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Proline and sodium nitroprusside increase the tolerance of *Physalis peruviana* L. plants to water deficit through chemical priming

Prolina e nitroprussiato de sódio aumentam a tolerância de *Physalis peruviana* L. ao déficit hídrico através do *priming* químico

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ABSTRACT

Water deficit is the main cause of abiotic stress to which plants are susceptible, whether cultivated or those that are underexploited, such as *Physalis peruviana* L. Searching for tools that contribute to the management of water deficit in plants is fundamental for the maintenance of agriculture, especially in arid and semi-arid areas around the world. Thus, the objective of this study was to evaluate the effect of chemical priming with proline and sodium nitroprusside as promoters of increased tolerance to water deficit in *P. peruviana* plants. Plants grown under well hydrated conditions (FC of 70±5%) were sprayed with proline (10 and 20 mM) or sodium nitroprusside (25 and 50 μM) and evaluated after exposure to water deficit (FC of 20±5%). Plants cultivated under water deficit without pretreatment showed reductions in the variables of water relations, gas exchange and growth. Proline and sodium nitroprusside were effective in maintaining less negative water potential, contributing to improvements in gas exchange, such as CO_2 assimilation, stomatal conductance and transpiration. Thus, it was possible to measure greater tolerance to water deficit in pretreated plants and, consequently, greater biomass accumulation. It appeared that pretreatment of proline and sodium nitroprusside can have a priming effect against water deficit in *P. peruviana* plants.

Index terms: Abiotic stress; water deficit mitigation; goldenberry.

RESUMO

O déficit hídrico é o principal promotor de estresse abiótico ao qual as plantas estão suscetíveis, sejam as cultivadas ou aquelas subexploradas, como as fisális. A busca por ferramentas que contribuam para a gestão do déficit hídrico em plantas é fundamental para a manutenção da agricultura, sobretudo em zonas áridas e semiáridas ao redor do mundo. Dessa forma, o objetivo deste trabalho foi avaliar o efeito do *priming* químico com prolina e nitroprussiato de sódio como promotores do aumento da tolerância ao déficit hídrico em plantas de *Physalis peruviana*. Assim, plantas cultivadas em condições bem hidratadas (FC de 70±5%) foram pulverizadas com prolina (10 e 20 mM) ou nitroprussiato de sódio (25 e 50 μM) e avaliadas após a exposição ao déficit hídrico (FC de 20±5%). Plantas cultivadas sob déficit hídrico sem pré-tratamento apresentaram reduções para as variáveis de relações hídricas, trocas gasosas e de crescimento. O uso de prolina e nitroprussiato de sódio foram eficazes na manutenção do potencial hídrico menos negativo, contribuindo para melhorias nas trocas gasosas, como assimilação de CO_2, condutância estomática e transpiração. Dessa forma, foi possível mensurar maior tolerância ao déficit hídrico nas plantas pré-tratadas e, consequentemente, maior acúmulo de biomassa. O uso de prolina e nitroprussiato de sódio em pré-tratamento podem ter efeito *priming* contra o déficit hídrico em plantas de *P. peruviana*.

Palavras-chave: Estresse abiótico; atenuação do déficit hídrico; fisális.

INTRODUCTION

In order to meet the food demands of a growing world population, it is necessary to improve agricultural production, an indispensable task in the face of climate change (Rodrigues et al., 2019), especially for intensifying abiotic stresses. Terrestrial plants are exposed to a variety of unfavorable conditions, such as temperature extremes, high salinity, and deficient or excessive water (He; He; Ding, 2018). Among these factors, water deficit is the main responsible for reducing yield in agroecosystems worldwide (Leite et al., 2021a).

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Plants can temporarily overcome water deficit through joint or combined responses, such as stomatal closure and morphophysiological changes, which include inhibition of leaf expansion, leaf abscission and modifications in root architecture (Simontacchi et al., 2015), in addition to biomolecular changes and changes in water relations. However, these responses can compromise plant production as a function of stress duration and intensity, making it a great challenge to maintain plant performance under these conditions.

Searching for tools that contribute to the management of water deficit in plants is fundamental for the maintenance of agriculture, especially in arid and semi-arid areas. In recent decades, research has sought to highlight the use of drought-tolerant species and genotypes, as well as the use of biostimulants and osmoprotectants to reverse the negative effects of stress (Nazari; Pakniyat, 2010; Terra et al., 2015; Irani; Valizadehkji; Naeini, 2021). More recently, the chemical priming technique has been shown to be efficient for increasing tolerance to abiotic stresses in various crops (Fleming et al., 2019; Gohari et al., 2020; Hameed et al., 2020). In this strategy, before exposure to stress, plants are treated with natural or synthetic chemical compounds, which act as signal transducers, activating plant defense systems (primed state) and promoting greater tolerance to the stress factor compared to untreated plants (Antoniou et al., 2020; Sako; Nguyen; Seki, 2021).

Different molecules are used to perform priming, among which nitric oxide and proline stand out. Nitric oxide (NO) is a gas that acts as a signaling molecule in plants under abiotic stress conditions (Rahimian Boogar; Salehi; Jowkar, 2014). The role of NO in mitigating the effects of water stress has been observed in several plant species, including grains, legumes, fruit trees, medicinal plants and underexploited species (Lau et al., 2021; Leite et al., 2021a), and its effects are related to the NO donor used, exposure time and the evaluated species.

