



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Exogenous nitric oxide improves sugarcane growth and photosynthesis under water deficit

Citation for published version:

Silveira, NM, Frungillo, L, Marcos, FCC, Pelegrino, MT, Miranda, MT, Seabra, AB, Salgado, I, Machado, EC & Ribeiro, RV 2016, 'Exogenous nitric oxide improves sugarcane growth and photosynthesis under water deficit', *Planta*, vol. 244, no. 1, pp. 181-190. <https://doi.org/10.1007/s00425-016-2501-y>

Digital Object Identifier (DOI):

[10.1007/s00425-016-2501-y](https://doi.org/10.1007/s00425-016-2501-y)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

Planta

Publisher Rights Statement:

This is a post-peer-review, pre-copyedit version of an article published in *Planta*. The final authenticated version is available online at: <https://doi.org/10.1007/s00425-016-2501-y>

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



1 **Exogenous nitric oxide improves sugarcane growth and photosynthesis**
2 **under water deficit**

3

4 Neidiquele M. Silveira^a, Lucas Frungillo^{b,c}, Fernanda C.C. Marcos^b, Milena T.

5 Pelegrino^d, Marcela T. Miranda^b, Amedea B. Seabra^d, Ione Salgado^b, Eduardo C.

6 Machado^a, Rafael V. Ribeiro^{b,*}

7

8 ^aLaboratory of Plant Physiology “Coaracy M. Franco”, Center R&D in Ecophysiology
9 and Biophysics, Agronomic Institute (IAC), Campinas SP, Brazil

10 ^bDepartment of Plant Biology, Institute of Biology, University of Campinas
11 (UNICAMP), Campinas SP, Brazil

12 ^cInstitute of Molecular Plant Sciences, School of Biological Sciences, University of
13 Edinburgh, Edinburgh, UK

14 ^dDepartment of Exact and Earth Sciences, Federal University of São Paulo (UNIFESP),
15 Diadema SP, Brazil

16 *Corresponding author: rvr@unicamp.br

17

18 **Abbreviations:** *A*, leaf CO₂ assimilation; *C_i*, intercellular CO₂ concentration; ETR,
19 apparent electron transport rate; *F_v/F_M*, maximum quantum efficiency of PSII; *g_s*,
20 stomatal conductance; GSH, glutathione; GSNO, *S*-nitrosoglutathione; *k*, instantaneous
21 carboxylation efficiency; LDM, leaf dry mass; NO, nitric oxide; NPQ, non-
22 photochemical quenching; PEG, polyethylene glycol; PPFD, photosynthetic photon flux
23 density; PSII, photosystem II; RDM, root dry mass; RWC, relative water content; RSNO,
24 *S*-nitrosothiol; WD, water deficit; ϕ_{PSII} , effective quantum efficiency of PSII.

25

26 **Abstract**

27 ***Main conclusion* NO-mediated redox signaling plays a role in alleviating the negative**
28 **impact of water stress in sugarcane plants by improving root growth and**
29 **photosynthesis.**

30 Drought is an environmental limitation affecting sugarcane growth and yield. The redox
31 active molecule nitric oxide (NO) is known to modulate plant responses to stressful
32 conditions. NO may react with glutathione (GSH) to form *S*-nitrosoglutathione (GSNO),
33 which is considered the main reservoir of NO in cells. Here, we investigate the role of
34 NO in alleviating the effects of water deficit on growth and photosynthesis of sugarcane
35 plants. Well-hydrated plants were compared to plants under drought and sprayed with
36 mock (water) or GSNO at concentrations ranging from 10 to 1000 μ M. Leaf GSNO
37 sprayed plants showed significant improvement of relative water content, and leaf and
38 root dry matter under drought compared to mock-sprayed plants. Additionally, plants
39 sprayed with GSNO (≥ 100 μ M) showed higher leaf gas exchange and photochemical
40 activity as compared to mock-sprayed plants under water deficit and after rehydration.
41 Surprisingly, a raise in the total *S*-nitrosothiols content was observed in leaves sprayed
42 with GSH or GSNO, suggesting a long-term role of NO-mediated responses to water
43 deficit. Experiments with leaf discs fumigated with NO gas also suggested a role of NO
44 in drought tolerance of sugarcane plants. Overall, our data indicate that the NO-mediated
45 redox signaling play a role in alleviating the negative effects of water stress in sugarcane
46 plants by protecting the photosynthetic apparatus and improving shoot and root growth.

47

48 **Keywords:** Drought; Photochemistry; *Saccharum* spp; *S*-nitrosoglutathione; Water
49 stress.

50

51 **Introduction**

52

53 Drought is considered the main abiotic stress for plants (Parry et al. 2004; Cruz de
54 Carvalho 2008), being the most important environmental constrain to sugarcane (Ramesh
55 2000). Under drought conditions, stomatal closure is a primary response to avoid water
56 loss through leaf transpiration. However, such response also reduces the CO₂ availability
57 for photosynthesis and then biomass production is inhibited (Machado et al. 2009; Ribeiro
58 et al. 2013). Additionally, decreases in leaf chlorophyll content, inhibition of
59 photochemical activity and photosynthetic enzymes of the C₄ metabolism have been
60 reported in drought-stressed sugarcane (Machado et al. 2009; Barbosa et al. 2015). As
61 consequence of low carboxylation capacity, there is an ineffective recycling of
62 coenzymes ATP and NADPH produced during the light reactions and plants face
63 excessive light energy and photoinhibition of photosynthesis, with reduction on quantum
64 efficiency of photosystem II (Sales et al. 2013, 2015).

65 Nitric oxide (NO) is a redox active molecule with well-established central roles in
66 plant development and responses to biotic and abiotic stresses (Santos-Filho et al. 2012;
67 Salgado et al. 2013; Frungillo et al. 2014; Kneeshaw et al. 2014; Simontacchi et al. 2015).
68 Intracellularly, NO may react with the antioxidant glutathione (GSH) to yield GSNO (Liu
69 et al. 2001). GSNO has been considered a natural reservoir of NO in cells (Stamler et al.
70 1992; Lindermayr et al. 2005) and several lines of evidence suggest that the NO and
71 GSNO signaling functions overlap. In fact, both NO and GSNO are able to post-
72 transcriptionally control protein activity and localization through *S*-nitrosylation (Salgado
73 et al. 2013; Yu et al. 2014). NO may also react with superoxide under oxidative stress and
74 produce the potent oxidant peroxynitrite that causes permanent nitration of tyrosine
75 residues in proteins (Radi 2004). This NO-mediated mechanism of protein modification

76 may also be induced during plant responses to biotic and abiotic stresses (Chaki et al.
77 2011). As transcription factors can also be targets of *S*-nitrosylation, NO/GSNO can
78 change gene expression (Besson-Bard et al. 2009; Begara-Morales et al. 2014).

