Characterization of nitrate reductase activity (NR) in foliar and radicular tissues of *Physalis angulata* L.: diurnal variations and protocol optimization

Tamara Torres Tanan[1]*, Marilza Neves do Nascimento[2], Alismário Leite da Silva[3], David Santana Guimarães[2], Romeu da Silva Leite[2], Larissa de Souza Pereira[3], Francisco dos Santos Neto[3]

[1]Postgraduate Program in Plant Genetic Resources, Universidade Estadual de Feira de Santana, Brazil
[2]Department of Biological Sciences, Universidade Estadual de Feira de Santana, Brazil
[3]Agronomy course, Universidade Estadual de Feira de Santana, Brazil

*Corresponding author: tamara.tanan@yahoo.com.br

Abstract

Nitrate reductase (NR) is the first enzyme in the nitrogen assimilation pathway. The determination of its activity requires modifications for each plant species. The goal of this work was to evaluate the variation of NR activity throughout the day and the optimization of assay conditions in foliar and radicular tissues of *Physalis angulata*. The analysis was done in plants cultivated in a hydroponic system at two months of age. The NR activity was based on the *in vivo* assay method. Enzyme activity was observed on leaf and root, indicating two sites of nitrate assimilation with a higher activity in the daylight in leaf. The NR activity in leaf was increased after 4h of luminosity. In the root, we observed a high activity during most of the day, especially in periods of higher solar radiation and temperature. To obtain the highest activity of NR in both tissues we suggest 1% n-propanol, 50mM of KNO_3^- in pH=7 phosphate buffer for 75 min incubation in water bath.

Keywords: nitrogen, enzyme activity, daylight rhythm, camapú.
Abbreviations: aRN_nitrate reductase activity, Hmax_maximum humidity, Hmin_minimum humidity, N_nitrogen, RN_nitrate reductase, Tmax_maximum temperature, Tmin_minimum temperature.

Introduction

Physalis angulata L. is an herbaceous plant, belonging to the Solanaceae family, popularly known as camapú (Lorenzi and Matos, 2008). It is a multipurpose species. Its fruits have a sweet and slightly acid taste. They have the potential to be commercially exploited (Curi et al., 2018). *P. angulata* also has a medicinal potential, attributed to its phytochemical composition, in which flavonoids, alkaloids and different steroids can be found. Moreover, physalins were found in leaves, stem and root. It has diverse pharmacological properties such as antiparasitic, antiviral and antineoplasic activities (Marin Sisley et al., 2017; Tomassini et al., 2000).
Despite its potential, the cultivation of *P. angulata* is still incipient and the researches about its management are scarce. Regarding its mineral nutrition, some studies were concluded with the species (Cruz et al., 2015; Tanan et al., 2013; Thomé and Osaki, 2010). Leite et al. (2017) evaluated the nitrogen (N) influence, observing a strong correlation between an increase in dosage and a greater growth and fruit production. Nitrogen is the macronutrient mostly required by plants, with direct effects in the distribution of photoassimilates to the vegetative and reproductive parts, related to photosynthesis, growth of the radicular system, ionic absorption of nutrients and cellular development (Queiroga et al., 2007).

The nitrate is considered one of the most important nitrogen sources to the vegetables and it is the main form of inorganic nitrogen available to plants. However, this ion needs to be reduced to be incorporated into organic molecules. The nitrate reductase (NR) is a flavoprotein that executes the first step in the nitrogen assimilation pathway, the reduction of nitrate to nitrite through the reducing agent NAD(P)H (Taiz and Zeiger, 2017), having a remarkable importance in plant metabolism (Delú-Filho et al., 1998). The NR possess several regulation mechanisms important to avoid nitrite accumulation, which is considered toxic. The main regulation mechanisms are at the transcriptional and post-transcriptional levels. The first occurs in the long term and can be induced by nitrate, light, cytokinins, sucrose and CO_2. The second mechanism is fast and occurs in minutes, being essential to metabolism adaptation to diurnal variations of light/darkness and luminous intensity (Matsumura, 2012).
The method for evaluation of NR activity can be done *in vivo* and *in vitro*. The *in vivo* method has been widely used as an indicator of nitrogen metabolism in plants and consists of the incubation of plant tissue in a place that provides ideal conditions for enzyme activity under dark conditions. The nitrite diffusion rate to the medium is used as an indicator of enzyme activity (Lee and Titus, 1992). This method is less

laborious, and it is considered by some authors as the most adequate for comparison of NR activity between different species or between different treatments (Crafts-Brandner and Harper, 1982; Singh, 1994; Cairo et al., 1994). However, this method has the disadvantage of requiring adaptations in assay conditions, according to the nature of the vegetal tissue (Jaworski, 1971).

