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Encapsulation of *S*-nitrosoglutathione into chitosan nanoparticles improves drought tolerance of sugarcane plants

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ABSTRACT

The entrapment of NO donors in nanomaterials has emerged as a strategy to protect these molecules from rapid degradation, allowing a more controlled release of NO and prolonging its effect. On the other hand, we have found beneficial effects of *S*-nitrosoglutathione (GSNO) – a NO donor – supplying to sugarcane plants under water deficit. Here, we hypothesized that GSNO encapsulated into nanoparticles would be more effective in attenuating the effects of water deficit on sugarcane plants as compared to the supplying of GSNO in its free form. The synthesis and characterization of chitosan nanoparticles containing GSNO were also reported. Sugarcane plants were grown in nutrient solution, and then subjected to the following treatments: control (well-hydrated); water deficit (WD); WD + GSNO sprayed in its free form (WDG) or encapsulated (WDG-NP). In general, both GSNO forms attenuated the effects of water deficit on sugarcane plants. However, the encapsulation of this donor into chitosan nanoparticles caused higher photosynthetic rates under water deficit, as compared to plants supplied with free GSNO. The root/shoot ratio was also increased when encapsulated GSNO was supplied, indicating that delayed release of NO improves drought tolerance of sugarcane plants. Our results provide experimental evidence that nanotechnology can be used for enhancing NO-induced benefits for plants under stressful conditions, alleviating the negative impact of water deficit on plant metabolism and increasing biomass allocation to root system.

1. Introduction

Water deficit is the main environmental factor limiting plant growth and productivity worldwide. The primary plant response to water deficit is stomatal closure in order to avoid excessive loss of water through leaf transpiration. However, this response also decreases the availability of CO_2 for photosynthesis and consequently biomass accumulation by sugarcane plants [1–3]. In addition, decreases in leaf chlorophyll content, inhibition of photochemical activity and low enzymatic activity in C4 metabolism were reported in sugarcane plants under water deficit [4,5].

Recent studies have shown that nitric oxide (NO) plays an important role in plants under stressful conditions [5–9]. NO is a reactive and gaseous molecule that interacts with cellular compounds and radicals [10], being considered an important signaling molecule with antioxidant potential [11]. In fact, NO participates in a complex cellular signaling network [12,13], affecting germination, growth and flowering [7] and regulating multiple responses to various abiotic stresses [14]. There is also evidence of NO acting as a signal transduction molecule leading to the induction of defense response against pathogen attack and to the programmed cell death [15,16]. NO synthesis has been reported in water-stressed plants [9,17] and its role as an important secondary messenger in plants was proposed [18].

Many NO donors have low molecular weight and are thermally and photochemically unstable, such as *S*-nitrosoglutathione (GSNO) [19]. In order to increase such stability, these donors are incorporated into

Abbreviations: *A*, leaf CO_2 assimilation; C_i, intercellular CO_2 concentration; CS, chitosan; NPs, nanoparticles; F_V/F_M, potential quantum efficiency of PSII; g_S, stomatal conductance; GSH, glutathione; GSNO, *S*-nitrosoglutathione; GSNO-CS-NPs, GSNO-containing chitosan nanoparticles; SDM, shoot dry mass; NO, nitric oxide; PEG, polyethylene glycol; PPFD, photosynthetic photon flux density; PSII, photosystem II; RDM, root dry mass; RWC, relative water content; SNO, *S*-nitrosothiol; WD, water deficit; ϕ_{PSII}, effective quantum efficiency of PSII

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polymeric matrices, such as chitosan-based nanoparticles [20], which change NO release. Regarding the matrices, chitosan (CS) is a biodegradable and biocompatible polysaccharide extensively used in drug delivery applications, particular as a nanocarrier system [21,22]. The use of nanoparticles in agriculture is a relatively new approach and has increased in the last decade [23]. The entrapment of NO donors in nanomaterials emerged as a strategy to protect these molecules from rapid degradation and allow more controlled release of NO, thereby prolonging its effects [24,25]. A recent study about nanoparticle application in plants demonstrated that the NO donor *S*-nitroso-mercaptosuccinic acid (*S*-nitroso-MSA) was more efficient in reducing salt-induced damage to photochemical activity of maize plants as compared to the same NO donor in its free form [26]. However, the potential of NO-releasing CS-NPs in alleviating abiotic stresses might be dependent on the chemical nature of the NO donor and the nanocarrier, plant species, and the nature and intensity of the abiotic stress such as water deficit, salinity, metal toxicity, extreme temperatures, and UV radiation [27].