Sodium nitroprusside is the main nitric oxide donor in plant experiments. The application of SNP in plants under water deficit acts in the maintenance of water status (Gupta; Srivastava; Seth, 2017), stomatal regulation (Shao; Wang; Shangguan, 2010; Solangi et al., 2022) and increase of photosynthesis whether by stimulating the transport of electrons in photosystem II (Procházková et al., 2013) or by increasing the activity of the enzyme Ribulose-1,5-Bisphosphate Carboxylase / Oxygenase – RuBisCO (Siddiqui et al., 2020). Other compounds, such as proline, are studied as potential promoters of increased tolerance in plants.

Proline is an amino acid with several functions in the plant-environment interaction, such as protection of cellular structure during dehydration, osmotic adjustment and removal of reactive oxygen species (Verslues; Sharma, 2010). Although proline is produced by the plant itself, recent studies have shown the beneficial role of its supply in several species under conditions of water deficit and other abiotic stresses (Merwad; Desoky; Rady, 2018; Hanif et al., 2021; Tonhati et al., 2020). The accumulation of proline induced by water deficit, observed in many plant species, has led to the hypothesis that additional increments in proline accumulation would promote drought tolerance (Bhaskara; Yang; Verslues, 2015). For example, proline application improves growth and photosynthetic efficiency in onions (Semida et al., 2020) and yield in beets (Ghaffari et al., 2021) under water deficit. Thus, its application was also proposed in this study as a possible priming agent in *Physalis peruviana* L. plants.

A promising way of evaluating the effect of priming treatments on plants is through spectral analysis. Infrared spectroscopy with Fourier Transform (FTIR) offers a fast and non-destructive way to obtain a biochemical fingerprint of the samples, where the main functional groups and connections can be identified, providing structural information about the chemical compounds present (Palacio et al., 2014). In addition, it is a viable tool to analyze variations in the proportions of the main organic compounds in plants as a function of environmental conditions (Durak; Depciuch, 2020). For example, changes on biomolecular composition in plant organs of *Physalis angulata* L. under nitrogen deficiency have already been evaluated (Leite et al., 2018a; 2021b)

Physalis L. is an American genus of agricultural importance, with *P. peruviana* L. (Figure 1) as the main cultivated species (Leite et al., 2021b; Vargas-Ponce et al., 2016). In addition to the commercialization of its fruits, *P. peruviana* is a medicinal plant capable of eliminating reactive oxygen species (ROS) and increasing the antioxidant system in the human body (El-Beltagi et al., 2019), acting as a natural product with anticancer potential (Yu et al., 2021). In addition, fruit calyxes can be used as herbal medicines, nutraceuticals or as low-cost cosmetic ingredients (Medina et al., 2019).

The cultivation of *P. peruviana* in Brazil is underexploited. In addition, research evaluating the performance of the species under water deficit conditions is scarce in the country, especially studies conducted with the non-cultivated native species *Physalis angulata* L. (Leite et al., 2018b; Leite et al., 2019). Thus, the objective of the present study was to evaluate the effect of proline and sodium nitroprusside as promoters of increased tolerance to water deficit through chemical priming in *P. peruviana* plants.

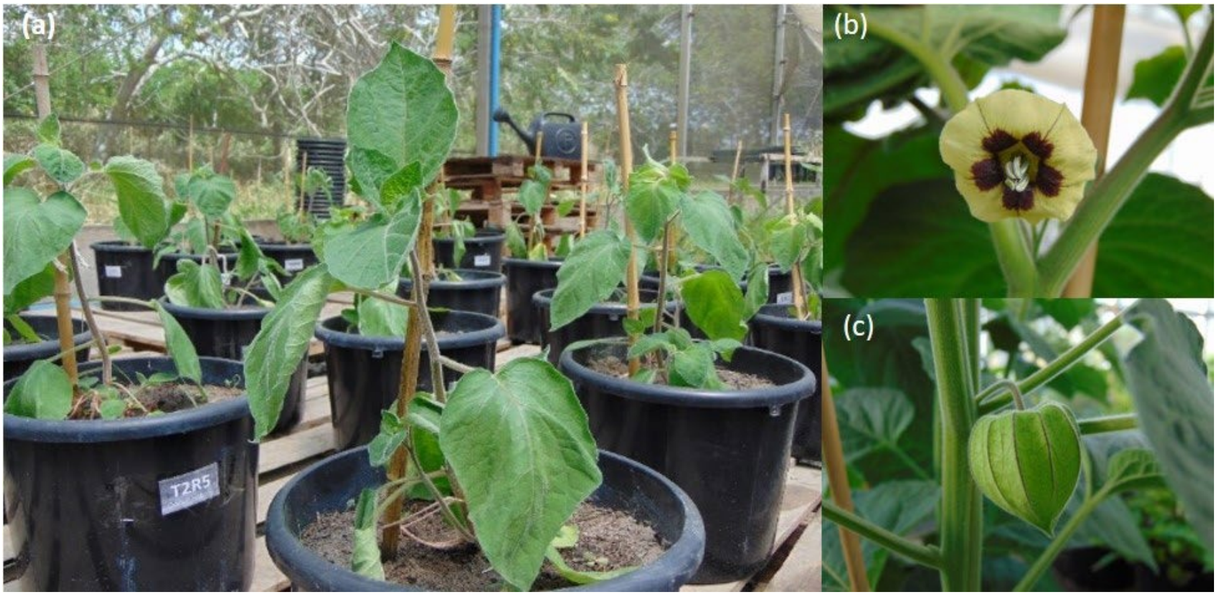

Figure 1: *Physalis peruviana* plants under water deficit (a) and details of the flower (b) and fruit enclosed by the calyx (c).