79 The phytohormone abscisic acid (ABA) is a key constituent of abiotic stress
80 responses in plants. During water stress, biosynthesis and activation of ABA mediates
81 stomatal closure to prevent water loss by transpiration, a processes modulated by the
82 activity of open stomata 1 (OST1)/sucrose nonfermenting 1 (SNF1)-related protein kinase
83 2.6 (SnRK2.6) (Lee et al. 2006). Recently, *S*-nitrosylation of SnRK2.6 at Cys 137 was
84 proposed to counteract ABA-induced stomatal closure in guard cells of *Arabidopsis*
85 *thaliana* (Wang et al. 2015). Additionally, pharmacological and genetic evidence indicate
86 that NO-mediated signaling increases tolerance to water stress in plants (Tian and Lei
87 2006; Cai et al. 2015; Foresi et al. 2015).

88 On the other hand, the studies regarding NO influence on the photosynthetic
89 apparatus are not easily conciliated. Metal-induced impairment of the electron transport
90 chain in photosynthesis was attenuated by NO in plants (Aftab et al. 2012; Yang et al.
91 2012). Additionally, NO was shown to induce a slow and continuous increase of the non-
92 photochemical quenching of fluorescence, a well-known photoprotective mechanism
93 (Ördög et al. 2013). Intriguingly, evidences suggest that NO reversibly inhibits the
94 photosynthetic electron transport in guard cells, reducing ATP and NADPH production,
95 starch formation and also the synthesis of malate and sucrose (Takahashi et al. 2002;
96 Wodala et al. 2008; Ördög et al. 2013; Misra et al. 2014). It has been proposed that the
97 protective functions of NO are likely dependent on a fine control of its cellular
98 homeostasis under different physiological conditions and stressful conditions (Salgado et
99 al. 2013).

100 Here, we have hypothesized that NO can attenuate the inhibition of growth and
101 photosynthesis in sugarcane plants under water deficit. In addition, the underlying
102 mechanisms leading to improved photosynthesis in NO-supplied plants under drought are
103 also addressed in this study.

104

105 **Materials and methods**

106

107 **Plant material and growth conditions**

108

109 Sugarcane plants (*Saccharum* spp.) cv. IACSP94-2094 were propagated by placing
110 mini-stalks from adult plants in trays containing commercial substrate (Carolina Soil of
111 Brazil, Vera Cruz RS, Brazil). Four-week-old plants with three to four leaves were
112 transferred to plastic pots (5 L) containing soil and irrigated daily under greenhouse
113 conditions, where the air temperature varied between 18 °C and 37 °C and the maximum
114 photosynthetic photon flux density (PPFD) was about 1100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Another group
115 of similar plants was transferred to modified Sarruge (1975) nutrient solution [0.31 g L⁻¹
116 KNO₃, 1.20 g L⁻¹ Ca(NO₃)₂, 0.50 g L⁻¹ MgSO₄, 0.08 g L⁻¹ NH₄NO₃, 0.14 g L⁻¹ KH₂PO₄,
117 0.06 g L⁻¹ KClO₃, 0.07 g L⁻¹ Na₂EDTA, 0.07 g L⁻¹ FeSO₄, 1.69 mg L⁻¹ H₃BO₃, 1.10 mg
118 L⁻¹ ZnSO₄, 0.16 mg L⁻¹ Cu₂SO₄, 0.92 mg L⁻¹ MnSO₄, 2.32 mg L⁻¹ (NH₄)₂MoO₄] and
119 maintained hydroponically in a growth chamber (PGR15, Conviron, Winnipeg MB,
120 Canada), at 30/20 °C (day/night), 80% relative humidity, 12 h photoperiod (7:00 to 19:00
121 h) and PPFD of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The pH of the nutrient solution was monitored with a
122 pHmeter Tec-3MPp (Tecnozon, Piracicaba SP, Brazil) and kept between 5.5 and 6.0 by
123 adding sulfuric acid or sodium hydroxide. The electrical conductivity of the nutrient
124 solution was also monitored (Tec-4MPp, Tecnozon, Piracicaba SP, Brazil) and the values

125 were kept between 1.53 and 1.70 mS cm⁻¹ by replacing the solution. Plants were grown
126 under the above conditions for 25 days prior to treatments.

127

128 **Synthesis of *S*-nitrosoglutathione (GSNO)**

129

130 GSNO was synthesized and characterized as previously described (Shishido et al.
131 2003; De Oliveira et al. 2002; Seabra and De Oliveira 2004; De Souza et al. 2006).
132 Reduced glutathione (GSH) was reacted with equimolar amount of sodium nitrite in
133 acidified aqueous solution, in an ice bath for 40 minutes, under magnetic stirring. The
134 obtained GSNO was precipitated by the addition of acetone, filtrated, and washed with
135 cold water. The obtained solid was freeze-dried for 24 h.

136

137 **Experiment I: Water deficit induced by PEG and GSNO spraying**

138

139 Sugarcane plants growing in nutrient solution were submitted to water deficit (WD)
140 by adding polyethylene glycol (CarbowaxTM PEG-8000, Dow Chemical Comp, Midland
141 MI, USA) to the solution. To prevent osmotic shock, PEG-8000 was added to the nutrient
142 solution to cause a gradual decrease in its osmotic potential as follows: -0.25 MPa with
143 20 mM PEG-8000 for one day; -0.50 MPa with 74 mM PEG-8000 for four days; and
144 finally -0.75 MPa with 111 mM PEG-8000. As we did not notice any significant change
145 in leaf gas exchange of plants grown in nutrient solution with -0.50 MPa of osmotic
146 potential, we considered the day 1 of water deficit when the osmotic potential of nutrient
147 solution reached -0.75 MPa. The osmotic potential of the nutrient solution was
148 determined by the hygrometric method, using a microvoltmeter (HR-33T) and C-52
149 measuring chambers (Wescor Inc., Logan UT, USA). After five days under PEG-induced

150 water deficit (-0.75 MPa), we transferred plants to the original nutrient solution (-0.15
151 MPa) for rehydration during two days.