Also, another important factor is the knowledge of the diurnal rhythm of NR, which has already been observed in some plants (Nievola and Mercier, 2001; Santos et al., 2014; Oliveira et al., 2005; Queiroz et al., 1993), indicating the increase of NR activity concomitant to luminous intensity. It is extremely important to determine the ideal schedule for performing the assay, coinciding with the period of NR maximum activity (Nievola and Mercier, 2001).

Each species has its anatomical and physiological particularities, so it is necessary to determine appropriate conditions to each species (Santos et al., 2014). Passos (2013) evaluated the NR activity in plants of *Physalis peruviana* L. and *Physalis pubescens* L. under different doses of nitrogen fertilizing. However, the knowledge on the diurnal cycle and the ideal conditions for performing *in vivo* assay is absent in the genus. In this context, the goal of this work was to evaluate variations of NR activity during the day and the optimization of assay conditions in foliar and radicular tissues of *P. angulata*.

Results and Discussion

Diurnal variations

The nitrate reductase activity (aNR) in plants of *P. angulata* was observed in radicular and foliar tissues, showing two sites of nitrogen assimilation. Since NR is an enzyme that has its activity influenced by different environmental factors, activity variations were observed throughout the day in both tissues (Figure 1).

In the root, the activity could be measured from the first hours in the morning, with two peaks at 10:00am and 02:00pm, which coincide with the period of highest solar radiation and temperature (Figures 1 and 2). The opposite occurred in the foliar tissues, in which a lower aNR was observed at the same moments. The absorbed nitrate can be reduced in the root or transported via xylem to be reduced in leaves. This transportation occurs by generation of negative pressures inside the xylem, because of the capillary forces in cellular walls of transpiring leaves (Taiz and Zeiger, 2017). In moments with high temperature and solar radiation, the plants tend to close their stomata to avoid water loss, reducing the translocation flow through the xylem; and consequently the transportation of nitrate from the root to the leaves. There is also a reduction of foliar NR activity and an increase of activity in the root, since the increase of nitrate content induces messenger RNA transcription involved with NR codification (Konishi and Yanagisawa, 2011).

Then, like nitrate, the light induces a high expression of NR genes (Tischner, 2000; Lillo et al., 2001), which was observed with the aNR reduction occurred at the end of the light period, mainly in leaves (Figure 2). The light may induce the enzyme directly, in a phytochrome-mediated pathway, or indirectly, through photosynthesis and its photoassimilates (Rajasekhar et al., 1988; Lillo et al., 2004). According to

Kaiser and Huber (2001), in normal conditions of activation and in the presence of light, NR activity would be in the order of 70% to 90%, reducing to 10% to 30% in the dark, corroborating with observation in this experiment. When nightfall occurred at (06:00pm), a 67.7% reduction in NR activity observed compared to the activity registered in periods with greater light intensity.

Nievola and Mercier (2001) also observed this performance in pineapple leaves, which presented greater aNR in the presence of light. Santos et al. (2014) observed maximum values of aNR at 01:00pm for sugarcane, indicating that the global solar radiation may be the climatic factor that most influences the enzyme activity. However, for coffee plants it was observed a gradual decline throughout the light period, followed by an increase of activity in the first hours of the night (Queiroz et al., 1993). The NR high sensitivity to variations of light/dark is controlled by a post-translational mechanism that involves the phosphorylation of a serine residue followed by the interaction with a signal-transducing protein, inactivating the enzyme. When exposed to light, this protein no longer interacts with NR, and the enzyme becomes active again (Lea et al., 2006; Souza et al., 2002).

Effect of incubation time on NR activity

Talking about the optimization of assay conditions, it was observed a linear growth of aNR with the increase of incubation time for both tissues, being more pronounced in leaves (Figure 3A). For the determination of aNR it is necessary to have a diffusion of available nitrate in the medium to the vegetal tissue and subsequently nitrite liberation. The highest enzymatic activity, with a prolonged incubation period may be related to a time requirement for complete infiltration of the reaction medium (Oliveira et al., 2005), being that period variable according to the species and tissues studied. For many plants such as coffee, peach-palm, pineapple, *Brachiaria* and macroalgae, similar performances of increased enzyme activity with longer incubation periods have been observed (Meguro and Magalhães, 1982; Nievola and Mercier, 2001; Oliveira et al., 2005; Martins et al., 2009; Cazetta and Villela, 2004). According to the obtained results, an incubation time of 75 minutes was adopted for all subsequent analyses.