MATERIAL AND METHODS

Plant material and cultivation conditions

Physalis peruviana plants were grown in a greenhouse at the State University of Feira de Santana (UEFS), Feira de Santana, Bahia, Brazil (12°16’7.99”S, 38°56’21.63”W, 258 m asl), from January to April 2020. During the experiment, maximum and minimum daily values of temperature and relative humidity were measured using a thermohygrometer installed inside the greenhouse (Figure 2), and the plants were maintained under 50% of light retention in natural photoperiod. The seedlings used were obtained from seeds supplied by the UEFS research collection and cultivated in soil with the following physical and chemical characteristics (Table 1). Other conditions of cultivation, cultural practices and fertigation were in accordance with previous studies (Leite et al., 2019).

Application of chemical priming

Soil water storage capacity (Fc) was calculated based on a test described by Leite et al. (2019) and Bonfim-Silva et al. (2011). The pots used had a volume of 8 dm^3 and were filled with 8 kg of air-dried soil, saturated, sealed with a polyvinyl chloride (PVC) film and suspended to drain excess water. The drainage time was 48 hours, and maximum water storage capacity was determined based on the difference of weights. Irrigation was performed by the gravimetric method based on the daily weights of the pots of each treatment and disregarding the increment of weight due to plants.

All plants were kept under hydrated conditions with Fc of 70±5% during the application of chemical priming at 10 days after transplanting. The plants were separated into different treatments and sprayed with 100 mL m^{-2} proline - Pro ($C_5H_9NO_2$) at 10 mM and 20 mM, sodium nitroprusside – SNP (Na^2 $[Fe(CN)_5NO]\cdot 2H_2O$) at 25 µM and 50 µM; and distilled water (H_2O) as control. After chemical sensitization, the soil was maintained with Fc of 70±5% for 5 days. Then, irrigation was suspended in sensitized plants and water availability was monitored until reaching Fc of 20±5% after 15 days, when plants exposed to water deficit were assessed for any priming effect. The following treatments were evaluated in *P. peruviana* plants: Fc of 70% + H_2O; Fc of 20% + H_2O; Fc of 20% + Proline at 10 mM; Fc of 20% + Proline at 20 mM; Fc of 20% + SNP at 20 µM; Fc of 20% + SNP at 50 µM.

Experimental determinations

Relative water content and leaf water potential

Leaf water potential ($\psi_{w\ leaf}$) was determined in the early morning period using a Scholander pressure chamber (PMS 1000, PMS Instrument, Corvallis, USA). For this, leaves were collected from the middle third of the plants and immediately subjected to the reading of equilibrium pressure (MPa) after pressurization with compressed N_2, until the sap exited at the open end of the petiole.

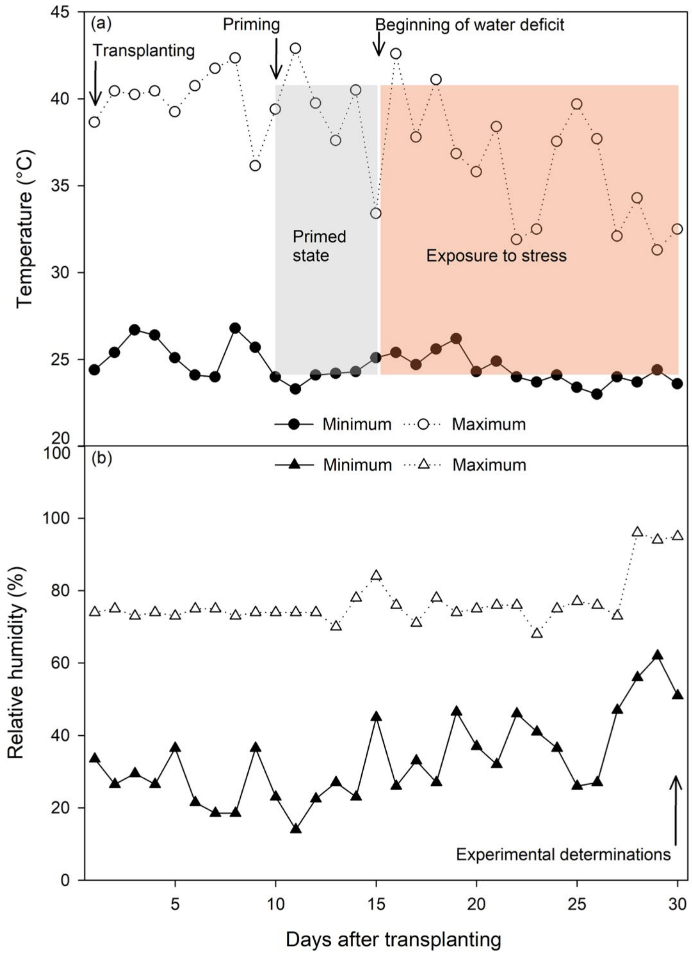

Figure 2: Maximum and minimum temperature and humidity during 30 days of conducting the experiment. The period of sensitization *(primed state)* and exposure to water deficit are indicated in the graph.

Table 1: Physical and chemical characteristics of the soil used in the experiment.

In water	mg dm^{-3}			Cmol c/dm^{-3}					%
pH	Organic matter	P	K	Ca+Mg	Al	Na	S	CEC	Base saturation
6.67	1.13	24	77	7.0	0.0	0.18	7.37	8.33	88.47

Relative water content was calculated based on the weight of leaf sections according to Weatherley (1950). The discs were collected from fully expanded leaves located in the middle third of the plants and immediately sealed in airtight packages. The time of hydration in distilled water was six hours at room temperature and the time in a forced air circulation oven at 60 °C to determine dry matter was 24 hours. For both variables, three plants were evaluated per treatment.