152 Sugarcane leaves were sprayed twice a day (at 12:00 and 18:00 h) with freshly
153 prepared GSNO solutions at 10, 100, 500 or 1000 μM . Leaves were sprayed as follows:
154 when the osmotic potential of nutrient solution reached -0.25 MPa; and at two consecutive
155 days under -0.50 MPa. In this way, the last GSNO spraying was done three days before
156 the nutrient solution reaches -0.75 MPa. GSNO spraying was done outside the growth
157 chamber to avoid undesirable interference in other treatments. As references, we had
158 control plants grown in original nutrient solution (-0.15 MPa) and plants subjected to
159 water deficit (nutrient solution with osmotic potential of -0.75 MPa) and sprayed with
160 water (WD + mock). Four plants composed each treatment, with each plant representing
161 one biological replicate. In all treatments plants were sprayed with similar volumes of
162 about 25 mL of GSNO solutions or water.

163

164 **Experiment II: Water deficit induced by leaf disc dehydration**

165

166 Leaf discs (2 cm of diameter) were detached from plants grown in pots and placed
167 on moistened (Milli-Q water) filter paper in Petri dishes. They were maintained under
168 22°C and PPFD of 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for three days for dehydration. Before detaching leaf
169 discs, plants were sprayed twice a day for three days with a freshly made GSNO or GSH
170 solutions at 100 μM . As reference, plants were sprayed with water (mock).
171 Approximately, 50 mL of GSNO and GSH solutions or water were sprayed on plants.

172 In another essay, leaf discs were taken as previously and submitted to an NO
173 atmosphere as done by Vitor et al. (2013). Briefly, leaf discs were placed on moistened
174 (Milli-Q water) filter paper in Petri dishes inside an acrylic fumigation chamber, which

175 was properly sealed with a transparent cover containing tubes for the gases to enter and
176 exit. A continuous flow of NO gas (60 mL min^{-1}) mixed with commercial air (240 mL
177 min^{-1}), equivalent to $60 \mu\text{mol mol}^{-1}$ of NO, was applied for 6 h. As reference, leaf discs
178 were exposed to a flow of commercial air (300 mL min^{-1}). The commercial air was
179 composed by oxygen (21%) and nitrogen (79%). After fumigation, the leaf discs were
180 transferred to moistened filter paper in Petri dishes and kept at $22 \text{ }^\circ\text{C}$ and PPFD of 80
181 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ for natural dehydration.

182

183 **Leaf gas exchange and photochemistry**

184

185 In plants growing in nutrient solution, gas exchange of the first fully expanded leaf
186 with visible ligule was measured daily using an infrared gas analyzer (Li-6400, Licor,
187 Lincoln NE, USA) attached to a modulated fluorometer (6400-40 LCF, Licor, Lincoln
188 NE, USA). Leaf CO_2 assimilation (A), stomatal conductance (g_s) and intercellular CO_2
189 concentration (C_i) were measured under PPFD of $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and air CO_2
190 concentration of $400 \mu\text{mol mol}^{-1}$. The measurements were performed between 10:00 and
191 13:00 h, following the procedures recommended by Long and Bernacchi (2003). The
192 vapor pressure difference between leaf and air (VPDL) was $2.2 \pm 0.3 \text{ kPa}$ and leaf
193 temperature was $29 \pm 1 \text{ }^\circ\text{C}$ during the evaluations. The instantaneous carboxylation
194 efficiency ($k=A/C_i$) was calculated according to Machado et al. (2009).

195 Chlorophyll fluorescence was evaluated simultaneously to the leaf gas exchange
196 and the apparent electron transport rate (ETR) estimated as $\text{ETR} = \phi_{\text{PSII}} \times \text{PPFD} \times 0.85 \times 0.4$,
197 in which ϕ_{PSII} is the effective quantum efficiency of photosystem II (PSII), 0.85 is the
198 light absorption and 0.4 is the fraction of light energy partitioned to PSII (Edwards and

199 Baker 1993; Baker 2008). Additionally, the non-photochemical quenching of
200 fluorescence (NPQ) was evaluated with the 6400-40 LCF.

201 The potential quantum efficiency of photosystem II (F_v/F_m) was estimated in leaf
202 discs by using the fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany) and
203 the chlorophyll content by using a portable chlorophyll meter SPAD-502 (Konica
204 Minolta, Tokyo, Japan), following the manufactory instructions.

205

206 **Relative water content**

207

208 The fresh (FW), turgid (TW) and dry (DW) weights of leaf discs were determined
209 and the relative water content (RWC) calculated according to Jamaux et al. (1997): RWC
210 = $100 * [(FW - DW) / (TW - DW)]$.

211

212 **Biometry**

213

214 At the end of the experiment I (nutrient solution), roots and all leaves were
215 harvested and the dry matter determined after drying samples in an oven (60 °C) with
216 forced-air circulation until constant weight.

217

218 **Estimation of leaf *S*-nitrosothiols content**

219

220 Total leaf protein was extracted in mili-Q water and the resulting homogenate used
221 for the amperometric estimation of *S*-nitrosothiol content as previous described (Santos
222 et al. 2016; Zhang et al. 2000). Measurements were carried out with the WPI
223 TBR4100/1025 amperometer (World Precision Instruments Inc., Sarasota FL, USA) and

224 a nitric oxide specific ISO-NOP sensor (2 mm). Aliquots of 0.2 mL of aqueous suspension
225 were added to the sampling compartment, which contained 10 mL of aqueous solution of
226 copper chloride (0.1 mol L^{-1}). This condition allowed for the detection of free NO
227 released from the *S*-nitrosothiol present in the leaf protein homogenate. The experiments
228 were performed in triplicate and the calibration curves were obtained with aqueous
229 solutions of freshly prepared GSNO (data not shown). Data was compared to a standard
230 curve obtained with GSNO and normalized against leaf FW.

231

232 **Data analysis**

233

234 Data was subjected to the ANOVA procedure and the Student's t-test ($P < 0.05$) was
235 used to compare treatments. The results presented are the mean \pm SD and the number of
236 replicates is stated in each figure legend.