Effect of n-propanol addition on NR activity

The nitrate input and the release of nitrite from the vegetal tissue may be increased with the addition of organic solvent to the medium, which allows for a general increase in membrane permeability, being the n-propanol one of the most effective (Jaworski, 1971). Regarding the solvent concentration used in leaves of *P. angulata* there were no significant differences in enzyme activity. However, for radicular tissue, the increase of n-propanol concentration led to a linear decrease on aNR (Figure 3B). The use of some organic solvents, in high concentrations, may lead to a disorganization of protein assemblies (Jamur and Oliver, 2010), and in foliar tissue the use of n-propanol in high concentrations may cause chlorophyll diffusion in the incubation medium and affect colorimetrically the estimation of NR activity (Streeter and Bosler, 1972).
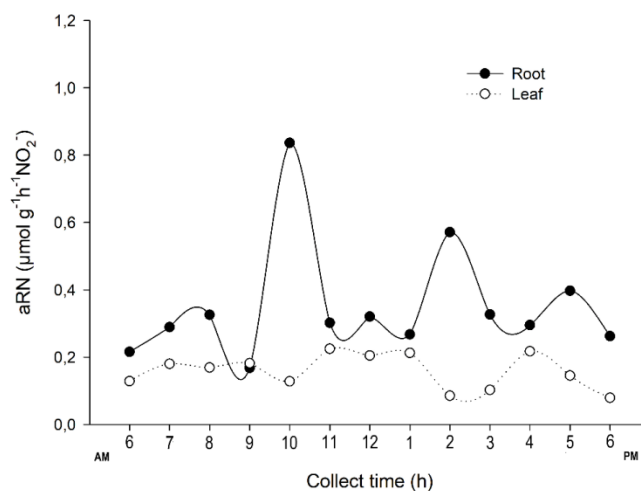
Fig 1. Diurnal variation of nitrate reductase activity in foliar and radicular tissues of *Physalis angulata* L.
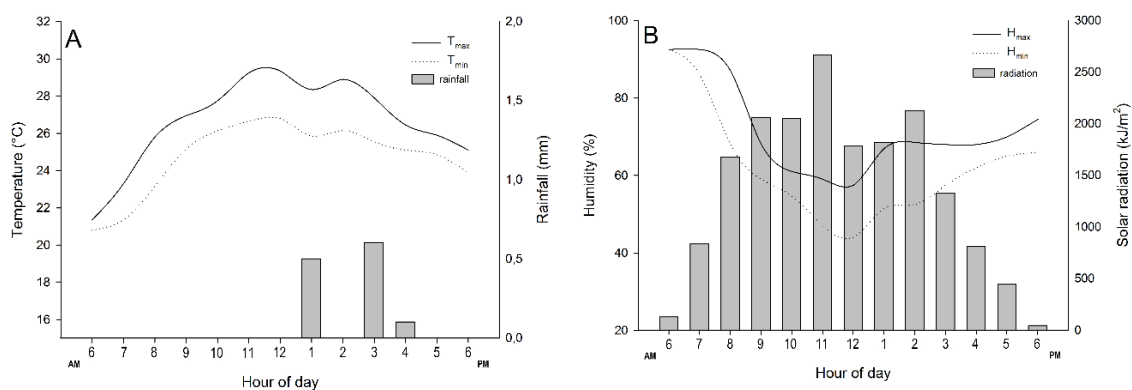