Among NO donors, GSNO is an important *S*-nitrosothiol, which spontaneously releases NO through cleavage of S-N bound [28]. Recent studies have shown that the GSNO spray in its free form increases drought tolerance of sugarcane plants [5,8]; however, the effects of encapsulated GSNO remain unknown in plants. In this context, we tested the hypothesis that the spray of encapsulated GSNO would be more efficient in attenuating the effects of water deficit on sugarcane plants when compared to non-encapsulated GSNO. We expected that the controlled release of NO by encapsulated GSNO would extend the benefits of this NO donor already reported in sugarcane plants [5,8]. In addition, the synthesis and characterization of chitosan nanoparticles containing GSNO were addressed.

2. Material and methods

2.1. Plant material and growth conditions

Mini-stalks from sugarcane plants (*Saccharum* spp.) cv. IACSP94-2094 were cultivated in trays containing commercial substrate composed of Sphagnum, rice straw and perlite in 7:2:1 ratio (Carolina Soil®, Vera Cruz RS, Brazil). IACSP94-2094 was developed by the ProCana Breeding Program (Agronomic Institute, IAC, Brazil), being a genotype with good performance under rainfed conditions and reasonable yield [1]. Three-week-old plants with two to three leaves were transferred to modified Sarruge [29] nutrient solution composed by 15 mmol L^{-1} N (7% as NH_4^+); 4.8 mmol L^{-1} K; 5.0 mmol L^{-1} Ca; 2.0 mmol L^{-1} Mg; 1.0 mmol L^{-1} P; 1.2 mmol L^{-1} S; 28.0 μmol L^{-1} B; 54.0 μmol L^{-1} Fe; 5.5 μmol L^{-1} Mn; 2.1 μmol L^{-1} Zn; 1.1 μmol L^{-1} Cu and 0.01 μmol L^{-1} Mo; and maintained hydroponically inside a growth chamber (model PGR14, Conviron, Winnipeg MB, Canada). Environmental conditions inside the growth chamber were 28/20 °C (day/night), 80% air relative humidity, 12 h photoperiod (7:00 to 19:00 h) and photosynthetic photon flux density (PPFD) of 700 μmol m^{-2} s^{-1}. The pH of nutrient solution was kept between 5.5 and 6.0 and its electrical conductivity between 1.53 and 1.70 mS cm^{-1} by daily monitoring. Plants were subjected to the above conditions for 20 days before imposing the treatments.

2.2. Synthesis of free S-nitrosoglutathione (GSNO)

GSNO was synthesized and characterized as previously described [8]. Reduced glutathione (GSH) was dissolved in hydrochloric acid (1 mol L^{-1}) at 1.2 mol L^{-1}. An equimolar amount of sodium nitrite ($NaNO_2$) was added into GSH solution in order to nitrosate GSH under ice bath for 30 min and magnetic stirring. The obtained GSNO was precipitated by the addition of acetone, filtrated, and washed several times with cold water. The obtained solid was freeze-dried for 24 h. The GSNO formation was confirmed at 336 and 545 nm using UV-VIS spectrometry (Agilent, model 8454, Palo Alto CA, USA).

2.3. Synthesis of chitosan nanoparticles containing S-nitrosoglutathione (GSNO)

Chitosan nanoparticles (CS-NPs) were prepared using the ionic gelation method [20–22,26,30]. Briefly, chitosan (CS) was dissolved in acetic acid (1%) and 1.3 mmol L^{-1} of GSH was added to the solution. After 90 min of magnetic stirring at room temperature, a sodium tripolyphosphate (TPP) solution at 0.6 mg mL^{-1} was dropwise added to the CS/GSH solution. The final mixture was magnetic stirred for at least 90 min. The final concentration of GSH was equal to 1.0 mmol L^{-1}. In order to obtain GSNO-containing chitosan nanoparticles (GSNO-CS-NPs), an equimolar amount of sodium nitrite ($NaNO_2$) related to GSH content was added to GSH-CS-NPs dispersion. The final solution was homogenized and the reaction was allowed for 90 min under darkness. Such GSNO-CS-NPs solution was used immediately after its preparation.

2.4. Water deficit induced by PEG and GSNO spraying

Sugarcane plants growing in nutrient solution were subjected to water deficit (WD) by adding polyethylene glycol (Carbowax™ PEG-8000, Dow Chemical Comp, Midland MI, USA) to the solution. To prevent osmotic shock, PEG-8000 was added to the nutrient solution to cause a gradual decrease in its osmotic potential. The osmotic potential of the nutrient solution was decreased as follows: −0.25 MPa at the first day; −0.50 MPa at the second day; and −0.80 MPa at the third day. After nine days under PEG-induced water deficit (−0.80 MPa), the plants were transferred to the original nutrient solution (−0.15 MPa) for recovery.