Gas exchange

Gas exchange was measured in fully expanded leaves located in the middle third of the plants with a portable photosynthesis meter – InfraRed Gas Analyzer (IRGA, CIRAS-3 model, PPSystems, Amesbury, USA). The parameters evaluated were: CO_2 assimilation (*A*, μmol CO_2 m^{-2} s^{-1}); stomatal conductance (*gs*, mmol H_2O m^2 s^{-1}) and transpiration rate (*E*, mmol H_20 m^{-2} s^{-1}); internal carbon concentration (*Ci*, μmol mol^{-1}) and water use efficiency (*WUE*, mmol CO_2 mol^{-1} H_2O). Measurements were performed in four plants per treatment between 09:00 and 10:00 hours, with the following configurations: photon flux of 800 µmol m^{-2} s^{-1}, reference CO_2 of 400 µmol mol^{-1} and constant temperature (25 °C).

Growth parameters and drought tolerance index

Plants were sectioned into fractions of leaves, stems and roots and kept in a forced air circulation oven at 60 °C until reaching constant weight, to obtain the dry matter. Absolute growth rate and relative growth rate were determined according to Cairo, Oliveira and Mesquita (2008). Leaf area was determined using the method of leaf discs (Leite et al., 2019). For growth variables, five plants were evaluated per treatment.

Drought tolerance index (DTI) (Blum; Jordan, 1985) was calculated using the data of total dry matter of plants (TDM) of water deficit treatments compared to well hydrated plants, according to the following equation: DTI= (TDM treatment under deficit / TDM well hydrated treatment).

Spectral analysis

Leaf dry matter samples were crushed in liquid nitrogen and homogenized in a 1 – mm – mesh sieve. The samples were characterized by Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy using Thermo Nicolet iS50 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The spectra were recorded in the medium infrared range (4000-400 cm^{-1}) at a spectral resolution of 4 cm^{-1}, with 64 scans per sample. Spectral analyses were performed at the Laboratory of Instrumental Techniques, Universidad de Valladolid, Spain.

Statistical analysis

The experimental design adopted was completely randomized with 8 replicates, considering each pot as an experimental unit. The data were subjected to analysis of variance (ANOVA) at 5% significance level. The results were expressed with the mean ± standard error (SE) and Tukey means comparison test. The obtained data of the analyzed parameters were statistically evaluated using the statistical program Sisvar.

Vibration data were analyzed with SigmaPlot 14.0 software (Systat Software Inc., Chicago, USA). Comparisons in relation to the FTIR spectral peaks and analysis of the corresponding functional groups were performed.

RESULTS AND DISCUSSION

Water deficit reduced the leaf water potential of the evaluated plants, but the pretreatment attenuated this effect (Figure 3; p<0.001). Despite that, there were no statistical differences between treatments for relative water content (p=0.2311). The most negative potentials were measured in plants under water deficit without treatment (-0.56 MPa) and pretreated with Pro 10 mM (-0.56 MPa) (Figure 3). Pretreatments with Pro 20 mM (-0.38 MPa), SNP 25 µM (-0.41 MPa) and SNP 50 µM (-0.35 MPa) were the most effective in maintaining a less negative water potential, indicating the priming effect of the substances used at these concentrations (Figure 3). In recent studies, micromolar concentrations of SNP and millimolar concentrations of proline have resulted in increased $\Psi_{w\ leaf}$ in *Physalis angulata* plants under water deficit (Leite et al., 2019, 2021a) as well as for other species, such as tomato and fennel (Jangid; Dwivedi, 2017; Gholami Zali; Ehsanzadeh, 2018). The supply of these substances can stimulate the accumulation of proline and promote osmotic adjustment (Verslues; Sharma, 2010), contributing to the maintenance of turgor and keeping the other physiological processes, such as gas exchange, stable.

In plants not sensitized by chemical agents, water deficit resulted in wilting and leaf abscission, evidencing the negative effects characteristic of this stress. Nevertheless, mitigating effects were observed in plants pretreated with Pro 20 mM and SNP 50 µM, evident by the maintenance of leaf turgor and color. However, plants pretreated with Pro 10 mM and SNP 25 µM exhibited symptoms similar to those of control plants under water deficit. It is important to mention that there was no expression of phenotypic characteristics of toxicity of the substances at the concentrations used. Leaf water potential data, in association with other physiological parameters evaluated, corroborate phenotypic observations (Figure 3, on top).

Water deficit and pretreatment with chemical agents significantly influenced the CO_2 assimilation (*A*, p<0.0001), stomatal conductance (*gs*, p<0.05) and transpiration (*E*, p <0.001) (Figure 4). However, there were no statistical differences between treatments for internal carbon concentration (p=0.9564) and water use efficiency (p=0.1119). The imposition of water deficit severely reduced CO_2 assimilation in *P. peruviana* plants, causing a reduction of 64.4% compared to plants under well hydrated conditions (17.95 µmol CO_2 m^{-2} s^{-1}; Figure 4a). Despite that, pretreatment with both doses of SNP was able to keep assimilation levels statistically equal to those of well hydrated plants. These plants showed mean values of *A* equal to 17.30 and 16.85 µmol CO_2 m^{-2} s^{-1}, with SNP at 25 and 50 µM, respectively. Increases in this variable were also observed in plants sensitized with proline (Figure 4a).