Fig 2. Meteorological data obtained from the Universidade Estadual de Feira de Santana Weather Station. Average values of evaluation days (A - pluviosity and maximum and minimum temperature; B - Solar radiation and maximum and minimum air humidity).
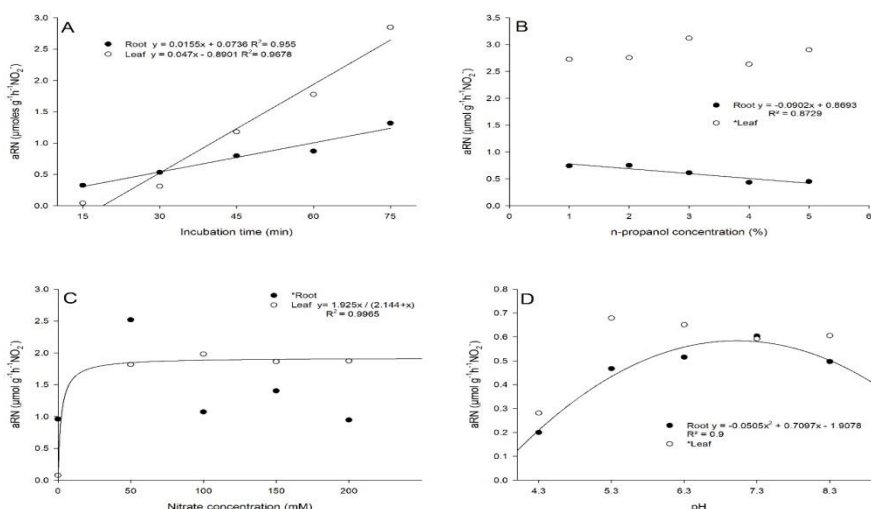

Fig 3. Method optimization for quantification of nitrate reductase (NR) activity. A - NR activity at different incubation time periods; B - NR activity at different n-propanol concentrations; C - NR activity at different nitrate concentrations; D - NR activity at different pH values.

1122

However, these effects varies according to cellular and anatomical characteristics of plants of different species.

In sugarcane leaves, the reduction of aNR was observed with the increase of n-propanol concentration (Santos et al., 2014). Regarding the radicular tissue of *Citrus sinensis* L. Osbeck, there was no difference between concentrations, but for foliar tissues a reduction was occurred starting at 3% (Dovis et al., 2014). In pineapple, Nievola and Mercier (2001) recommend the use of n-propanol at 3% and 4% for radicular and foliar tissues, respectively. These observed differences justify the need of method optimization to each species. In this work, with *P. angulata,* a concentration of 1% was adopted, since there were no differences between the different concentrations tested.

Effect of nitrate addition on NR activity

The activity data of foliar NR in relation to nitrate supply were adjusted to a rectangular hyperbola, a model used to describe the saturation kinetics of a ligand to its substrate (Motulsky and Christopoulos, 2004). The enzyme activity in the leaf was observed when exogenous nitrate was supplied, being 50mM the adopted concentration for subsequent analyses, since there were no differences between the higher concentrations tested (Figure 3C). This stabilization tendency was observed by other authors to sugarcane, *Brachiaria* and pineapple leaves (Santos et al., 2014; Cazetta and Villela, 2004; Nievola and Mercier, 2001). The initial increase in aNR in response to nitrate addition to the medium has been linked to a *de novo* enzyme synthesis as well as its activation (Solomonson and Barber, 1990).

Differently from what was observed to the leaves, an expressive activity of NR was detected even without addition of exogenous KNO_3^-, indicating the presence of readily available nitrate accumulated in cellular vacuoles (Dovis et al, 2014). However, the addition of 50mM nitrate increased the enzyme activity by more than 100%, followed by a reduction of activity in higher concentrations of nitrate in the incubation medium (Figure 3C). The reduction of aNR caused by higher nitrate concentrations probably occurs due to alterations in the equilibrium of ionic charges caused by a high ionic concentration, to the reduction of osmotic potential (Cairo et al., 1994) and to the increase of salt concentration in the reaction medium (Meguro and Magalhães, 1982).

Effect of pH on NR activity

Among the pH range chosen to this study, the lowest NR activity was at pH 4.3 for both tissues. In the leaf, with other pH values, there were no significant differences, allowing for the use of a relatively wide range of pH. The radicular tissue presented a quadratic response with a maximum aNR at pH 7. The highest enzyme activity at a pH close to neutral has been observed by some authors in coffee plants (Meguro and Magalhães, 1982), sugarcane (Santos et al., 2014), peach-palm (Oliveira et al., 2005), pineapple (Nievola and Mercier, 2001) and in *Brachiaria* (Cazetta and Villela, 2004), indicating that this is the enzyme's optimum pH, at which it is most active. The pH may influence enzyme activity in several ways, altering the charge distribution of an active site, altering the enzyme's structural conformation (Nelson and Cox, 2014) or modifying the translocational velocity of

nitrate and nitrite ions through cellular membranes (Oliveira et. al. 2005).