237

238 **Results**

239

240 **GSNO alleviates negative effects of water deficit in sugarcane phenotype**

241

242 The water deficit caused significant reduction in leaf (-62%) and root (-47%) dry
243 matter of sugarcane plants (Fig. 1a,b). Accordingly, the leaf relative water content was
244 also reduced (-13%) in water-stressed plants as compared to well-hydrated ones (Fig. 1c).
245 Interestingly, we found a protective effect on plants that were sprayed with GSNO when
246 considering biomass accumulation and leaf water status (Fig. 1). Such effect was found
247 even after 11 days of the last GSNO application. Plants subjected to water deficit and
248 sprayed with 100 μM GSNO solution presented similar ($P > 0.05$) root and leaf dry matter

249 and leaf relative water content to plants under well-watered conditions (Fig. 1). GSNO
250 concentrations lower or higher than 100 μ M caused mild protective effects in root growth.
251 These findings suggest a role of GSNO in alleviating the negative effects of dehydration
252 in sugarcane plants.

253

254 **Protective role of GSNO on leaf gas exchange**

255

256 As plant growth was improved under water deficit by GSNO spraying, we
257 hypothesized that leaf GSNO spray affects the leaf gas exchange. Whereas water deficit
258 induced a large reduction (-79%) in leaf CO₂ assimilation in sugarcane plants as compared
259 to the control, spraying plants with 100 μ M GSNO or higher concentrations significantly
260 restored leaf CO₂ assimilation (Fig. 2a). For instance, leaf CO₂ assimilation of GSNO
261 sprayed plants (> 100 μ M) under water deficit was similar ($P>0.05$) to one found in
262 control plants at the 4th day of water deficit and at the 1st and 2th day of rehydration
263 (recovery). Stomatal conductance was nearly suppressed in sugarcane plants under water
264 deficit (-83%) and strongly inhibited during the rehydration (-73%); however, spraying
265 plants with 100 μ M GSNO or higher concentrations kept the stomatal conductance of
266 plants under water deficit similar ($P>0.05$) to one found in control plants (Fig. 2b). The
267 instantaneous carboxylation efficiency, given by the rate between leaf CO₂ assimilation
268 and intracellular CO₂ partial pressure, was significantly reduced by water deficit (Fig.
269 2c). Such negative effect was partially alleviated by spraying 1000 μ M GSNO and no
270 differences ($P>0.05$) between treatments were found after two days of rehydration (Fig.
271 2c). Overall, these data suggest that GSNO plays a role in alleviating the negative effect
272 of water deficit on leaf photosynthesis, stimulating the stomatal aperture during both
273 water shortage and rehydration.

274 **GSNO improves photochemistry in sugarcane plants under water deficit**

275

276 The apparent electron transport rate and the effective quantum efficiency of PSII
277 were drastically reduced (-51% and -41%, respectively) in plants under water deficit as
278 compared to well-hydrated ones, indicating inhibition of the primary photochemistry in
279 sugarcane (Fig. 3a,b). However, such deleterious effects of water deficit were completely
280 offset by GSNO spraying (Fig. 3a,b). The non-photochemical quenching was increased
281 by water deficit (+62%) as compared to plants under well-hydrated conditions (Fig. 3c).
282 Notably, leaf spraying with 100 μ M GSNO or higher concentrations reduced the non-
283 photochemical quenching under water deficit (Fig. 3c), suggesting that GSNO was
284 effective in protecting sugarcane plants of excessive light energy at the PSII. Taken
285 together, these data indicate that leaf GSNO spraying has positive effects on sugarcane
286 by improving photochemistry under water deficit. At the 2th day of rehydration
287 (recovery), the photochemical activity was similar ($P>0.05$) in plants previously exposed
288 to water deficit and sprayed with GSNO and well-hydrated plants (data not shown).

289

290 **Effects of the redox active molecules GSH and GSNO during leaf dehydration**

291

292 Non-enzymatic catabolism of GSNO may yield the antioxidant GSH and the free
293 radical NO. To test a possible role of GSH on the protective effects found when spraying
294 GSNO on sugarcane plants, we followed the dehydration of leaf discs taken from plants
295 sprayed with GSH or GSNO. As a biological NO donor, GSNO is known to cause *S*-
296 nitrosylation of proteins. We first estimated the level of *S*-nitrosylated proteins in leaf
297 extracts of plants sprayed with water (mock), GSH or GSNO solutions. There was a sharp
298 increase in *S*-nitrosothiol concentration of leaf discs taken from GSNO sprayed plants

299 (Fig. 4a). Surprisingly, increase in *S*-nitrosothiol concentration was also found in plants
300 sprayed with GSH (Fig. 4a). Although not expected, we may argue that increasing GSH
301 availability due to leaf spraying may shift the equilibrium towards GSNO formation, thus
302 causing increased *S*-nitrosothiol content in GSH sprayed plants. Further analysis revealed
303 that the chlorophyll content was higher in plants sprayed with GSNO as compared to
304 water or GSH sprayed ones (Fig. 4b).

305 To assess the leaf disc functionality, the potential quantum efficiency of PSII was
306 measured during dehydration and significant increase in this physiological index was
307 observed in leaf discs taken from plants sprayed with GSNO as compared to those ones
308 sprayed with water or GSH (Fig. 5a). In accordance to the possible long-term protective
309 role of GSH, the potential quantum efficiency of PSII was higher in plants sprayed with
310 GSH than in ones sprayed with water at the 3rd day of dehydration (Fig. 5a). Importantly,
311 when we exposed the leaf discs to a NO atmosphere, similar results were obtained when
312 considering the protective role of NO on photochemistry (Fig. 5b). These findings
313 highlight the NO-mediated signaling in alleviating the negative effects of dehydration in
314 sugarcane plants.

315

316 **Discussion**

317

318 Due to the sugarcane importance as a bioenergy crop, physiological strategies
319 aiming to improve sugarcane growth and development are of great interest, mainly under
320 limiting environmental conditions. Field-grown sugarcane plants commonly face periods
321 of water shortage that negatively affects plant growth and reduces sucrose production
322 (Ribeiro et al. 2013; Barbosa et al. 2015). Our findings show that leaf GSNO spray
323 improves sugarcane tolerance to water deficit by improving plant growth and

324 photosynthetic rate. We also sprayed GSNO on well-hydrated plants (Suppl. Fig. S1), but
325 the beneficial effects of GSNO on photosynthesis were found only in sugarcane plants
326 under water deficit (Fig. 2a), indicating that the role of NO is dependent on stress
327 occurrence.