Sugarcane leaves were sprayed twice a day at the same three days when PEG was added to the nutrient solution. Plants were then subjected to the following treatments: nutrient solution with osmotic potential of −0.15 MPa + water spray (Control); nutrient solution with osmotic potential of −0.80 MPa + water spray (water deficit, WD); WD + GSNO spray, in which free GSNO solution was sprayed (WDG); and WD + encapsulated GSNO, in which encapsulated GSNO solution was sprayed (WDG-NP). GSNO spraying was performed outside the growth chamber to avoid undesirable interference among treatments. Based on our previous results [5], GSNO 100 μM was used in both free and encapsulated forms. Considering the beginning of the experiment, the maximum water deficit was reached at the 12^{th} day of treatment and the 15^{th} day was the third day of rehydration, when the experiment ended.

2.5. Determination of hydrodynamic size, polydispersity index (PDI) and zeta potential of GSNO-CS-NPs

The average hydrodynamic size, polydispersity index (PDI), and zeta potential of GSNO-CS-NPs were evaluated by dynamic light scattering (DLS) using a Nano ZS Zetasizer (Malvern Instruments Co, Malvern, UK) [31]. Measurements were performed in three independent experiments at 25 °C using a fixed angle of 173° in disposable folded capillary zeta cells with a 10 mm path length in aqueous suspension.

2.6. Kinetics of NO release from free and encapsulated GSNO

The kinetics of NO release from free and encapsulated GSNO forms was monitored over a period of three days at room temperature. The initial GSNO concentration was 1 mmol L^{-1} for both forms (Agilent, model 8454, Palo Alto CA, USA). The kinetics was followed by the spectral changes at 336 nm related to $\pi \rightarrow \pi^*$ transition (ε = 980.0 mol L^{-1} cm^{-1}), which are solely associated with S-N bond cleavage and free NO release [21,22,26].

2.7. Leaf gas exchange and photochemistry

Leaf gas exchange was measured daily using an infrared gas analyzer (Li-6400, Licor, Lincoln NE, USA) attached to a modulated fluorometer (6400-40 LCF, Licor, Lincoln NE, USA). Leaf CO_2 assimilation (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration (E) were measured under PPFD of 2000 μmol m^{-2} s^{-1} and air CO_2 concentration of 400 μmol mol^{-1}. The instantaneous carboxylation efficiency (k) was given by the rate between leaf CO_2 assimilation and intracellular CO_2 partial pressure (A/C_i). The measurements were performed between 10:00 h and 13:00 h, as done previously [5,8]. The vapor pressure difference between leaf and air (VPDL) was 2.2 ± 0.3 kPa and leaf temperature was 29 ± 1 °C during the evaluations. The A and E values were integrated during the experimental period to estimate the total CO_2 gain (A_i) and the total water vapor loss (E_i) in each treatment.

Chlorophyll fluorescence was evaluated simultaneously to the leaf gas exchange and the effective quantum efficiency of photosystem II (ϕ_{PSII}) was estimated by the pulse saturation method [32,33]. Following the same method, the potential quantum efficiency of photosystem II (F_v/F_m) was measured in dark-adapted leaves (30 min). The chlorophyll content was evaluated with a portable chlorophyllmeter model SPAD-502 (Konica Minolta, Tokyo, Japan), following the manufacturer's instructions.

2.8. Relative water content (RWC) and biomass analysis

The leaf relative water content (RWC) was calculated using the fresh (FW), turgid (TW) and dry (DW) weight of leaf discs according to Weatherley [34]: $RWC = 100 \times [(FW - DW)/(TW - DW)]$. This measure was performed at the day of maximum water deficit. At the end of experiment, shoots and roots were harvested and the dry matter determined after drying samples in an oven with forced-air circulation (model MA032, Marconi, Piracicaba SP, Brazil) at 60 °C until constant weight. The root/shoot ratio was calculated.

2.9. Estimation of leaf and root S-nitrosothiol contents

Total protein in leaf and root samples was extracted in Mili-Q water and the resulting homogenate used for the amperometric estimation of *S*-nitrosothiol content [35]. Measurements were carried out with the WPI TBR4100/1025 amperometer (World Precision Instruments Inc., Sarasota FL, USA) and a nitric oxide specific ISO-NOP sensor (2 mm) was used. Data was compared to a standard curve obtained with GSNO and normalized against leaf fresh weight. Calibration curves were obtained with aqueous solutions of freshly prepared GSNO.

2.10. Data analysis

The data were subjected to the analysis of variance (ANOVA) and mean values of each treatment (n = 3 to 5) were compared by the Scott-Knott test when significance was detected ($P < 0.05$).