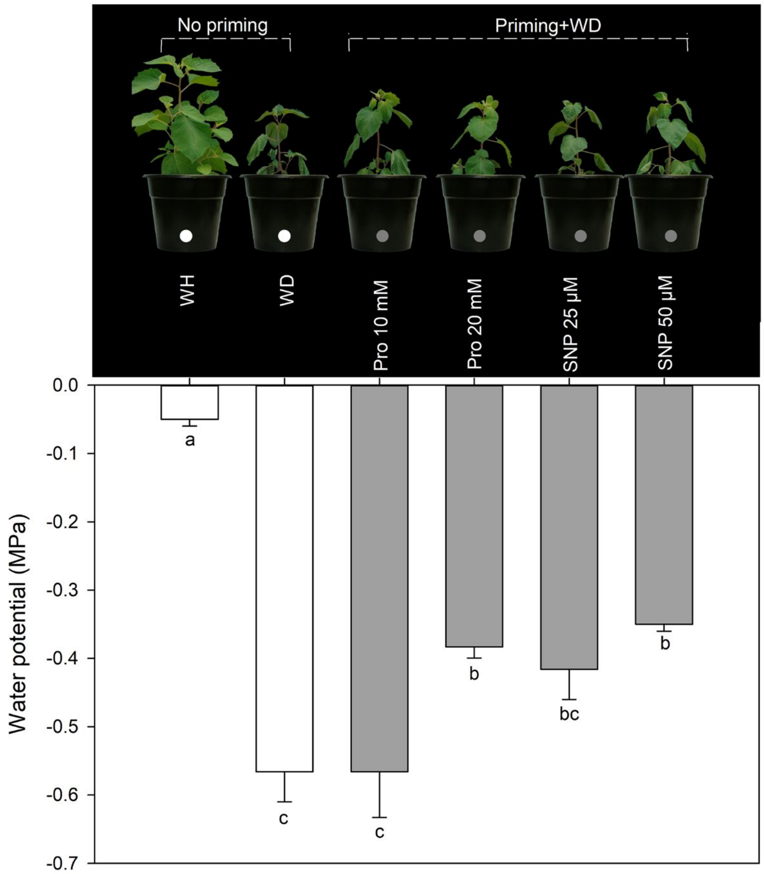

Figure 3: Leaf water potential in *Physalis peruviana* plants under well hydrated conditions (WH) and exposed to water deficit (WD) after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20mM) and sodium nitroprusside at 25 and 50 µM (SNP 25 µM and SNP 50 µM). The data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

The reduction of CO_2 assimilation in plants under water deficit conditions occurs, among other factors, due to stomatal closure. This finding has been demonstrated in several studies in the recent literature (Pazzagli; Weiner; Liu, 2016; Wang; Wang; Shangguan, 2016; Leite et al., 2018b). However, the use of SNP in pretreatment attenuated the negative effects of water deficit on CO_2 assimilation. This is because SNP is considered the only NO donor capable of stimulating electron transport during the photochemical phase of photosynthesis (Procházková et al., 2013; Antoniou et al., 2020). In addition, the exogenous supply of NO promotes increments in the activity of the enzyme ribulose-1,5-bisphosphate carboxylase / oxygenase - RuBisCO (Siddiqui et al., 2020), contributing to the improvement of CO_2 assimilation.

The mean stomatal conductance measured in well hydrated plants was 68.1% higher than that of untreated plants under water deficit, which indicates the state of stress for this second group. Despite that, both priming treatments used stimulated the increase in stomatal conductance, acting in the maintenance of *gs* at levels statistically equal to those of plants under well hydrated conditions, ranging from 352.25 to 525 mmol $H_2O\ m^{-2}\ s^{-1}$ (Figure 4b). Similar performance for the use of chemical agents was observed in leaf transpiration, which was maintained at levels between 5.37 and 5.65 mmol $H_2O\ m^{-2}\ s^{-1}$ (Figure 4c). Although the results show that the chemical agents stimulated stomatal opening, it is important to highlight that there was also improvement in the water status of the plants (Figure 3). The use of proline and SNP is reported to improve gas exchange in plants under abiotic stresses, for instance increasing stomatal conductance (Faraji; Sepehri, 2020) and transpiration in plants (Zouari et al., 2016).

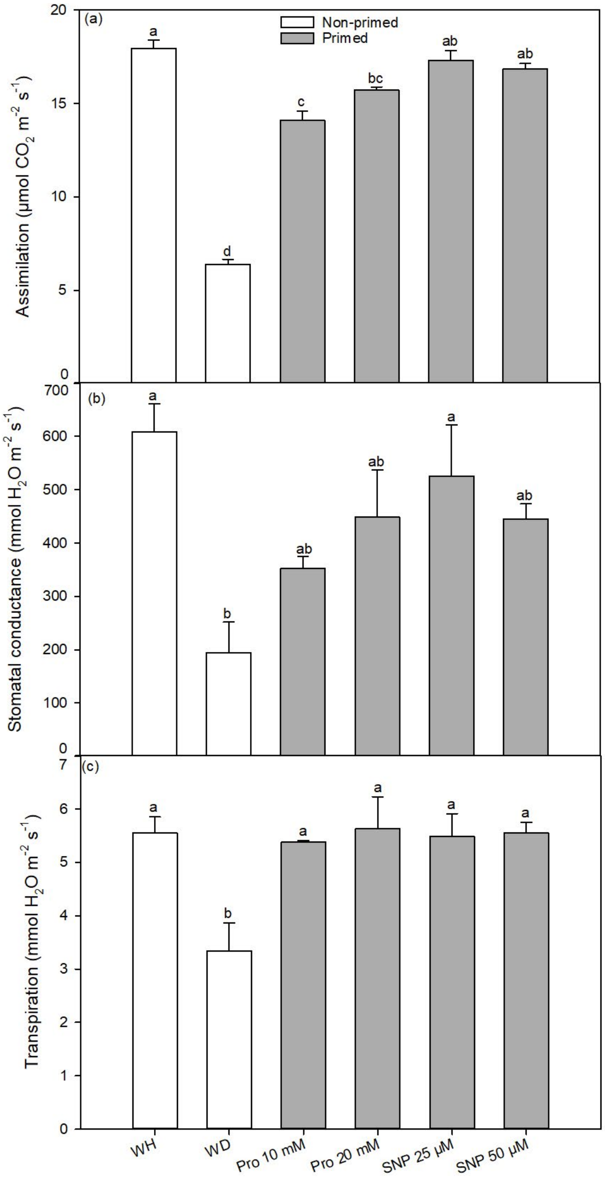

Figure 4: CO_2 assimilation (a), stomatal conductance (b) and transpiration (c) of *Physalis peruviana* plants under well hydrated conditions (WH) and exposed to water deficit (WD) after spraying with proline at 10 and 20 mM of (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 μM (SNP 25 μM and SNP 50 μM). Data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