Materials and Methods

Location of study

The study was carried at an experimental facility (Unidade Experimental Horto Florestal) located at the State University of Feira de Santana (UEFS), in the period of July to October, 2017.

Plant materials

The plants were obtained by sowing in plastic cups containing soil from the same experimental facility. The seedlings were transplanted to a hydroponic system when they presented two pairs of true leaves and approximately 10cm.

Evaluations were done using 15 plants at two months of age, when they started the reproductive stage. Samples of root and leaf from plants were collected and immediately taken to the laboratory, where they were washed with distilled water. The radicular tissue was fragmented in smaller uniform pieces using scissors and from the foliar tissue 8mm disks were removed using a piercer.

Conduction of study

The experiment was carried in hydroponic system containing Sarruge solution modified for *Physalis* cultivation (Leite et al., 2017), composed of the following macronutrients, in mg L^{-1}: N=162; P=31; K=234.6; Ca=200.4; Mg=48.6; S=64.1.

The electrical conductivity (EC) was measured daily and the solution changed when the EC reduced by 30%. The pH of the solution was maintained at 6.5. For pest control, 5% solution of Neem (*Azadirachta indica*) was applied weekly.

Laboratorial analysis

The *in vivo* assay for NR activity was done following the methodology proposed by Jaworski (1971).

To evaluate diurnal variations of NR activity, samples of foliar tissue were collected from 06:00am to 06:00pm, at 1-hour intervals. 300mg of radicular tissue and 200mg (approximately 20 disks) of foliar tissue were transferred to test tubes containing 5mL of incubation medium containing 0.1M phosphate buffer, pH 7.5, 1% (v/v) n-propanol and 0.1M potassium nitrate. The tubes were wrapped in aluminum foil to be protected from light and subjected to vacuum for 2 min, followed by a 30-minute incubation in a water bath at 30°C with constant shaking. For the radicular tissue, a 1-minute N_2 bubbling was previously performed. At the end of the experiment, an aliquot of 500uL of medium was taken and transferred to tubes containing a reaction medium composed of 1mL of 1% sulfanilamide in HCl 1.5N, 1mL of 0.02% N-2-naphthyl-ethylene and 1.5mL of distilled water (Jaworski, 1971). The climatological data from the evaluation days were obtained in the Climatological Station of Feira de Santana, BA, WMO code: 86658 (INMET, 2017) (Figure 2).

For the optimization of assay conditions, sample collections were done at 11 hours for foliar tissue and 14 hours for

radicular tissue, using the same amounts of material from the previous experiment. The procedure consisted of sequential assays similar to the first one, modifying one factor at a time, using the highest enzyme activity registered from the previous assay as reference.

The absorbance of the samples was determined using a quartz cuvette in a double-beam spectrophotometer (FEMTO 800XI), adjusted to 540nm. The NR activity was estimated in μmol of nitrite liberated by 1g of fresh tissue per incubation hour ($\mu mol\ g^{-1}h^{-1}\ NO^{2-}$) and it was calculated based on a linear equation obtained from a previously prepared nitrite standard curve.

Analyzed variables

The variation of the activity of the RN was evaluated throughout the daylight (6:00am to 06:00pm). The effects of varying incubation time (15, 30, 45, 60 and 75 minutes), n-propanol concentration in the incubation medium (1, 2, 3, 4 and 5%), KNO_3 concentration (0, 50, 100, 150 and 200mM) and pH (4.3, 5.3, 6.3, 7.3 and 8.3) were analyzed.

Statistical analysis

The experimental design was completely randomized, with six repetitions. The obtained data were submitted to variance analysis and comparison of means by Tukey's test with 5% of significance using the software Sisvar v.5.3 (Ferreira, 2008). A regression analysis was also performed, using the software SigmaPlot v.11.0 (Systat Software Inc., Chicago, USA).

Conclusion

The nitrate reductase activity in plants of *Physalis angulata* is influenced by environmental factors, with the highest activity in the light period. In the foliar tissues, the analysis should be done after the first four hours of luminosity, when the enzyme activity is increased. In the root, the activity may be evaluated for a longer period during the day, preferentially in the periods of highest solar radiation and temperature. For the evaluation of NR activity in *P. angulate*, it is recommended the use of n-propanol 1% and addition of 50mM KNO^{3-} in the incubation medium, in a pH 7 phosphate buffer. The incubation should be carried out in a water bath for 75min to determine the maximum activity.

