Nitric oxide improves gas exchange and growth in *Physalis angulata* plants under water deficit

Romeu da Silva Leite¹,²*, Marilza Neves do Nascimento¹, Daniele de Brito Trindade², Alismário Leite da Silva³, Uasley Caldas de Oliveira¹, Ianna Kamyla Freitas Lima³

¹State University of Feira de Santana, Biological Sciences Department, Feira de Santana 44.036-900, Brazil
²Baiano Federal Institute of Science and Technology, Campus Guanambi, Guanambi 46.430-000, Brazil
³*Corresponding author: leiteromeu@hotmail.com

Abstract

Plant sensitization with nitric oxide (NO) donors may improve the tolerance to abiotic stresses such as water deficit. *Physalis angulata* is a genetic resource growing in semiarid areas of Brazil, with the potential for fruit growing and medicinal uses. In this experiment sodium nitroprusside, a NO donor was sprayed at three concentrations (0, 50 and 100 µM) at 25 and 49 days after transplantation in well-watered plants and under water deficit to evaluate the NO mitigating role. The gas exchange, photosynthetic pigments, water relations, growth and productivity parameters were evaluated. The water deficit negatively influenced most of the variables analyzed. However, the SNP spray was able to attenuate, reverse or act in the recovery of stress effects. There was an improvement in gas exchange, especially carbon assimilation, stomatal conductance and transpiration; as well as an increase in total chlorophyll content. The donated NO was able to influence the plant water status. Besides, it promoted an increase in growth parameters, especially in photoassimilates incorporation and yield. However, the NO supply to plants under well-watered conditions does not seem to affect the physiology parameters. The NO supply in micromolar concentrations can attenuate or even reverse the water deficit effects on this species, being an important tool for promoting tolerance to this abiocstic stress.

Keywords: biomass incorporation; photosynthesis; sodium nitroprusside; water relations; water stress mitigation.

Abbreviations: AGR_absolute growth rate; A_leaf assimilation; LA_leaf area; LDM_leaf dry mass; WLDW_leaf water potential; LWR_leaf weight ratio; NO_nitric oxide; PSII_photosystem II; RGR_relative growth rate; RLWC_relative leaf water content; RDM_root dry mass; SNP_sodium nitroprusside; SLA_specific leaf area; gs_stomatal conductance; Chltotal_total chlorophyll; E_transpiration; WSCi_total water storage capacity; WD_water deficit conditions; WH_well-watered conditions.

Introduction

Physalis fruits have traditionally been used for human consumption since ancient times (Vargas-Ponce et al., 2016), which probably contributed to the selection of more productive plants and adapted to unfavorable environmental conditions, such as long water deficit periods observed in Brazilian semiarid areas, the naturally occurring region of *Physalis angulata*. This species is native to Brazil and occurs in tropical and subtropical areas around the world (Sun et al., 2011). However, it is an underexploited genetic resource, with uses in traditional medicine and potential for its insertion in the fruit market of small fruits, making this species a food alternative.

The production of nutritious food in sufficient quantities in a scenario of intensified climate change, rapid population growth, and urbanization, is one of the great challenges facing society in this century (Roa et al., 2016). In this sense, it is necessary to develop technologies that enable agricultural production in unfavorable edaphoclimatic conditions, such as drought. Drought is one of the most limiting factors for agricultural production around the world (Hussain et al., 2018).

Plants can temporarily overcome water deficit through rapid responses through stomatal closure and some morphological changes that include inhibition of leaf expansion, leaf abscission and changes in root architecture (Simmontacchi et al., 2015). These physiological responses can lead to reductions in crop growth and yield due to reduced CO₂ uptake. However, modern techniques have made it possible to increase tolerance to abiotic stress effects, such as water deficit, by sensitizing plants with nitric oxide donors.

Nitric oxide (NO) is a gaseous molecule produced by plant cells which recent research shows that exogenous NO can attenuate the effects of water deficit in several species (Silveira et al., 2016; Batista et al., 2018), although the mechanisms of the action are not completely clarified. In recent years, studies involving the role of NO in abiotic stresses have been conducted with underexploited species such as *Isatis cappadocica* (Souri et al., 2019) and *P. angulata* (Leite et al., 2019) in addition to large crops.

There is no doubt that NO is an important participant in plant cell metabolism and signaling, however, there are still some questions to be answered, especially about its effects on underexplored crops. The hypothesis is that NO is able to improve the water deficit tolerance in *P. angulata* plants. Thus, this work aimed to evaluate how nitric oxide donated by sodium nitroprusside can mitigate the water deficit effects
or improves their tolerance on *P. angulata* plants, in addition to understanding its role in plants without water restriction.