Priming treatments stimulated the growth of *P. peruviana* plants when exposed to water deficit (Figures 5, 6 and 7). There was no statistical difference for the mean absolute growth rate (AGR) between plants that were exposed to water deficit and sensitized with chemical agents (Figure 5a). Nevertheless, plants pretreated with SNP at 25 and 50 µM showed an increase in AGR, incorporating 0.14 and 0.15 g.day^{-1}, respectively, corresponding to increments of 31% and 36% compared to untreated plants under water deficit ($p<0.0001$; Figure 5a). Pretreatment with proline and SNP maintained the relative growth rate (RGR) with average values above 0.19 g g^{-1} day^{-1} ($p<0.0001$) (Figure 5b). Thus, the improvement of physiological processes promoted by the pretreatments, such as in water relations and gas exchange, contributed to the maintenance of plant growth under water deficit, although at lower levels compared to plants under well hydrated conditions.

Water deficit reduced the leaf area (LA) of non-sensitized plants by 81.9%, compared to well hydrated plants (Figure 6), which is a common response in plants under this stress (Shawon et al., 2020; Leite et al., 2021a). Pretreatment with both doses of proline was not able to mitigate the effects of water deficit for this variable, while plants pretreated with SNP at 25 and 50 µM showed increments in LA of 46.2% and 34.4%, respectively, compared to non-sensitized plants, indicating efficiency in the application of priming ($p<0.0001$; Figure 6).

Biomass accumulation was reduced by water deficit in *P. peruviana* plants (Figure 7), as reported in other studies (Ozaslan et al., 2016; Leite et al., 2019). Leaf dry matter showed a drastic reduction with the imposition of water deficit, decreasing by 72.9% compared to well hydrated plants. This effect was attenuated in plants pretreated with both doses of SNP and Pro 20 mM ($p<0.0001$; Figure 7a). However, the use of these substances was not able to mitigate the effects of water deficit on the stem dry matter of the evaluated plants, differing only from those that were well hydrated ($p<0.0001$; Figure 7b). For root dry matter, pretreatment with SNP 25 mM was the most effective for biomass accumulation in plants under water deficit ($p<0.0001$; Figure 7c). The total dry matter in plants sprayed with SNP 25 µM and 50 µM differed from that of plants without pretreatments under water deficit and maintained mean values above 2.86 g, showing the priming effect ($p<0.0001$; Figure 7 d, e).

Pretreatments with NO donor (25 and 50 µM) and proline (20 mM) were able to improve hydration in plants under water deficit, acting in the maintenance of the photosynthetic process, and consequently stimulated biomass accumulation in plants under water deficit (Figure 7) and increased tolerance to stress (Figure 8). Reduced plant growth is one of the main processes caused by water deficit, as it reduces cell expansion (Salisbury; Ross, 2012). The maintenance or improvement, natural or induced, in the growth rates of plants when grown under adverse conditions is a characteristic that may represent greater tolerance to the stressful factor.

Water deficit and the application of pretreatments also altered infrared spectra in *P. peruviana* leaves (Figure 9). Figure 9a shows the FTIR spectrum in the region of 4000-400 cm^{-1} typical of the leaves of *P. peruviana* plants cultivated under well hydrated conditions and under water deficit without pretreatment. Strong absorptions were observed in the spectra of plants under water deficit, with peaks common to well hydrated plants. In the region of 3500-3000 cm^{-1}, the peaks found were associated with the stretching of O-H bands (Jones, 2012); lipid bands due to C-H stretching vibration occurred in the region of 3000-2800 cm^{-1} (Skotti et al., 2014), peaking at 2914 cm^{-1}, and in the range of 1800-800 cm^{-1}, which is the fingerprint region, in which most variations in infrared absorption occur (Carrión-Prieto et al., 2017) (Figure 9b).