Acknowledgements

References

Cairo PAR, Oliveira LEM, Delú Filho N (1994) Determinação das condições ótimas para o ensaio in vitro da redutase do nitrato em algumas espécies arbóreas. Rev Árvore. 18:87-95.

Cazetta JO, Vilella LCV (2004) Nitrate reductase activity in leaves and stems of tanner grass (*Brachiaria radicans* Napper). Sci Agric. 61(6):640-648.

Crafts-Brandner SJ, Harper JE (1982) Nitrate reduction by roots of soybean (*Glycine max* [L.] Merr.) seedlings. Plant Physiol. 69:1928-1303.

Cruz JL, Souza Filho LFS, Pelacani CR (2015) Influência da adubação fosfatada sobre o crescimento do camapu (*Physalis angulata* L.). Rev bras plantas med. 17(3):360-366.

Curi PA, Carvalho CS, Salgado DL, Pio R, Silva DF, Pinheiro ACM, Souza VR (2018) Characterization of different native american physalis species and evaluation of their processing potential as jelly in combination with brie-type cheese. Food Sci Technol. 38(1):112-119.

Delú-Filho N, Oliveira LEM, Alves JD (1998) Atividade da redutase do nitrato em plantas jovens de seringueira (*Hevea brasiliensis* Muell.Arg): otimização das condições de ensaio e ritmo circadiano. Rev Árvore. 21:329-336.

Dovis VL, Hippler WR, Silva KI, Ribeiro RV, Machado EC, Mattos Jr D (2014) Optimization of the nitrate reductase activity assay for citrus trees. Braz J Bot. 37(4):383-390.

Ferreira DF (2008) Sisvar: um programa para análises e ensino de estatística. Rev Symposium. 6:36-41.

Instituto Nacional de Meteorologia (INMET) (2017) Estações e Dados: Estação Meteorológica de Observação de Superfície Automática. Available in http://www.inmet.gov.br/portal/.

Jamur MC, Oliver C (2010) Permeabilization of cell membranes. In: Oliver C, Jamur MC (Ed.). Immunocytochemical methods and protocols. 3rd edn. Totowa: Humana Press, 2010.

Jaworski EG (1971) Nitrate reductase assay in intact plant tissues. Biochem Biophys Res Commun. 43:1274-1279.

Kaiser WM, Huber SC (2001) Post-translational regulation of nitrate reductase: mechanism, physiology relevance and environmental triggers. J Exp Bot. 52(363):1981-1989.

Konishi M, Yanagisawa S (2011) The regulatory region controlling the nitrate-responsive expression of a nitrate reductase gene, NIA1, in Arabidopsis. Plant Cell Physiol. 52(5):824-836.

Lea US, Leydecker MT, Quilleré I, Meyer C, Lillo C (2006) Posttranslational regulation of nitrate reductase strongly affects the levels of free amino acids and nitrate, whereas transcriptional regulation has only minor influence. Plant Physiol. 140(1):1085-1094.

Lee HJ, Titus JS (1992) Factors affecting the in vivo nitrate reductase assay from MM106 apple trees. Commun Soil Sci Plant Anal. 23:981-991.

Leite RS, Tanan TT, Nascimento MN, Oliveira LM, Abreu PAS (2017) Hydroponic cultivation of *Physalis angulata* L.: growth and production under nitrogen doses. Pesq Agropec Trop. 47(2):145-151.

Lillo C, Meyer C, Ruoff P (2001) The nitrate reductase circadian cicle system. The central clock dogma contra multiple oscillatory feedback loops. Plant Physiol. 125:1554-1557.

Lillo C, Meyer C, Lea US, Provan F, Oltedal S (2004) Mechanism and importance of post-translational regulation of nitrate reductase. J Exp Bot. 55:1275-1282.

Lorenzi H, Matos M (2008) Plantas medicinais no Brasil: nativas e exóticas cultivadas. 2nd edn. Nova Odessa, SP: Plantarum, 512 p.

Marin Sisley GM, Max Horna FY, Ríos Isern F, Aranda-Ventura J, Villacrés Vallejo J (2017) Actividad inmunoestimulante del extracto acuoso liofilizado de la planta entera de *Physalis angulata* L. en ratas albinas cepa Holtzman. Rev Peru Med Int. 2(1):38-46.