**Results**

**Gas exchange and total chlorophyll content**

Water deficit reduced carbon assimilation (A), stomatal conductance (gs) and transpiration (E) in *P. angulata* plants under water deficit (Fig. 1; Supplementary 1). Sodium nitroprusside application at a concentration of 50 µM kept A above 14 µmol CO₂ m⁻² s⁻¹, contributing to the attenuation of water deficit effects, whereas plants sprayed with 100 µM had the lowest mean value (8.57 µmol CO₂ m⁻² s⁻¹) for this variable under the same soil water conditions (Fig. 1 a). Similar to carbon assimilation, the application of SNP at 50 µM reversed the effects of water deficit on gs. In plants sprayed at this concentration, there was a 33.4% increase in gs (126.33 mmol H₂O m² s⁻¹) in relation to untreated plants (0 µM SNP + WD, 94.67 mmol H₂O m² s⁻¹). For E, there was no improvement with the NO application under water restriction conditions. Well-watered plants when sprayed at both evaluated doses showed reductions in E and gs (Fig. 1 b, c), although there were no statistical differences. The highest average value of water use efficiency (WUE, 5.40 mmol CO₂ mol⁻¹ H₂O) was observed in plants under water deficit sprayed with 50 µM (Fig. 1 d). SNP promoted the increase of total chlorophyll (Chltotal) content in plants under water deficit (Fig. 1 e; Supplementary 2). Plants sprayed with 50 µM SNP + WD showed an average increase of 27.4% in total chlorophyll content (40.54 µg cm⁻²), while for plants with 100 µM + WD the increase was 32.4% (42.15 µg cm⁻²), compared to plants sprayed with 0 µM SNP + WD (31.82 µg cm⁻²), respectively.

**Water relations**

Water deficit reduced leaf water potential (Ψwleaf) in *P. angulata* plants; however, the NO supply through SNP was able to attenuate its symptoms (Fig. 2). Plants under water deficit treated with 50 µM SNP showed an increase in Ψwleaf (-0.55 MPa) compared to plants that were not treated with NO under the same water availability conditions (-0.93 MPa). However, the application was not able to attenuate the water deficit effects in plants sprayed at 100 µM. There were no statistical differences for relative leaf water content (p>0.05; Supplementary 3).

**Growth and yield**

The SNP application influenced the growth and yield parameters of *P. angulata* plants under water deficit cultivation conditions (Fig. 3 and Fig. 4; Supplementary 4 and 5). Plants under water deficit treated with 50 µM SNP showed an increase in absolute growth rate (AGR), being incorporated 0.10 g day⁻¹, which corresponds to an increase of 66.6% in comparison to non-sprayed plants with SNP (0.06 g day⁻¹) (Fig. 3 a). SNP spraying did not influence the relative growth rate (RGR) in plants under water deficit, with averages ranging from 0.08 g g⁻¹ day⁻¹ to 0.09 g g⁻¹ day⁻¹ (Fig. 3 b). Plants sprayed with 100 µM of SNP had the highest weight leaf ratio (LWR) mean, with 0.28 g g⁻¹ (Fig. 3 c). The water deficit reduced the specific leaf area (SLA) of *P. angulata* plants and the SNP provision was not able to attenuate its effects (Fig. 3 d). Overall, SNP application at both doses did not influence these growth rates in well-watered plants.

The leaf area (LA) was reduced with the water deficit imposition (average values ranged from 335.89 cm² to 465.57 cm²), however, it was observed in sprayed plants with both SNP doses similar values to well-watered plants not treated with the NO donor (Fig. 4 a). A similar performance was observed for leaf dry mass (LDM) (Fig. 4 b). Stem dry mass (SDM) was reduced with water deficit, however, SNP applications promoted an increase of 34.2% and 12.3% to 50 µM and 100 µM of SNP, respectively (Fig. 4 c). In plants subjected only to water deficit, there was a 37.8% reduction in root dry mass (RDM), however, SNP application at 50 µM reversed the effects of water deficit, keeping the RDM above 1.44 g (Fig. 4 d). Similarly, the same dosage attenuated the water deficit effects on shoot dry mass (SDM) (Fig. 4 e). Plants under water deficit treated with 50 µM of SNP showed an increase in SDM (3.46 g) compared to plants that were not treated with nitric oxide under the same water availability conditions (2.32 g). However, the application of SNP was not able to attenuate the water deficit effects in plants sprayed at 100 µM. When provided, both SNP doses promoted the increase of LA, LDM and RDM in well-watered plants. Water deficit also reduced fruit yield (Fig. 4 f). Nevertheless, 50 µM of SNP supply promoted an increase of 80.9% in yield compared to untreated plants, with an average value of 1.23 g m⁻². Well-watered plants with or without NO supply showed similar performances, with values above 2.92 g m⁻².