3. Results

3.1. Synthesis of GSNO-CS-NPs

Chitosan nanoparticles were synthesized by ionotropic gelation method, which is a simple and effective route for nanoparticle preparation. Positive charged CS chains were crosslinked with the polianion TPP. As a NO precursor molecule, GSH was incorporated into CS-NPs, followed by its nitrosylation, yielding GSNO-CS-NPs. DLS analyses demonstrated that the NPs have average hydrodynamic size of 104.8 ± 1.7 nm, PDI of 0.345 ± 0.009, and a positive zeta potential of 17.5 ± 0.9 mV. The NPs were found to be at the nanoscale with moderate polydispersity and a positive zeta potential due to the presence of protonated amino groups on their surface. These results are in accordance with previous data reported for the encapsulation of low molecular NO donors in CS-NPs [21,22].

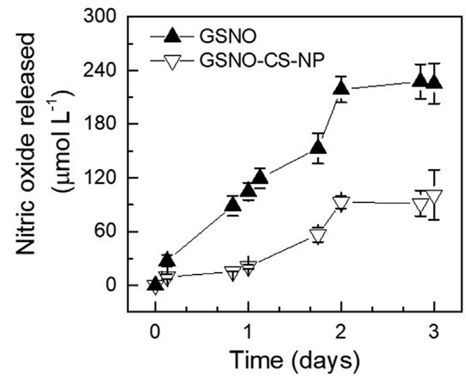

Fig. 1. Temporal kinetics of NO release from free or encapsulated GSNO in chitosan nanoparticles (GSNO-CS-NP). Each symbol represents the mean value of three replications ± standard deviation.

3.2. Kinetics of NO release from free and encapsulated GSNO

Both free and encapsulated GSNO underwent decomposition, releasing free NO. However, the encapsulation of GSNO into CS-NP decreased the rate of GSNO decomposition in comparison with free GSNO (Fig. 1). After 24 h, the decomposition of free GSNO was 3-fold higher as compared with the encapsulated GSNO. After three days, free GSNO released 225.5 μmol L^{-1} of NO, while the decomposition of encapsulated GSNO released around 100 μmol L^{-1} (Fig. 1). These results indicate that the incorporation of GSNO into CS-NP protects the NO donor from degradation.

3.3. Leaf gas exchange and photochemistry

As expected, the water deficit decreased leaf CO_2 assimilation of sugarcane plants and the supply of free or encapsulated GSNO was able to attenuate such negative effects on leaf physiology (Fig. 2). When considering the integrated CO_2 gain throughout the experimental period, the encapsulated GSNO (WDG-NP) was more effective in alleviating the water stress than free GSNO (Fig. 2A). While water loss through transpiration was decreased in sugarcane plants under water deficit (−58%), such decreases were less intense in plants sprayed with free or encapsulated GSNO (Fig. 2B). Although reduced by water deficit, stomatal conductance tended to increase in plants sprayed with GSNO, regardless of its form (Fig. 2C). The instantaneous carboxylation efficiency (k) was significantly reduced by water deficit and this negative effect was alleviated on the 4^{th} and 11 th days of water deficit by spraying encapsulated GSNO (Fig. 2D).

Regarding the photochemistry, the potential quantum efficiency of PSII was significantly reduced under low water availability, with such effects being attenuated by free and encapsulated GSNO at the maximum water deficit (Fig. 3A). Water deficit also reduced the effective quantum efficiency of PSII (−70%) when compared to well-hydrated plants (Fig. 3B). However, such decrease was attenuated in plants sprayed with either encapsulated or free GSNO (Fig. 3B).

3.4. Relative water content and chlorophyll content

The water deficit caused reduction in leaf relative water content (RWC) of sugarcane plants at the maximum water deficit (Fig. 4A). However, plants sprayed with encapsulated or free GSNO presented higher RWC than the other plants under water deficit without GSNO supplying. Low water availability also reduced the chlorophyll content