328 By decreasing the water potential of the nutrient solution through the sequential
329 addition of PEG, we imposed a water deficit to sugarcane plants hydroponically
330 cultivated, avoiding any osmotic shock. This protocol is an advantageous strategy to study
331 plant responses to water deficit because of its similarity to the actual desiccation that
332 occurs in field, where the water potential is gradually reduced and plants are able to
333 trigger metabolic acclimation (Farrant et al. 2015). At the end of the experiment, we
334 observed a significant reduction in biomass accumulation and leaf relative water content
335 of plants not supplied with GSNO (Fig. 1), indicating that plants were facing water
336 shortage. Interestingly, we found a significant alleviation of water stress on biomass
337 accumulation of plants by spraying GSNO several days prior the water deficit imposition.

338 Plants trigger several physiological processes in response to water deficit (revised
339 by Fang and Xiong 2015; Santisree et al. 2015) and the stomatal closure is a well
340 established and primordial response aiming to protect plants from water loss through
341 transpiration (García-Mata and Lamattina 2001). Although reduction in stomatal
342 conductance protects plants from desiccation, it negatively affects photosynthesis by
343 reducing the CO₂ availability to carboxylation processes (Sales et al. 2015). Under water
344 deficit, we observed an inhibition of photochemistry accompanied by decreases in
345 stomatal conductance in plants not sprayed with GSNO. While sugarcane photosynthesis
346 seems to be limited by photochemical reactions and stomatal closure under water deficit
347 (Figs. 2b and 3a,b), our data revealed that spraying 100 μM GSNO was able to protect
348 plants from those negative effects of water stress. Protein S-nitrosylation is an important

349 post-translational modification, affecting the activity of proteins. Kato et al. (2013) have
350 found *S*-nitrosylated proteins associated with photosynthesis (small and large subunits of
351 Rubisco and oxygen-evolving system) and cellular redox status in potato leaves treated
352 with GSNO. In fact, GSNO was effective in recovering the photosynthetic rates of water-
353 stressed plants, and plants sprayed with GSNO presented photosynthesis similar to one
354 found in well-hydrated plants after four days under water shortage (Fig. 2a).

355 It has been proposed that GSNO acts as both NO reservoir and donor in biological
356 systems (revised by Salgado et al. 2013; Yu et al. 2014). In fact, non-enzymatic cleavage
357 of GSNO yields GSH and NO (Liu et al. 2001). NO is a redox active molecule that acts
358 mainly through *S*-nitrosylation of proteins (Lindermayr et al. 2005; Yun et al. 2011;
359 Frungillo et al. 2013; Kneeshaw et al. 2014; Wang et al. 2015). The covalent addition of
360 a NO moiety to a cysteine residue in proteins, called *S*-nitrosylation, is known to
361 frequently alter protein activity and localization (Spadaro et al. 2010; Frungillo et al.
362 2014). GSNO is able to directly transfer its NO moiety to thiol groups, a process referred
363 as *S*-transnitrosylation (Salgado et al. 2013).

364 In this sense, the protective effect observed after leaf GSNO spraying could be
365 caused by NO release or transfer, increase in GSH availability or both synergistically. We
366 sought to test these possibilities by spraying plants with GSNO, GSH or mock solution
367 and follow the dehydration of leaf discs. Surprisingly, our analyses done at the 3rd day of
368 dehydration (at the end of the experiment) revealed similar increases in the total level of
369 *S*-nitrosothiol in plants sprayed with GSNO and GSH (Fig. 4a). The potential quantum
370 efficiency of PSII indicated a significant protective effect of GSNO during the first three
371 days of dehydration compared to control and GSH sprayed plants (Fig. 5a). Interestingly,
372 a significant protective effect of GSH was found at the 3rd day of dehydration as compared
373 to mock discs. Such unexpected protective effect of GSH may be explained by changes

374 in GSH and NO reactions towards the formation of the product GSNO. Although further
375 analysis are necessary, we hypothesize that GSH spray indirectly increase NO half-life
376 and bioavailability in cells over time (Salgado et al. 2013), which would justify the
377 protective effect of GSH observed only after three days of dehydration (Fig. 5a). The
378 increase in NO bioavailability would then be reflected in the protective effect of GSH
379 spray on the potential quantum efficiency of PSII (Fig. 5a). It is worthy to mention that
380 the determination of the total *S*-nitrosothiols content was carried out 3 days after spraying
381 the plants. Although the levels of leaf *S*-nitrosothiols are comparable in plants sprayed
382 with GSH or GSNO, the kinetics of *S*-nitrosylation may differ. Unlike the GSH, the
383 GSNO is able to *S*-nitrosylate proteins indirectly by the release of NO or through *S*-
384 transnitrosylation.

385 Several reports indicate an intimate and complex interplay between NO signaling
386 and plant hormones. For instance, overlapping roles of the NO and the phytohormone
387 abscisic acid (ABA) have been reported in plants under water stress (García-Mata and
388 Lamattina 2001; Bright et al. 2006; Wang et al. 2015). Recently, it has been found
389 that open stomata 1 (OST1)/sucrose nonfermenting 1 (SNF1)-related protein kinase 2.6
390 (SnRK2.6) is targeted by an inhibitory *S*-nitrosylation in *Arabidopsis thaliana* guard cells
391 that led to the inhibition of the ABA-induced stomatal closure *in vivo* (Wang et al. 2015).
392 Remarkably, evidences suggest that a reactive thiol group is highly conserved throughout
393 the SnRK2 family in the plant kingdom (Wang et al. 2015). Thus, it is tempting to
394 speculate that the NO released or transferred by GSNO targets protein kinases that
395 ultimately affect the stomatal conductance in sugarcane plants sprayed with GSNO and
396 subjected to water deficit. Specifically, it can be fruitful to investigate the role of the
397 SnRK2.6 in sugarcane plants under water stress. Due the wide extent of possible targets
398 of NO in cells, we cannot exclude that the GSNO spray may impact in other process to

399 promote drought tolerance in sugarcane. Regarding plant tolerance to abiotic stresses,
400 Foresi et al (2015) reported that transgenic plants expressing OtNOS accumulated higher
401 NO concentrations compared with siblings transformed with the empty vector and
402 displayed enhanced salt, drought and oxidative stress tolerance. Moreover, transgenic
403 OtNOS lines exhibited increased stomatal development compared with plants
404 transformed with the empty vector.