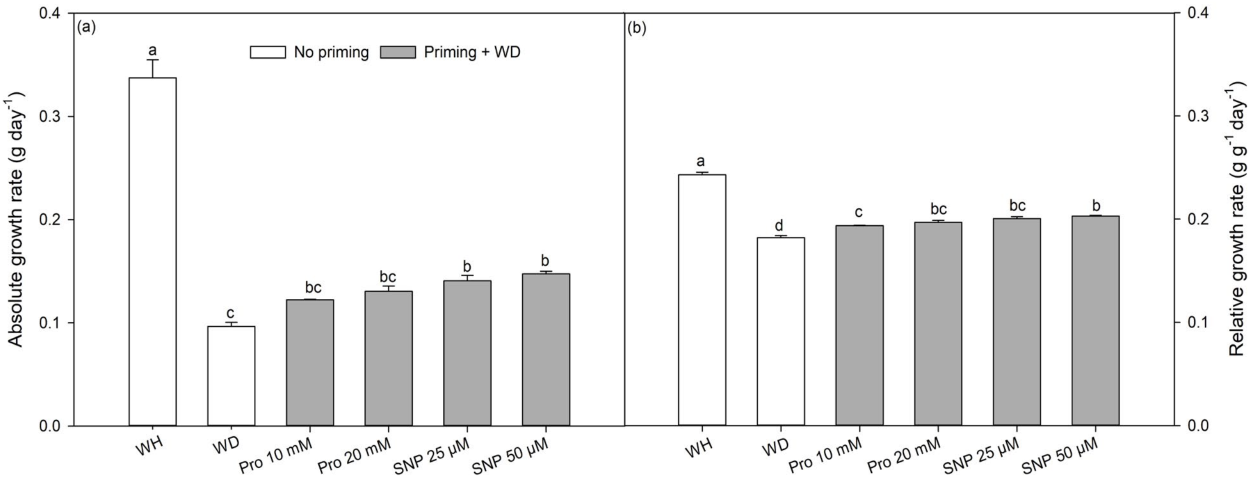

Figure 5: Absolute growth rate (a) and relative growth rate (b) of *P. peruviana* plants under well hydrated conditions (WH) and exposed to water deficit (WD) after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 μM (SNP 25 μM and SNP 50 μM). Data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

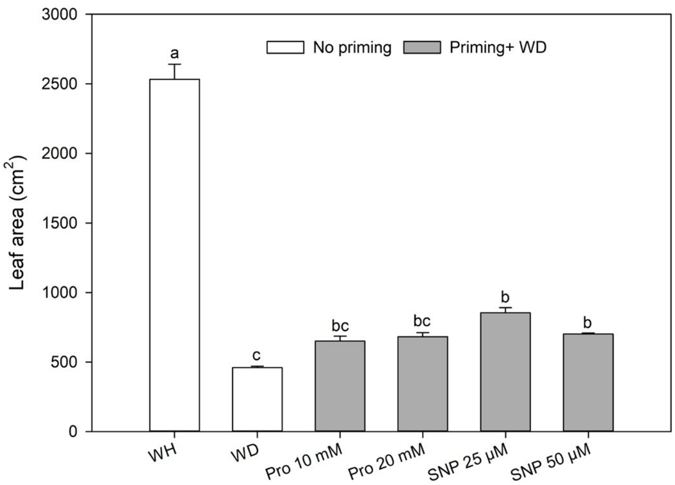

Figure 6: Leaf area of *P. peruviana* plants under well hydrated conditions (WH) and exposed to water deficit (WD) after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 μM (SNP 25 μM and SNP 50 μM). Data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

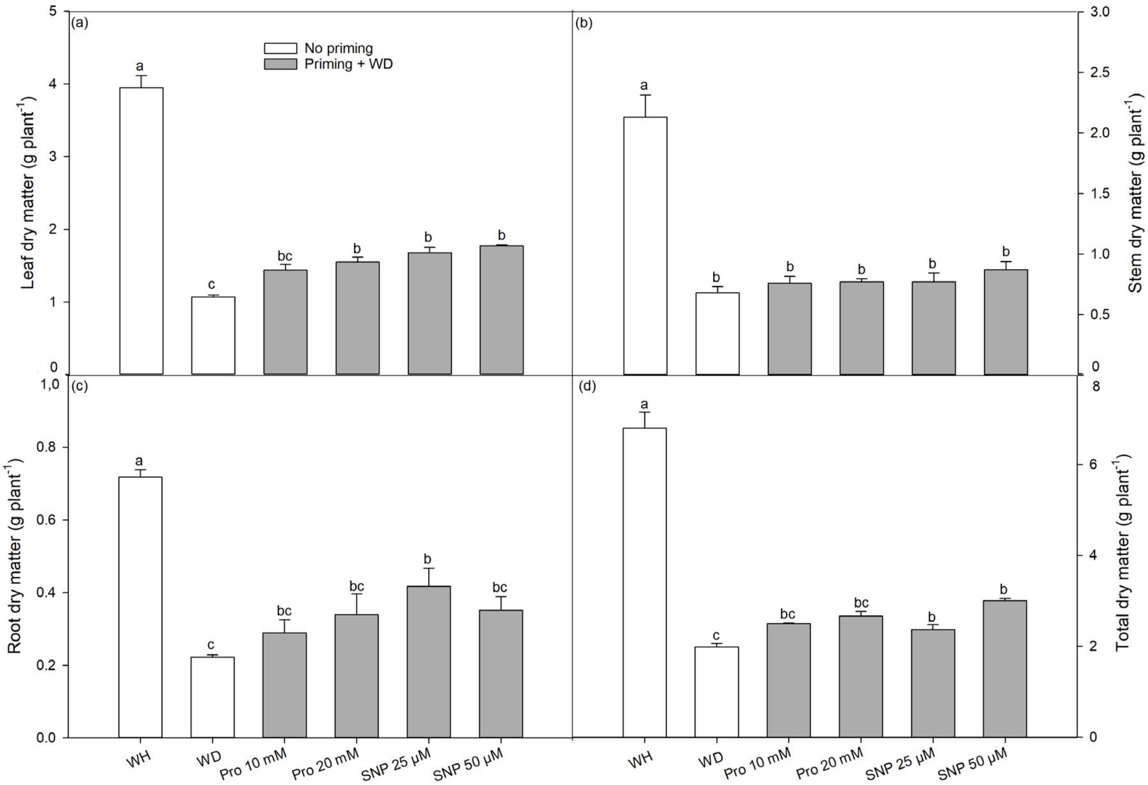

Figure 7: Leaf dry matter (a), stem dry matter (b), root dry matter (c) and total dry matter (d) and heat-map (e) of *P. peruviana* plants under well hydrated conditions (WH) and exposed to water deficit (WD) after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 μM (SNP 25 μM and SNP 50 μM). Data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