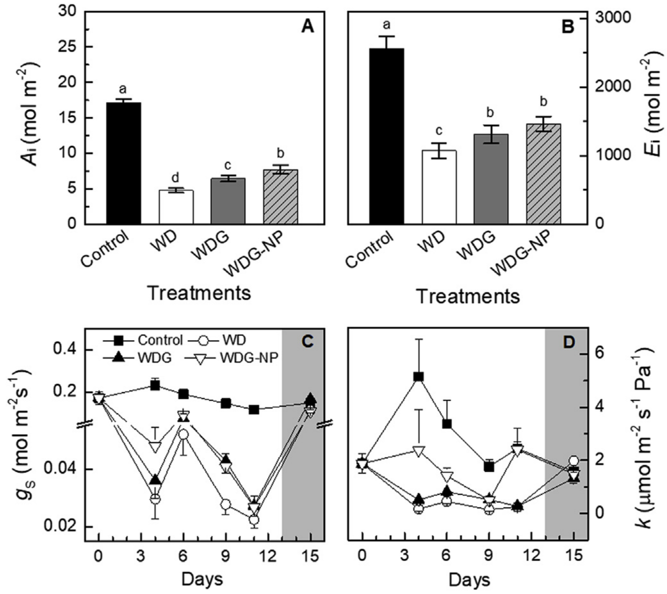

Fig. 2. Integrated leaf CO_2 assimilation (A_i, in A) and transpiration (E_i, in B) and time-course of stomatal conductance (g_s, in C) and instantaneous carboxylation efficiency (k, in D) of sugarcane plants maintained well-hydrated (Control), subjected to water deficit and sprayed with water (WD) or subjected to WD and sprayed with free (WDG) or encapsulated (WDG-NP) GSNO at 100 μM. The shaded area (in C and D) indicates recovery period, when plants were moved to nutrient solution used in control treatment. Data represent the mean values of five replications ± standard deviation. In A and B, different lowercase letters indicate statistical difference among treatments (Scott-Knott test, $P < 0.05$).

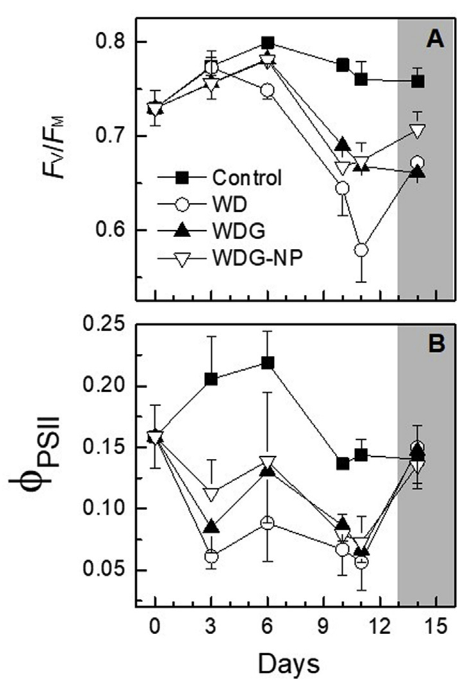

Fig. 3. Potential (F_V/F_M, in A) and effective (ϕ_{PSII}, in B) quantum efficiency of PSII in sugarcane plants maintained well-hydrated (Control), subjected to water deficit and sprayed with water (WD) or subjected to WD and sprayed with free (WDG) or encapsulated (WDG-NP) GSNO at 100 μM. The shaded area indicates recovery period, when plants were moved to nutrient solution used in control treatment. Each symbol represents the mean value of five replications ± standard deviation.

of sugarcane plants and again the encapsulated or free GSNO spraying attenuated such effect, with GSNO-supplied plants under water deficit presenting values similar to those of control plants (Fig. 4B).

3.5. Plant biomass

Shoot dry mass (SDM) was reduced under water deficit, with plants showing similar values regardless of GSNO spraying (Fig. 5A). However, decreases in root dry mass (RDM) due to water deficit were found only in plants that did not receive GSNO spraying (Fig. 5B). As consequence, the root/shoot ratio was increased 2.8 times in WDG treatment and 5.8 times in WDG-NP treatment compared to WD treatment (Fig. 5C).

3.6. Leaf and root S-nitrosothiois concentrations

The level of *S*-nitrosylated proteins was evaluated in both leaf and root extracts of plants sprayed with water (control and WD), encapsulated and free GSNO solutions. The highest *S*-nitrosothiol concentrations in both plant organs were found in plants sprayed with GSNO, regardless of how this donor was supplied (Fig. 6). Interestingly, *S*-nitrosothiol concentration was not changed due to water deficit.

4. Discussion

Our data revealed that both encapsulated and free GSNO forms were efficient in mitigating the damage caused by water deficit on sugarcane plants. As novelty, the encapsulation of this donor into chitosan nanoparticles (GSNO-CS-NPs) increased leaf CO_2 assimilation under water deficit, as compared to those ones supplied with free GSNO (Fig. 2A). This can be explained by the release dynamics, which was slower in encapsulated GSNO than in free one (Fig. 1) and then allowed sustainable release of NO for a longer period of time. Then, we may argue that NO-releasing by CS-NPs was more efficient in alleviating the effects of water stress on sugarcane photosynthesis. This finding has practical importance for agricultural/horticultural applications because long-term effect of NO would be warranted by GSNO encapsulation. The improvement of CO_2 assimilation in plants under water deficit supplied with GSNO was associated, in part, with higher stomatal conductance and higher relative leaf water content (RWC) (Figs. 2C and 4A). Such increase in RWC would be a consequence of increased root system (Fig. 5B), which likely favored water uptake.

