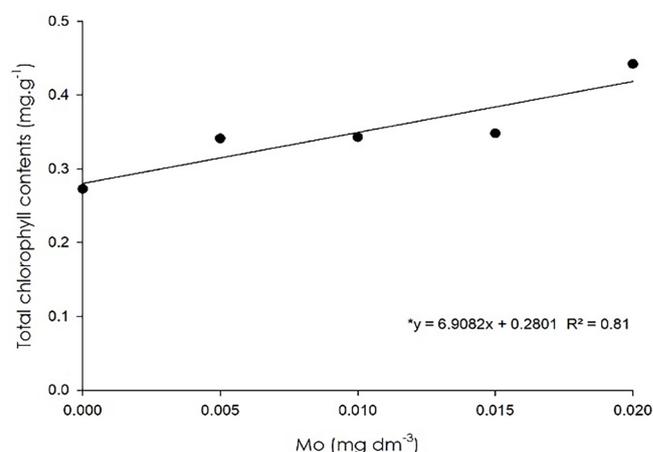


Figure 7 - Total chlorophyll contents in leaves of *Physalis angulata* plants subjected to different molybdenum (Mo) rates in the nutrient solution in a hydroponic system. *Significant at 5% level.

photochemical phase of photosynthesis (Rodrigues et al., 2016) and probably generate gains for process of formation of ATP and NAD(P)H. Contrastingly, large reductions can cause greater excitation in chlorophyll molecules, which can cause formation of free radicals and photo-oxidation, affecting other membranes in the photosynthetic process, resulting in plant death (Oliveira et al., 2018). These findings are important indicators of the function of molybdenum in chlorophyll biosynthesis, as this micronutrient promotes the stabilization of chlorophyll structure and an increase in the number and volume of chloroplasts, consequently resulting in a better performance for chlorophyll synthesis (Iannone et al., 2015).

Regarding the gas exchange parameters, the treatments had significant effects ($p < 0.05$) on stomatal conductance (g_s), transpiration rate (E), internal carbon concentration (C_i), photosynthetic rate (A), and water use efficiency (WUE) (Figure 8).

The parameter g_s (related to stomatal opening) decreased linearly as the Mo rate was increased ($R^2 = 0.78$) (Figure 8a). The higher mean g_s ($409.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was found in plants grown without Mo (0 mg dm^{-3}) in the nutrient solution, denoting that stomata opening was under no functional restrictions; however, this result did not cause increases in CO_2 assimilation (Figure 8d). Additionally, the photosynthetic rate followed the NR activity (Figure 5), i.e., the decrease in enzyme activity in leaves decreased the photosynthesis (Figure 8d) and increased internal CO_2 concentrations (Figure 8c). This was probably due to restrictions in the photosynthetic apparatus connected to the synthesis of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO).

The transpiration process (Figure 8b) is determined and controlled by stomatal performance (Lima et al., 2010), which depends on environmental conditions, such as relative air humidity and temperature, for its regulation. High relative humidity can cause decreases in the transpiration rate, whereas increases in temperature result in a higher transpiration rate and may also increase respiration and a concomitant increase in intercellular carbon dioxide concentrations, which can cause stomatal closure (Taiz et al., 2017).

Regarding the crop conditions over the experimental period, the temperature increased over the period and the relative humidity was constant (Figure 1). High temperatures can have harmful effects on plants, mainly on their gas exchange and photosynthetic functions, such as disrupting stomatal opening and

closure and affecting primary photochemical reactions (Salisbury & Ross, 2012). Transpiration rate (E) (Figure 8b) followed the same dynamics of g_s (Figure 8a). The higher E means were found with the lower Mo rates. The lowest mean E ($4.3 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was found with the highest Mo rate (0.02 mg dm^{-3}).