405 Additionally to its role in stomatal closure, ABA is known to promote root growth
406 under dehydrating conditions by inhibition of ethylene production (Sharp and LeNoble
407 2002). By spraying sugarcane plants with GSNO under water deficit, we found a
408 significant increase in root biomass and likely increment of water absorption area, which
409 may allow plants to maintain their water status. In fact, the leaf relative water content was
410 not changed by water deficit in plants sprayed with GSNO at 10, 100 and 1000 μ M (Fig.
411 1c). This increase in root:shoot ratio can represent a strategy to explore more efficiently
412 the soil and it aids plants to cope with water stress (Sharp 2002). In addition, it is known
413 that NO has been appointed as an intermediate in the signaling cascade regulated by
414 auxin, influencing the morphology and physiology of roots (Correa-Aragunde et al.
415 2007). Studies show that NO modulates the metabolism, transport and signaling of
416 auxins, by raising the levels of 3-indoleacetic acid in alfalfa seedlings (Sanz et al. 2014)
417 and promoting root growth (Gouvea et al. 1997) and the formation of adventitious
418 (Pagnussat et al. 2002) and side (Correa-Aragunde et al. 2004) roots. Thus, it is likely that
419 NO-mediated modulation of ABA and/or auxin signaling is shaping sugarcane responses
420 to water stress in our experimental conditions.

421 In a scenario of climate changes and decreasing water resources, water shortage has
422 become a severe bottleneck in crop yield worldwide. The development of novel
423 agriculture practices and concepts about drought tolerance is of utmost importance to

424 improve crop yield and understand how plants cope with environmental challenges. Our
425 data indicate that the NO-mediated redox signaling plays a role in promoting shoot and
426 root growth and improving the photosynthesis in sugarcane plants under water deficit.

427

428 **Acknowledgements**

429

430 The authors acknowledge the financial support (BIOEN Program, Grant no. 2008/57519-
431 2) provided by the São Paulo Research Foundation (FAPESP, Brazil) as well as the
432 scholarships to NMS and MTP (Grant no. 2012/19167-0 and 2015/00393-8). LF is a
433 European Molecular Biology Organization (EMBO) - Long Term Fellow (no. 420/2015).
434 The authors also acknowledge the fellowships (ABS; IS; ECM; RVR) and scholarships
435 (FCCM and MTM) granted by the National Council for Scientific and Technological
436 Development (CNPq, Brazil).

437

438 **Authors' contribution**

439

440 NMS, LF, IS, ABS, ECM and RVR designed the experiments. NMS and FCCM
441 performed the measurements of photosynthesis and plant growth. MTP and ABS prepared
442 the GSNO and GSH solutions and measured *S*-nitrosothiol concentration in leaf samples.
443 NMS, MTM and IS carried out the experiment with NO fumigation. NMS, LF and RVR
444 wrote the manuscript and all authors contributed in data discussion and edited the final
445 version of the manuscript.

446

447

448

449

450 **References**

451

452 Aftab T, Khan MM, Naeem M, Idrees M, Moinuddin, Teixeira da Silva JA, Ram M
453 (2012) Exogenous nitric oxide donor protects *Artemisia annua* from oxidative stress
454 generated by boron and aluminum toxicity, *Ecotox Environ Safe* 80:60-68.

455 Baker NR (2008) A probe of photosynthesis: in vivo. *Annu Rev Plant Biol* 59:89-113.

456 Barbosa AM, Guidorizi KA, Catuchi TA, Marques TA, Ribeiro RV, Souza GM (2015)
457 Biomass and bioenergy partitioning of sugarcane plants under water deficit. *Acta Physiol*
458 *Plant* 37:137-142.

459 Begara-Morales JC, Sánchez-Calvo B, Luque F, Leyva-Pérez MO, Leterrier M, Corpas
460 FJ, Barroso, JB (2014) Differential transcriptomic analysis by RNA-Seq of GSNO-
461 responsive genes between *Arabidopsis* roots and leaves. *Plant Cell Physiol* 55:1080-1095.

462 Besson-Bard A, Astier J, Rasul S, Wawer I, Dubreuil-Maurizi C, Jeandroz S,
463 Wendehenne D (2009) Current view of nitric oxide-responsive genes in plants. *Plant*
464 *Science* 177:302-309.

465 Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation
466 and stomatal closure in *Arabidopsis* are dependent on H₂O₂ synthesis. *Plant J* 45:113-122.

467 Cai W, Liu W, Wang WS, Fu ZW, Han TT, Lu YT (2015) Overexpression of rat neurons
468 nitric oxide synthase in rice enhances drought and salt tolerance. *PLoS One*. doi:
469 10.1371/journal.pone.0131599.

470 Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV,
471 López-Jaramillo J, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas
472 FJ, Barroso JB (2011) High temperature triggers the metabolism of S-nitrosothiols in
473 sunflower mediating a process of nitrosative stress which provokes the inhibition of
474 ferredoxin–NADP reductase by tyrosine nitration. *Plant Cell Environ* 34:1803-1818.

475 Correa-Aragunde N, Graziano M, Lamattina L (2004) Nitric oxide plays a central role in
476 determining lateral root development in tomato. *Planta* 218:900-905.

477 Correa-Aragunde N, Lanteri ML, García-Mata C, Have AT, Laxalt AM, Graziano M,
478 Lamattina L (2007) Nitric oxide functions as intermediate in auxin, abscisic acid and lipid
479 signaling pathways, in: L. Lamattina, J. Polacco, (Eds.), *Nitric Oxide in Plant Growth,*
480 *Development and Stress Physiology, Plant Cell Monographs, Vol. 5, Springer.* 113-130.

481 Cruz de Carvalho MH (2008) Drought stress and reactive oxygen species: production,
482 scavenging and signaling. *Plant Signal Behav* 3:156-165.

483 De Oliveira MG, Shishido SM, Seabra AB, Morgon, NH (2002) Thermal stability of
484 primary S-nitrosothiols: roles of autocatalysis and structural effects on the rate of nitric
485 oxide release. *Phys Chem A* 106:8963-8970.

486 De Souza GFP, Yokoyama-Yasunaka JKU, Seabra AB, Miguel DC, de Oliveira MG,
487 Uliana SRB (2006) Leishmanicidal activity of primary S-nitrosothiols against
488 *Leishmania major* and *Leishmania amazonensis*: Implications for the treatment of
489 cutaneous leishmaniasis. *Nitric Oxide* 15:209-216.