Although the encapsulation had not affected chlorophyll content, GSNO avoided the degradation of chlorophyll under water deficit (Fig. 4B), confirming our previous results [5,8]. In relation to the potential quantum efficiency of PSII, improvements caused by GSNO supplying were similar regardless of its form (Fig. 3A). Curiously, higher chlorophyll content in plants under WDG and WDG-NP treatments did not cause higher effective quantum efficiency of PSII (Figs. 3B and 4B). In fact, photochemical activity is not defined solely by chlorophyll content. While chlorophyll molecules are needed to light absorption and drive energy to the PSII reactions centers, photochemical activity from charge separation at PSII level to the production of ATP and NADPH is dependent on and regulated by other physiological processes such as alternative electron sinks activated under stressful conditions [36–40]. Then, plants may exhibit similar photochemical activity in spite of their chlorophyll contents being different, as found herein.

The absence of improvements in photochemistry due to GSNO encapsulation is not in agreement with Oliveira et al. [26], who compared the effects of NO donor *S*-nitroso-mercaptosuccinic acid (*S*-nitroso-MSA) in its encapsulated and free forms on maize plants under salt stress. In fact, the best responses in terms of photochemistry were found in plants supplied with encapsulated S-nitroso-MSA. However, any extrapolation of those previous results must be careful as photosynthesis and plant growth are driven not only by light conversion at PSII level

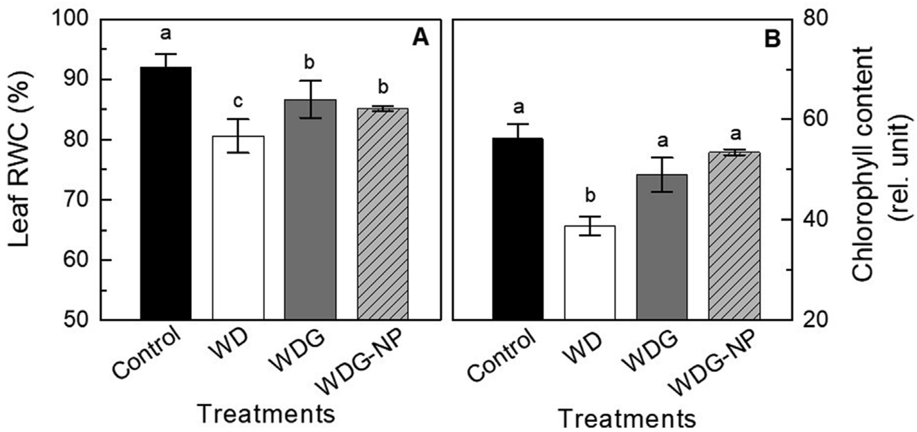

Fig. 4. Leaf relative water content (RWC, in A) and chlorophyll content (in B) in sugarcane plants maintained well-hydrated (Control), subjected to water deficit and sprayed with water (WD) or subjected to WD and sprayed with free (WDG) or encapsulated (WDG-NP) GSNO at 100 μM. Measurements done at maximum stress (12th day). Data represent the mean values of four replications ± standard deviation. Different lowercase letters indicate statistical difference among treatments (Scott-Knott test, $P < 0.05$).