In this manuscript, we show that low micromolar concentrations of sodium nitroprusside (a nitric oxide donor) are able to attenuate the negative effects of water deficit in *Physalis angulata* plants and promotes the improvement in photosynthetic rates, maintenance of leaf water potential and growth parameters, but has little influence on physiological and growth conditions when well-watered conditions.

**Discussion**

Species grown in arid and semi-arid regions are constantly confronted with water deficit, resulting in yield reductions (Santisreeze et al., 2015). Thus, the use of new tools that promote tolerance to water deficit becomes important, whether for traditionally cultivated crops or underexploited species, promoting yield improvement and aggregating information on physiological changes in face of adverse environmental conditions. Recent research shows that the nitric oxide supply can improve the tolerance of plants to water deficit. In this experiment, spraying plants with sodium nitroprusside (SNP), a nitric oxide donor, promoted attenuation and / or reversal of water deficit effects on *P. angulata* plants, on gas exchange variables, water relations, growth rates and yield (Fig. 5).

Prolonged reduction in water availability reduced gas exchange in *P. angulata* plants, especially CO₂ assimilation and transpiration. This reduction is indicative of the deleterious effects of water deficit and species acclimatization, similarly to other crops such as *Crambe abyssinica* (Batista et al., 2018) and sugarcane (Silveira et al., 2019), since the reduction of leaf transpiration is an important mechanism to prevent water loss under water deficit conditions. Consequently, this response decreases the CO₂ availability for photosynthesis and biomass incorporation in *P. angulata* plants (Leite et al., 2019). Even so, the SNP application was able to attenuate the water deficit effects on A, even under low transpiration levels.

How has NO application improved photosynthesis on *P. angulata* plants under water deficit conditions? Water deficit reduces messenger RNA synthesis (mRNAs) and promotes photosystem protein (PS) II degradation (Yuan et al., 2005),
Fig 1. Gas exchange in *Physalis angulata* plants under well-watered conditions (WW) and water deficit (WD) sprayed with sodium nitroprusside: assimilation (a); transpiration (b); stomatal conductance (c); water use efficiency (d) and total chlorophyll (e). Values are mean ± standard error. Values sharing the same letters are not significantly different at 5% significance level.

Fig 2. Leaf water potential in *Physalis angulata* plants under well-watered conditions (WW) and water deficit (WD) sprayed with sodium nitroprusside. Values are mean ± standard error. Values sharing the same letters are not significantly different at 5% significance level.
**Fig 3.** Growth indices in *Physalis angulata* plants under well-watered conditions (WW) and water deficit (WD) sprayed with sodium nitroprusside: absolute growth rate (a); relative growth rate (b); leaf mass ratio (c) and specific leaf area (d). Values are mean ± standard error. Values sharing the same letters are not significantly different at 5% significance level.

**Fig 4.** Plant growth parameters in *Physalis angulata* plants under well-watered (WW) and water deficit (WD) sprayed with sodium nitroprusside: leaf area (a); leaf dry mass (b); stem dry mass (c); root dry mass (d); shoot dry mass (e) and yield (f). Values are mean ± standard error. Values sharing the same letters are not significantly different at 5% significance level.
Fig 5. Main effects of sodium nitroprusside application on Physalis angulata plants under well-watered and water deficit conditions.

Fig 6. Sodium nitroprusside (SNP) application in plants to recover from the water deficit effects. WSC$_{soil}$: water storage capacity; T$_i$: initial time; T$_f$: final time.

and a NO-mediated regulation mechanism for photosynthesis has been partially attributed to the protection of critical functional proteins in PS II complex during water deficit (Santisree et al., 2015). In addition, the NO donor used in this study, SNP, is the only one capable of stimulating electron transport through PS II (Procházková et al., 2013). Improvement of photosynthesis by NO supply under water deficit conditions has been reported for other species (Silveira et al., 2016). Added to this, nitric oxide treatments promote increased photosynthetic pigment content in various species under abiotic stresses.