This result may be connected to an intensification of the ammonium conversion process catalyzed by nitrate reduction due to increases in Mo rates. Ammonium can have a negative effect on stomatal conductance, directly affecting transpiration (Silva et al., 2010), which may explain the decreases found for these variables as response to the higher Mo rates tested. However, Mo can contribute to the synthesis of abscisic acid (ABA), which has an essential function in regulating the water status of guard cells and inducing gene expressions that are involved in cellular dehydration tolerance.

Wu et al. (2014) studied the effects of Mo on WUE, antioxidant defense, and osmotic adjustment in winter wheat (*Triticum aestivum*) plants under water stress. They found that decreases in g_s and E , induced by using Mo, contributed for the maintenance of an increased water status. Despite the decreases found for photosynthetic rate from the Mo rate of 0.013 mg dm^{-3} , no negative effect on dry biomass accumulation was found (Figure 3). Leite et al. (2018) found similar results for g_s and E in *P. angulata* plants grown in soils with no water restrictions: approximately $270 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and $2.6 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, respectively. Regarding C_i (Figure 8c), the treatments with Mo rates of 0 and 0.005 mg dm^{-3} caused a linear decrease with increasing the micronutrient rate. The highest means found were 305.25 and $295.17 \text{ } \mu\text{mol mol}^{-1}$, denoting the presence of carbon in the substomatal chamber. The presence of carbon dioxide in the substomatal chamber is enough to increase the activity of carboxylative enzymes, but this relationship is not always direct, as several other factors are regulatory, such as the number of enzymes and coenzymes in the carbon assimilation process.

Decreases in C_i were found for the highest rates of the micronutrient, mainly for the recommended rate for hydroponics (0.01 mg dm^{-3} of Mo), presenting a mean of $278.17 \text{ } \mu\text{mol mol}^{-1}$ (Figure 8 c). The limitation in C_i due to stomatal closure, caused by the higher Mo rates, may have induced RuBisCo to use the carbon that was in the stomatal chamber, which probably was not enough to saturate all the active sites; this may have caused a small decrease in photosynthesis (A) (Figure 8d) due to lack of CO_2 .