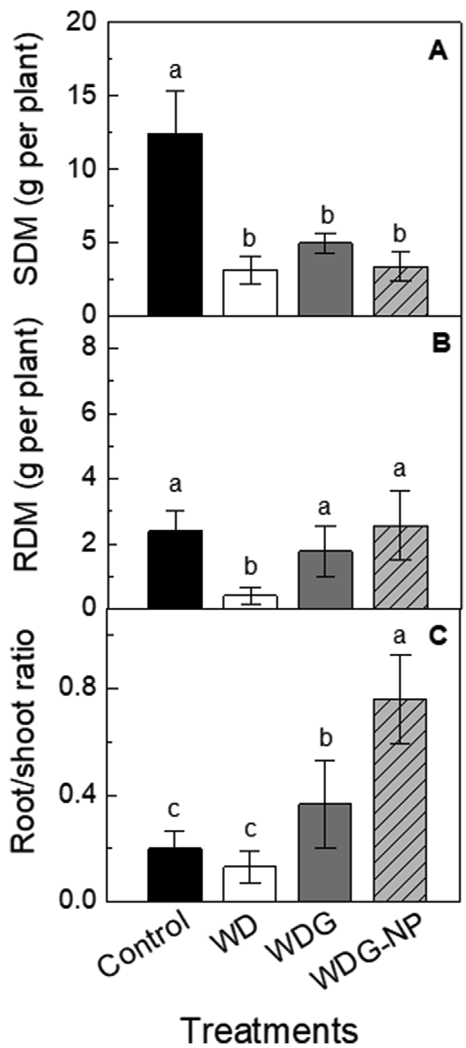

Fig. 5. Shoot (SDM, in A) and root (RDM, in B) dry mass and root/shoot ratio (in C) in sugarcane plants maintained well-hydrated (Control), subjected to water deficit and sprayed with water (WD) or subjected to WD and sprayed with free (WDG) or encapsulated (WDG-NP) GSNO dose at 100 μM. Measurements done at the end of the experiment (15th day). Data represent the mean values of four replications ± standard deviation. Different lowercase letters indicate statistical difference among treatments (Scott-Knott test, $P < 0.05$).

but also by CO_2 diffusion from atmosphere to carboxylation sites and consequent assimilation through biochemical reactions. Herein, we reported for the first time the impact of GSNO-containing CS-NPs on sugarcane plants under water deficit, considering stomatal, metabolic and photochemical limitations of the photosynthetic process and also the impact on biomass production. Comparing our results with previous ones using CS-NPs [26], it is evident that the impacts of GSNO-CS-NPs on sugarcane under water deficit were different when compared to S-nitroso-MSA-CS-NPs on maize under salinity and these findings show us that plant species can respond significantly different to NO-releasing CS-NPs. At this time, it is important to emphasize that NO donors differ in composition, half-life, stability and release rate [41,42] as well as plant sensitivity to stressful conditions and donors are likely species- and dose-dependent, respectively.

NO can interact directly or indirectly with several targets and then modulate protein function and gene expression. The bioactivity transfer of NO can occur through several mechanisms, with *S*-nitrosylation being the main one based on redox and post-translational modifications. *S*-nitrosylation targets regulatory proteins involved in responses to water deficit, including both protein kinases and transcription factors [43]. The *S*-nitrosylation of cysteine residues is known to promote or inhibit the formation of disulfide bonds, inducing changes in protein conformation and altering its activity [44]. As higher instantaneous carboxylation efficiency and leaf CO_2 assimilation were found in GSNO-supplied plants (Fig. 2A,D) and the level of nitrosothiols was higher in those plants, we may suggest that GSNO supplying caused *S*-nitrosylation of proteins involved in photosynthetic metabolism. Recently, we found increases in Rubisco activity in GSNO-supplied plants [5], which is in agreement with the results reported herein. However, GSNO form did not affect the level of nitrosothiols after 12 days of water deficit (Fig. 6). As compared to WDG, our data revealed that the photosynthetic improvement in plants sprayed with encapsulated GSNO (WDG-NP) was not caused by higher stomatal conductance and higher photochemical activity. The other limiting process to photosynthesis is related to carboxylation and this process was affected by nano-encapsulation, as shown in Fig. 2D. It seems that delayed release of NO was able to improve carboxylation activity when plants faced water deficit, being Rubisco protein a possible target of nitrosylation. This hypothesis must be explored in future research, as well as other proteins involved in both C3 and C4 cycles.

Despite the shoot and root biomasses were similar in plants supplied with GSNO, the root/shoot ratio was significantly higher when encapsulated GSNO was used (Fig. 5C). This increase in root/shoot ratio may represent a strategy to explore more efficiently the root medium, balancing water uptake and loss and helping plants cope with water stress [45]. Studies using mutants infer that NO acts downstream of hormone auxin through a direct signaling pathway during root growth and development, in close cooperation with other molecules, such as