In this study, NO application promoted an increase in Chl$_{total}$ content in P. angulata plants under water deficit, which may also contribute to photosynthesis improvement, since Chl participates in essential processes, like the light energy capture and electrons transfer. In addition to negatively regulating the genes expression levels of Chl catabolic pathway, NO plays a positive role in maintaining the thylakoids membranes stability (Liu and Guo, 2013). Underwater deficit, the encapsulation of S-nitrosoglutathione, a NO donor, in chitosan nanoparticles increased chlorophyll content in sugarcane plants (Silveira et al., 2019). Leaf application of SNP significantly improved the chlorophyll a and chlorophyll b content of broccoli plants under water stress (Munawar et al., 2019).

SNP application has been shown to regulate stomatal responses under well-watered and water deficit conditions. In plants under water restriction, it is common to observe a reduction in stomatal conductance (Batista et al., 2018; Castro et al., 2019), since gs is associated with the osmotic state of plant cells, however, when NO is supplied, the stomatal opening is induced (Hasanuzzaman et al., 2018). P. angulata plants sprayed with SNP at 50 µM + WD had the highest mean gs value, while the E remained statistically equal to the other treatments under water deficit. Thus, in line with A, the NO supply in plants under water deficit promoted an improvement in water use efficiency (WUE) (Fig. 3), contributing to the cellular water maintenance.

The observation that SNP applications attenuate the water relations of P. angulata plants shows that the NO donated also served to maintain leaf water potential (Fig. 2). Underwater deficit, leaf water potential variation is common in some species, following environmental fluctuations (Martínez-Vilalta et al., 2014). Comparatively, micromolar SNP concentrations resulted in an increased of Ψ$_{leaf}$ in P. angulata plants under water deficit (Leite et al., 2019). Nevertheless, these applications did not influence the relative leaf water content. However, other studies have found that the NO supply by different donors improves RLWC under water deficit in cucumber (Arasimowicz-Jelonek et al., 2009), tomato (Jangid and Dwivedi, 2017) and canola (Akram et al., 2018). Exposure to water deficit promoted a significant reduction in growth parameters (Fig. 4 and Fig. 5). Under these conditions, the highest values of absolute growth rate, which represents the speed of plant growth, were observed in plants sprayed with SNP. Similar to leaf mass ratio LMR. LMR is a physiological component that reflects how much of what the plant invested in its production (via photosynthesis)
remained in the leaf (Costa et al., 2006). Thus, these data reinforce the role of nitric oxide in improving the plant’s assimilatory system under water deficit, from photosynthesis to photoassimilates incorporation.

Growth reductions in water-restricted herbaceous species, such as *P. angulata* plants, are commonly reported (Ozaslan et al., 2016; Lima et al., 2016). Under these adverse conditions, some physiological disorders are commonly observed, like a reduced cell expansion and cell death (Taiz et al., 2017), as well as reduced carbon assimilation (Fig. 2), which may reduce growth and crop yield (Fig. 5). Interestingly, despite marked reductions in growth, nitric oxide supply was able to attenuate symptoms of water deficit on leaf area, root, leaf and shoot dry mass as well as yield. Increases in dry mass incorporation and yield induced by exogenous nitric oxide in *P. angulata* plants under water deficit are related to factors already presented, such as improved CO₂ assimilation, protection of photosynthetic pigments and increased leaf water potential. In addition, NO plays an important role in root growth, as evidenced in this work (Fig. 6), as it mediates auxin response and modulates the expression of cell cycle regulatory during lateral roots formation (Pagnussat et al., 2002; Correa-Aragunde et al., 2006). Thus, NO can be used to promote increased tolerance to water deficit in plants of *P. angulata*.

**Materials and Methods**

**Plant materials and growth conditions**

Seeds of *P. angulata* were produced from the collection of seeds collected in the semi-arid region of Bahia (Freitas, 2004) and multiplied by the research group. The seedlings were transplanted to individual pots at 20 days after sowing in commercial substrate, with a spacing of 0.6 (rows) x 0.3 (plants). The pots were filled with soil collected from the 0-20 cm deep layer and at the time of collection had the following characteristics, in g kg⁻¹: sand=851; clay=90; silt=59; in g dm⁻³: M.O.= 16; pH= 4.7; in mg dm⁻³: P=16; S= 6; B=0.06; Cu=0.8; Fe=49; Mn=21.2 and Zn=2.6; in mmol dm⁻³: Al⁺³=1; K=1.8; Ca=11; Mg=5; H⁺Al=23.