490 Edwards GE, Baker NR (1993) Can CO₂ assimilation in maize leaves be predicted
491 accurately from chlorophyll fluorescence analysis? *Photosynth Res* 37:89-102.

492 Fang Y, Xiong L (2015) General mechanisms of drought response and their application
493 in drought resistance improvement in plants. *Cell Mol Life Sci* 72:673-689.

494 Farrant JM, Cooper K, Hilgart A, Abdalla KO, Bentley J, Thomson JA, Dace HJW, Peton
495 N, Mundree SG, Rafudeen MS (2015) A molecular physiological review of vegetative
496 desiccation tolerance in the resurrection plant *Xerophyta viscosa* (Baker). *Planta* 242:407-
497 426.

498 Foresi N, Mayta ML, Lodeyro AF, Scuffi D, Correa-Aragunde N, García-Mata C,
499 Casalongué C, Carrillo N, Lamattina L (2015) Expression of the tetrahydrofolate-

500 dependent nitric oxide synthase from the green alga *Ostreococcus tauri* increases
501 tolerance to abiotic stresses and influences stomatal development in *Arabidopsis*. The
502 *Plant Journal* 82:806-821.

503 Frungillo L, Skelly MJ, Loake GJ, Spoel SH, Salgado I (2014) S-nitrosothiols regulate
504 nitric oxide production and storage in plants through the nitrogen assimilation pathway.
505 *Nat. Commun* 5:5401-5410.

506 Frungillo L, De Oliveira JF, Saviani EE, Oliveira HC, Martínez MC, Salgado I (2013)
507 Modulation of mitochondrial activity by S-nitrosoglutathione reductase in *Arabidopsis*
508 *thaliana* transgenic cell lines. *Biochim Biophys Acta* 1827:239-247.

509 García-Mata CG, Lamattina L (2001) Nitric oxide induces stomatal closure and enhances
510 the adaptive plant responses against drought stress. *Plant Physiol* 126:1196-1204.

511 Gouvea CMCP, Souza JF, Magalhães ACN, Martins IS (1997) NO-releasing substances
512 that induce growth elongation in maize root segments. *Plant Growth Regul* 21:183-187.

513 Jamaux I, Steinmetz A, Belhassen E (1997) Looking for molecular and physiological
514 markers of osmotic adjustment in sunflower. *New Phytol* 137:117-127.

515 Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of S-nitrosylated proteins
516 in potato plant. *Physiol Plant* 148:371-386.

517 Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH (2014) Selective protein
518 denitrosylation activity of thioredoxin-*h5* modulates plant immunity. *Mol Cell* 56:153-
519 162.

520 Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I, Kwak JM, Lee IJ,
521 Hwang I. (2006) Activation of glucosidase via stress-induced polymerization rapidly
522 increases active pools of abscisic acid. *Cell* 126:1109-1120.

523 Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of S-nitrosylated
524 proteins in *Arabidopsis*. *Plant Physiol* 137:921-930.

525 Liu L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS (2001) A metabolic enzyme
526 for S-nitrosothiol conserved from bacteria to humans. *Nature* 410:490-494.

527 Long SP, Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about
528 the underlying limitations to photosynthesis? Procedures and sources of error, *J Exp Bot*
529 4:2393-2401.

530 Machado RS, Ribeiro RV, Marchiori PER., Machado DFSP, Machado EC, Landell MGA
531 (2009) Respostas biométricas e fisiológicas ao déficit hídrico em cana-de-açúcar em
532 diferentes fases fenológicas. *Pesq Agropec Bras* 44:1575-1582.

533 Misra NA, Vladkova R, Singh R, Misra M, Dobrikova AG, Apostolova EL (2014) Action
534 and target sites of nitric oxide in chloroplasts. *Nitric Oxide* 39:35-45.

535 Ördög A, Wodala B, Rózsavölgyi T, Irma Tari, Horváth F (2013) Regulation of guard
536 cell photosynthetic electron transport by nitric oxide. *J Exp Bot* 64:1357-1366.

537 Pagnussat GC, Simontacchi M, Puntarulo S, Lamattina L (2002) Nitric oxide is required
538 for root organogenesis. *Plant Physiol* 129:954-956.

539 Parry MAJ, Habash D, Araus JL (2004) Optimisation of water use by plants. *Ann Appl*
540 *Biol* 144:125-126.

541 Radi R (2004) Nitric oxide, oxidants, and protein tyrosine nitration. *PNAS* 101:4003-
542 4008.

543 Ramesh P (2000) Effect of different levels of drought during the formative phase on
544 growth parameters and its relationship with dry matter accumulation in sugarcane. *J*
545 *Agron Crop Sci* 85:83-89.

546 Ribeiro RV, Machado RS, Machado EC, Machado DFSP, Magalhães Filho JR, Landell
547 MGA (2013) Revealing drought-resistance and productive patterns in sugarcane
548 genotypes by evaluating both physiological responses and stalk yield. *Exp Agr* 49:212-
549 224.

550 Sales CRG, Marchiori PER, Machado RS, Fontenele AV, Machado EC, Silveira JAG,
551 Ribeiro RV (2015) Photosynthetic and antioxidant responses to drought during the
552 sugarcane ripening. *Photosynthetica* 53:547-554.

553 Sales CRG, Ribeiro RV, Silveira JAG, Machado EC, Martins OM, Lagôa AMMA (2013)
554 Superoxide dismutase and ascorbate peroxidase improve the recovery of photosynthesis
555 in sugarcane plants subjected to water deficit and low substrate temperature. *Plant Physiol*
556 *Biochem* 73:326-336.

557 Salgado I, Martínez MC, Oliveira HC, Frungillo L (2013) Nitric oxide signaling and
558 homeostasis in plants: a focus on nitrate reductase and S-nitrosoglutathione reductase in
559 stress-related responses. *Braz J Bot* 36:89-98.

560 Santisree P, Bhatnagar-Mathur P, Sharma KK (2015) NO to drought-multifunctional role
561 of nitric oxide in plant drought: Do we have all the answers? *Plant Sci* 239:44-55.

562 Santos MC, Seabra AB, Pelegrino MT, Haddad PS (2016) Synthesis, characterization and
563 cytotoxicity of glutathione- and PEG-glutathione-superparamagnetic iron oxide
564 nanoparticles for nitric oxide delivery. *Appl Surf Sci* 367:26–35.