Martins AP, Chow F, Yokoya NS (2009) Ensaio in vitro da enzima nitrato redutase e efeito da disponibilidade de nitrato e fosfato em variantes pigmentares de *Hypnea musciformis* (Wulfen) J. V. Lamour. (Gigartinales, Rhodophyta). Braz J Bot. 32(4):635-645.

Matsumura AT (2012) Controle da atividade da reductase do nitrato em plantas de abacaxizeiro submetidas a baixas temperaturas em diferentes fases do ciclo diurno. (Master's Dissertation in Science – Botany) – Institute of Biosciences, University of São Paulo.

Meguro NE, Magalhães AC (1982) Atividade da redutase de nitrato em cultivares de café. Pesq agropec bras. 17:249-257.

Motulsky HJ, Christopoulos A (2004) Fitting models to biological data using linear and non-linear regression. A practical guide to curve fitting. In Oxford University Press, UK: Oxford University, 351p.

Nelson DL, Cox MM (2014) Princípios de bioquímica de Lehninger. 6th edn. Porto Alegre: Artmed.

Nievola CC, Mercier H (2001) Variações diurnas da atividade in vivo da redutase do nitrato em abacaxizeiro (*Ananas comosus* (L.) Merr. - Bromeliaceae). Braz J Bot. 24(3):295-301.

Oliveira MAJ, Bovi MLA, Machado ECJ, Rodrigues JD (2005) Atividade da redutase de nitrato em mudas de pupunheira (*Bactris gasipaes*). Cienc Rural. 359(3):515-522.

Passos AI (2013) Adubação e assimilação de nitrogênio em duas espécies de fisális. (Master's Dissertation in Agronomy) – Federal Technological University of Paraná, Pato Branco.

Queiroga RCF, Puiatti M, Fontes PCR, Cecon PR, Finger FL (2007) Influência de doses de nitrogênio na produtividade e qualidade do melão *Cantalu pênsis* sob ambiente protegido. Hortic Bras. 25(4):550-556.

Queiroz CGS, Rena AB, Cordeiro AT, Alves JD (1993) Ritmo diurno na atividade da redutase do nitrato em folhas e raízes de *Coffea arábica* L. Pesq agropec bras. 28(7):787-795.

Rajasekhar VK, Gowri G, Campbell WH (1988) Phytochrome-mediated light regulation of nitrate reductase expression in squash cotyledons. Plant Physiol. 88:242-244.

Santos CLR, Cazetta JO, Saran LM, Sanches A (2014) Otimização da análise da atividade da redutase do nitrato e sua caracterização em folhas de cana-de-açúcar. Pesq agropec bras. 49(5):384-394.

Singh VK (1994) Optimum conditions for measurement of nitrate reductase activity in the leaves of blast fibre yielding plants. Proc Natl Acad Sci India. 64:389-398.

Solomonson LP, Barber MJ (1990) Assimilatory nitrate reductase: functional properties and regulation. Annu Rev Plant Physiol Plant Mol Biol. 41:225-253.

Souza SR, Stark EMLM, Fernandes MS (2002) Enzimas de assimilação de nitrogênio em plantas. 1st edn. Rio de janeiro: Artware Projetos Culturais. 55p.

Streeter JG, Bosler ME (1972) Comparison of in vitro and in vivo assays for nitrate reductase in soybeans leaves. Plant Physiol. 49:448- 450.

Taiz L, Zeiger E, Moller IM, Murphy A (2017) Fisiologia e desenvolvimento vegetal, 6th edn. Artmed, Porto Alegre.

Tanan TT, Nascimento MN, Passos AR, Leite RS, Guimarães DS (2013) Produção de biomassa de *Physalis angulata* L. cultivadas sob níveis crescentes de adubação. Rev Magistra. 25(esp):378-379.

Thomé M, Osaki F (2010) Adubação de nitrogênio, fósforo e potássio no rendimento de *Physalis* spp. Rev Acad Ciênc Agrár e Ambient. 8(1):11-18.

Tischner R (2000) Nitrate uptake and reduction in higher and lower plants. Plant Cell Environ. 21:1005-1024.

Tomassini TCB, Barbi NS, Ribeiro IM, Xavier DCD (2000) Gênero *Physalis*: uma revisão sobre vitaesteróides. Quím Nova. 23(1):47-57.