**Soil water availability and treatments application**

To apply water deficit, the maximum soil water storage capacity (WSC_{soil}) and water replacement were determined from a test according to Leite et al. (2019). The initial cultivation was maintained with 60% of WSC_{soil} in the pot (well-watered plants) until plant canopy establishment, when treatments were applied. The 60% value of WSC_{soil} was considered ideal for species cultivation according to previous studies (Leite et al. 2018). Then, the treatments were applied to well-watered plants and plants under water deficit, both sprayed with an aqueous solution of sodium nitroprusside (SNP, Na₂[Fe(CN)₆]NO·2H₂O) at concentrations of 0 μM (controls), 50 μM and 100 μM of SNP (Fig. 6). Well-watered plants and underwater deficit treatment controls were sprayed with distilled water (H₂O). It was applied to 100 mL m⁻² of different solutions during the flowering starts (25 days after transplantation) and fruit filling stage (49 days after transplantation) (Leite et al., 2019).

Water availability for well-watered (WW) and under water deficit (WD) plants was maintained at 60% and 20% of the maximum WSC_{soil}, respectively. Water deficit was started simultaneously with the first application of SNP, with the following treatments: WW + H₂O (0 μM SNP); WD + H₂O (0 μM SNP); WW + 50 μM (50 μM SNP); WW + 100 μM (100 μM SNP); WD + 100 μM (100 μM SNP).

**Experimental measurements**

Experimental measurements were performed after 25 days under water deficit, at 50 days after transplantation (DAT) to individual pots.

**Measurement of gas exchange and total chlorophyll content**

Gas exchanges were assessed using an InfraRed Gas Analyzer equipment (IRGA, model CIRAS-3, PPSystems, Amersbury, USA). Three plants per treatment were evaluated, recording two measurements for each plant for 60 seconds, and obtained the parameters: CO₂ assimilation, (μmol CO₂ m⁻² s⁻¹); transpiration rate (mmol H₂O m⁻² s⁻¹); stomatal conductance (mmol H₂O m⁻² s⁻¹) and water use efficiency (mmol CO₂ mol⁻¹ H₂O). Measurements were performed between 08:00 and 10:00 hours with a photon flux of 800 μmol m⁻² s⁻¹, reference CO₂ of 400 μmol mol⁻¹ reference CO₂ and constant temperature (~25 °C). For total chlorophyll content determination (Chl_{total}), discs of 0.785 cm² were used. The discs were immersed in 5 mL of dimethyl sulfoxide (DMSO, C₃H₇OS) in test tubes wrapped with aluminum. Chlorophyll contents were expressed in μg.cm⁻² according to Wellburn (1994) equations for DMSO extracts.

**Measurement of water relations**

The determination of leaf relative water content was made based on the weight of nine leaf discs according to Weatherley’s (1950) methodology. In this work, the hydration time in distilled water was six hours and 24 hours in a forced air circulation oven at 60 °C to obtain the dry mass. Leaf water potential was determined in the early morning using a Scholander chamber (PMS 1000, PMS Instrument, Corvallis, USA) in leaves collected in the middle third of the plants were used and immediately read. For both measurements, three plants per treatment were evaluated.

**Growth parameters**

For growth evaluations, five plants per set randomly used treatment. The following parameters were evaluated: leaf area (cm²) using a leaf area meter (model LI-3100C, Li-Cor, Lincoln, Nebraska, USA); fruit production (g plant⁻¹) and yield. The plants were separated into leaves, stems and roots to obtain the dry mass after drying in a forced air circulation oven at 60 °C until it reaching constant weight. The following growth rates were determined: absolute growth rate, relative growth rate, leaf mass ratio and specific leaf area (Cairo et al., 2008).

**Statistical analysis**

The experimental design adopted was completely randomized with 15 replications, considering each pot an experimental unit. After initial analysis, the data were subjected to the analysis of variance (ANOVA) at 5% of significance to verify the possible differences between treatments. The assumptions (independence, normality and homoscedasticity) of ANOVA were checked. Results were expressed as mean ± standard error (SE). Tukey and Kruskal-Wallis tests were used, and data obtained from the analyzed parameters were statistically evaluated using R statistical software (R Core Team, 2015).
Conclusions

Nitric oxide donated by sodium nitroprusside has little influence on the physiological and growth characteristics of Physalis angulata plants grown without water restriction. On the other hand, in micromolar concentrations can attenuate or even reverse the water deficit effects, being an important tool for promoting tolerance to this abiotic stress. Sodium nitroprusside applications can attenuate the water deficit effects in P. angulata by improving gas exchange, protection of photosynthetic pigments and plant growth.

Acknowledgement

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. Romeu da Silva Leite would also like to thank the support provided by the Cynthia Fernandes Inácio, Francisco dos Santos Neto and Maryelle Vanilla de Abreu Cerqueira.

References