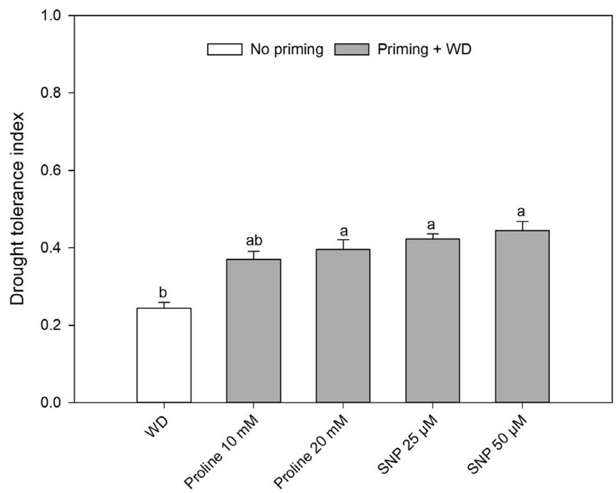

Figure 8: Drought tolerance index in *P. peruviana* plants exposed to water deficit (WD) and after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 μM (SNP 25 μM and SNP 50 μM). Data are expressed by the mean ± standard error. Means sharing the same letters show no significant differences at 5% significance level.

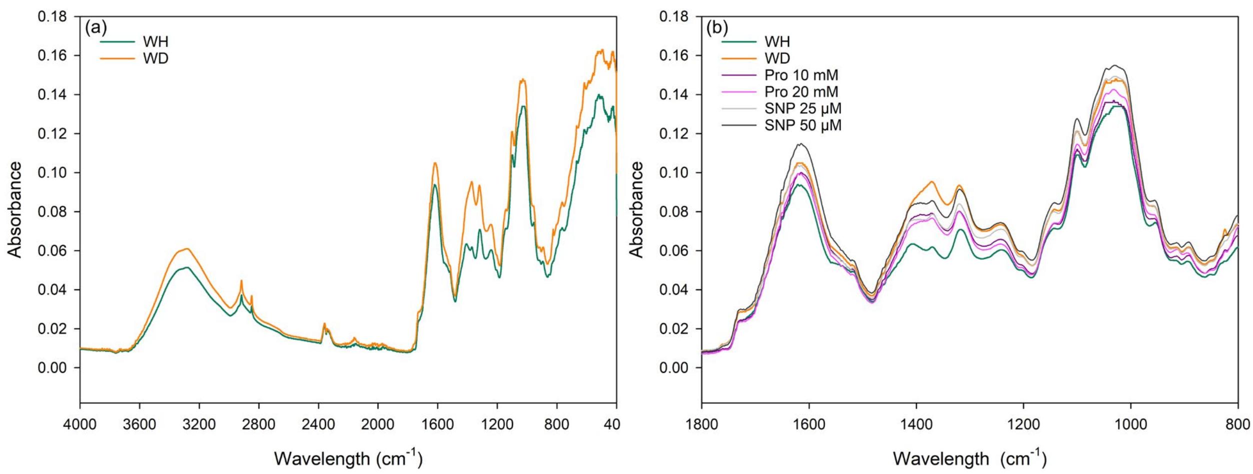

Figure 9: Infrared spectra with Fourier transform of *Physalis peruviana* leaves: (a) well hydrated plants (WH) and plants exposed to water deficit (WD); (b) characterization of the fingerprint region of plants under WH, WD and after spraying with proline at 10 and 20 mM (Pro 10 mM and Pro 20 mM) and sodium nitroprusside at 25 and 50 µM (SNP 25 µM and SNP 50 µM).

According to the fingerprint region, the bands from 1750 to 1250 cm^{-1} and from 1250 to 900 cm^{-1} correspond to amides and carbohydrates (Ogbaga et al., 2017), respectively, which had higher absorptions in plants under water deficit and other pretreatments (Figure 9b). Thus, variations induced in plant metabolism by environmental factors, such as water deficit, or any stressful situations may affect spectral peaks (Kumar et al., 2016). It is possible to notice that among plants subjected to pretreatments, the use of both doses of SNP induced higher absorption in the spectra corresponding to amides and carbohydrates, which may indicate greater accumulation of these substances in physiological processes such as osmoregulation. The spectra of leaves of plants pretreated with proline showed intermediate absorptions between plants under water deficit without pretreatment and plants under well hydrated conditions, indicating that this substance reduced the effects of water stress.

CONCLUSIONS

The use of proline and sodium nitroprusside has a priming effect on *Physalis peruviana* plants cultivated under water deficit, being indicated for increased tolerance to this stress factor. Foliar spraying with these substances in pretreatment, under the conditions indicated in this study, can promote improvements in water relations, gas exchange, growth rates, biomass and biomolecular composition. Given the emergence of the concept of sustainability, studies with underexploited species, especially those with food potential, stand out. Thus, the use of strategies that contribute to the maintenance and conservation of plant genetic resources are important. In this perspective, the use of chemical priming may emerge as an effective and strategic resource in agricultural cultivation, especially in places where severe water deficit is verified, such as semiarid regions.

AUTHOR CONTRIBUTION

Conceptual Idea: Leite, R. S.; Methodology design: Leite, R. S.; Nascimento, M. N.; Hernández-Navarro, S.; Potosme, N. M. R.; Data collection: Leite, R. S.; Silva, A. L.; Santos, R. J.; Data analysis and interpretation: Leite, R. S.; Hernández-Navarro, S.; Nascimento, M. N.; Potosme, N. M. R.; Silva, A. L.; Santos, R. J., and Writing and editing: Leite, R. S.; Hernández-Navarro, S.; Nascimento, M. N.; Potosme, N. M. R.; Silva, A. L.; Santos, R. J.

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