The results found for A (Figure 8d) and WUE (Figure

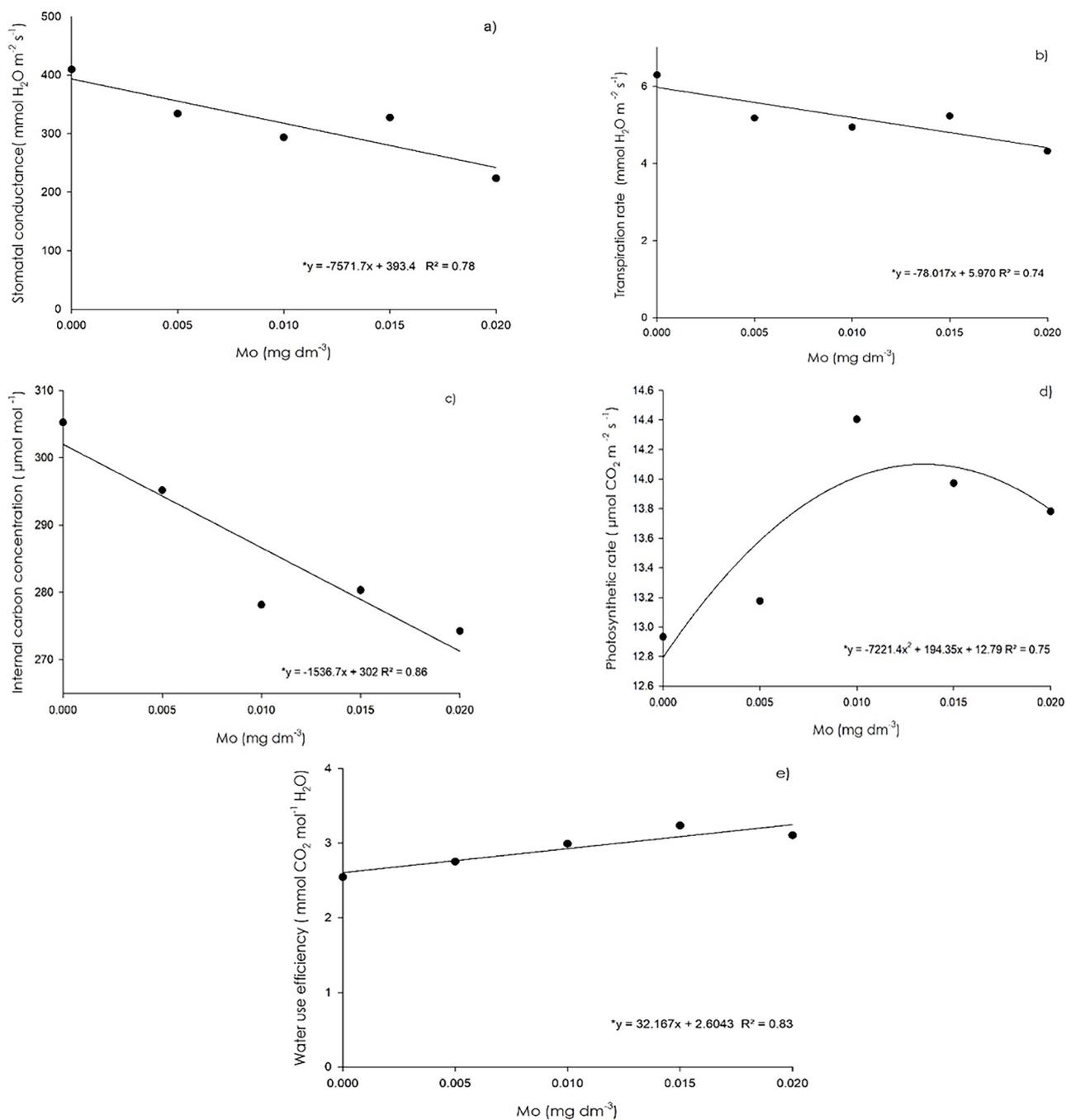


Figure 8 - Stomatal conductance - g_s (a), transpiration rate - E (b), internal carbon concentration - C_i (c), photosynthetic rate - A (d), and water use efficiency - WUE (e) of *Physalis angulata* plants subjected to different molybdenum (Mo) rates in the nutrient solution in a hydroponic system. *Significant at 5% level.

8e) differed from those found for the other gas exchange variables ($p < 0.05$). A data presented a quadratic response ($R^2 = 0.75$); the lower means (12.93 and 13.17 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) were found for the Mo rates of 0.0 and 0.005 mg dm^{-3} , corroborating the lower NR activity found in the leaves with the same rates (Figure 5). Photosynthesis is necessary for activation of the NR enzyme in leaves, possibly through assimilates that act as chloroplast signals, causing rapid changes in the enzymatic activity (Kaiser & Huber, 2001).

WUE is connected to the amount of carbon

fixed by the plant per unit of transpired water (Campos et al., 2016). WUE presented a linear response ($R^2 = 0.83$), with 3.23 and 3.10 $\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$ for the highest Mo rates: 0.015 and 0.02 mg dm^{-3} , respectively (Figure 8e). According to these results, increasing the Mo rate increases WUE in *P. angulata* plants, resulting in minimal losses of available water. Similar dynamics was found for g_s (Figure 8a), during CO_2 assimilation in the photosynthesis for biomass production (Iqbal et al., 2011), as shown in (Figure 3).

Conclusions

The use of molybdenum (Mo) as a fertilizer in the nutrient solution at a rate of 0.020 mg dm⁻³ provided gains in biomass and increases in the contents of photosynthetic pigments in *Physalis angulata* plants grown in hydroponic system.

The photosynthetic rate and water use efficiency were favored by the increases in Mo rates.

Acknowledgements

The authors thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) - Financing Code 001".

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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