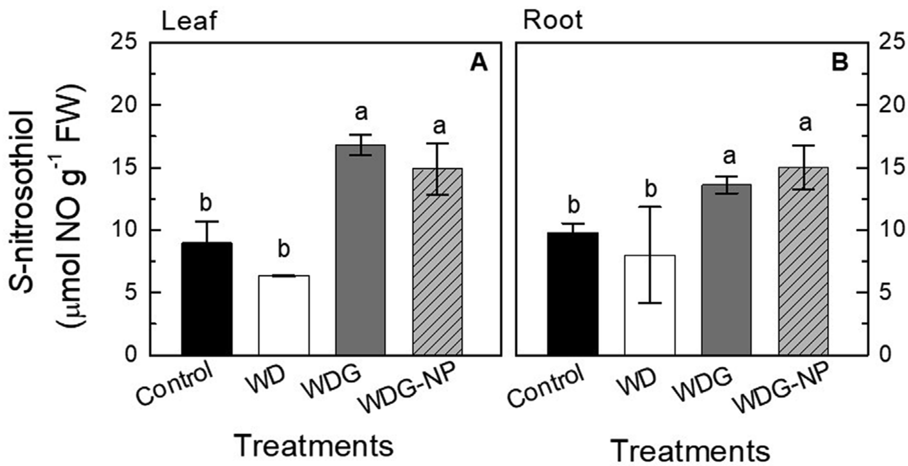

Fig. 6. The *S*-nitrosothiol concentration in leaves (in A) and roots (in B) of sugarcane plants maintained well-hydrated (Control), subjected to water deficit and sprayed with water (WD) or subjected to WD and sprayed with free (WDG) or encapsulated (WDG-NP) GSNO dose at 100 μM. Measurements done at the maximum water deficit (12th day). Data represent the mean values of four replications ± standard deviation. Different lowercase letters indicate statistical difference among treatments (Scott-Knott test, $P < 0.05$).

cGMP [46,47]. NO has also been shown to influence auxin content in plants by modulating the synthesis, transport and degradation of this hormone, and influencing growth and development of lateral and adventitious roots [46,48]. Accordingly, Silveira et al. [8] found stimulation of root growth when sugarcane plants were supplied with GSNO in its free form.

S-nitrosoglutathione (GSNO) reductase (AtGSNOR1) is a key regulator of cellular *S*-nitrosothiol (SNO) levels in *Arabidopsis thaliana* and the impact of loss- and gain-of-function mutations in AtGSNOR1 on plant growth and development were studied by Kwon and colleagues [49]. While loss of AtGSNOR1 function increased SNO levels and disabled plant defense responses, increased AtGSNOR1 activity reduced SNO formation and enhanced protection against virulent microbial pathogens. In fact, SNO formation and turnover are regulated in multiple ways and have influence on plant responses to both abiotic and biotic stresses [49,50]. We noticed increases in root SNO levels when plants were supplied with GSNO either encapsulated or free (Fig. 6B). This finding suggests the existence of NO-modified proteins in roots and then signaling between above and belowground organs. Although the root architecture is shaped by auxin and NO [51], how those factors interact and affect growth and developmental processes is not well understood. In *Arabidopsis thaliana* F-box proteins TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNAL-ING F-BOX (TIR1/AFB) are auxin receptors that mediate degradation of AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) repressors to induce auxin-regulated responses. TIR1 *S*-nitrosylation has been reported to enhance TIR1–Aux/IAA interaction, indicating an important role for a redox-based mechanism mediated by NO [52].

In general, spraying plants with encapsulated GSNO caused higher photosynthesis under water deficit, without affecting the total biomass of plants but increasing the root/shoot ratio (Figs. 2 and 5). This could be partly explained by increases in plant respiration, a process that deserves some attention in future research. NO is able to induce the alternative oxidase pathway (AOX) by inhibiting cytochrome oxidase [53]. AOX1 expression is strongly induced in Arabidopsis cell cultures treated with NO, resulting in increased respiration through the alternative pathway [54]. In plants, excessive mitochondrial energy can be minimized by the action of AOX and non-phosphorylative pathways related to the electron transport in mitochondria may have a role in plant acclimation to environmental stresses [55].

Although NO-releasing nanomaterials have been extensively explored in different biomedical applications, their uses in plants are still poorly explored. To the best of our knowledge, this is the first report to describe the use of NO-releasing nanoparticles in alleviating the deleterious effect of water deficit in sugarcane plants, considering not only leaf gas exchange and primary photochemistry but also biomass production. Such approach might have important applications for improving plant responses to abiotic stresses by controlling the rates and the amounts of NO release from the encapsulated NO donors into the nanomaterials. In conclusion, the spraying of both encapsulated and free GSNO – a NO donor – reduced sugarcane sensitivity to water deficit. However, leaf CO_2 assimilation and root/shoot ratio were increased when encapsulated GSNO was provided, indicating that delayed release of NO improves sugarcane response to water deficit. Although NO and nanomaterials have potential for using in management of both field-grown and potted plants, further studies are needed to investigate how nanotechnology affects NO accumulation and signaling in plants under stressful conditions.

Authors' contribution

NMS, ABS, ECM and RVR designed the experiments. NMS and FCCM performed the measurements of photosynthesis and plant biometry. MTP and ABS prepared the free and encapsulated GSNO solutions and measured *S*-nitrosothiol levels. NMS, ABS and RVR wrote the manuscript and all authors contributed in data discussion and edited the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.niox.2019.01.004.

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