565 Santos-Filho PR, Vitor SC, Frungillo L, Saviani EE, Oliveira HC, Salgado I (2012)
566 Nitrate reductase and nitric oxide-dependent activation of sinapoylglucose:malate
567 sinapoyltransferase in leaves of *Arabidopsis thaliana*. *Plant Cell Physiol* 53:1607-1616.

568 Sanz L, Fernández-Marcos M, Modrego A, Lewis DR, Muday GK, Pollmann S, Dueñas
569 M, Santos-Buelga C, Lorenzo O (2014) Nitric oxide plays a role in stem cell niche
570 homeostasis through its interaction with auxin. *Plant Physiol* 166:1972-1984.

571 Sarruge JR (1975) Soluções nutritivas. *Summa Phytopathol* 3:231-233.

572 Seabra AB, de Oliveira MG (2004) Poly (vinyl alcohol) and poly (vinyl pyrrolidone)
573 blended films for local nitric oxide release. *Biomaterials* 25:3773–3782.

574 Sharp RE (2002) Interaction with ethylene: changing views on the role of abscisic acid in
575 root and shoot growth responses to water stress. *Plant Cell Environ* 25:211-222.

576 Sharp RE, LeNoble ME (2002) ABA, ethylene and the control of shoot and root growth
577 under water stress. *J Exp Bot* 53:33-37.

578 Shishido SM, Seabra AB, Loh W, De Oliveira MG (2003) Thermal and photochemical
579 nitric oxide release from S-nitrosothiols incorporated in Pluronic F127gel: potential uses
580 for local and controlled nitric oxide release. *Biomaterials* 24:3543-3553.

581 Simontacchi M, Galatro A, Ramos-Artuso F, Santa-María GE (2015) Plant survival in a
582 changing environment: the role of nitric oxide in plant responses to abiotic stress. *Front*
583 *Plant Sci* 6:977-996.

584 Spadaro D, Yun BW, Spoel SH, Chu C, Wang YQ, Loake GJ (2010) The redox switch:
585 dynamic regulation of protein function by cysteine modifications. *Physiol Plant* 138:360-
586 371.

587 Stamler JS, Singel DJ, Loscalzo J (1992) Biochemistry of nitric oxide and its redox-
588 activated forms. *Science* 258:1898-1902.

589 Takahashi S, Yamasaki H (2002) Reversible inhibition of photophosphorylation in
590 chloroplasts by nitric oxide. *FEBS Lett* 512:145-148.

591 Tian X, Lei Y (2006) Nitric oxide treatment alleviates drought stress in wheat seedlings.
592 *Biol Plant* 50:775-778.

593 Vitor SC, Duarte GT, Saviani EE, Vincentz MGA, Oliveira HC, Salgado I (2013) Nitrate
594 reductase is required for the transcriptional modulation and bactericidal activity of nitric
595 oxide during the defense response of *Arabidopsis thaliana* against *Pseudomonas*
596 *syringae*. *Planta* 238:475-486.

597 Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK
598 (2015) Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-
599 nitrosylation of OST1, Proc Natl Acad Sci USA 112:613-618.

600 Wodala B, Deák Z Vass I, Erdei L, Altorjay I, Horváth F (2008) In vivo target sites of
601 nitric oxide in photosynthetic electron transport as studied by chlorophyll fluorescence in
602 pea leaves. Plant Physiol 146:1920-1927.

603 Yang LT, Qi YP, Chen LS, Sang W, Lin XJ, Wu YL, Yang CJ (2012) Nitric oxide
604 protects sour pummelo (*Citrus grandis*) seedlings against aluminum-induced inhibition
605 of growth and photosynthesis. Environ Exp Bot 82:1-13.

606 Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a
607 redox cue in deconvolution, New Phytol 202:1142-1156.

608 Yun BW, Feechan A, Yin M, Saidi NBB, Bihan TL, Yu M, Moore JW, Kang JG, Kwon
609 E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates
610 cell death in plant immunity. Nature 478:264-268.

611 Zhang X, Cardoso L, Broderick M, Fein H, Davies IR (2000) Novel calibration method
612 for nitric oxide microsensors by stoichiometrical generation of nitric oxide from SNAP.
613 Electroanal 12:425-428.

614

615 **Figure captions**

616

617 **Fig. 1.** Leaf (LDM, in a) and root (RDM, in b) dry mass and leaf relative water content
618 (RWC, in c) in sugarcane plants maintained well-hydrated (Control) and subjected to
619 water deficit (WD) and sprayed with water (mock) or GSNO doses (10, 100, 500 or 1000
620 μM). Data represents the mean value of four replications + standard deviation. Asterisks

621 indicate statistical differences between a specific condition and the WD+mock treatment
622 (Student's t-test, $P < 0.05$).

623

624 **Fig. 2.** Changes in leaf CO_2 assimilation (A , in a), stomatal conductance (g_s , in b) and the
625 instantaneous carboxylation efficiency (k , in c) in sugarcane plants maintained well-
626 hydrated (Control) and subjected to water deficit (WD) and sprayed with water (mock)
627 or GSNO doses (10, 100, 500 or 1000 μM). Data represents the mean value of four
628 replications \pm standard deviation. In b and c, we show measurements taken after four days
629 of water deficit and two days of rehydration (recovery). Asterisks indicate significant
630 differences between a specific condition and the WD+mock treatment (Student's t-test,
631 $P < 0.05$).

632

633 **Fig. 3.** The apparent electron transport rate (ETR, in a), effective quantum efficiency of
634 PSII (ϕ_{PSII} , in b) and non-photochemical quenching (NPQ, in c) in sugarcane plants
635 maintained well-hydrated (Control) and subjected to water deficit (WD) and sprayed with
636 water (mock) or GSNO doses (10, 100, 500 or 1000 μM). Data represents the mean value
637 of four replications + standard deviation. Measurements were taken after four days of
638 water deficit. Asterisks indicate significant differences between a specific condition and
639 the WD+mock treatment (Student's t-test, $P < 0.05$).

640

641 **Fig. 4.** The *S*-nitrosothiol concentration (a) and chlorophyll content (b) in leaf discs of
642 sugarcane plants under dehydration. Plants were sprayed with water (mock), 100 μM
643 GSNO and 100 μM GSH. The data represents the mean value + standard deviation. The
644 number of replications varied as follows: $n=6$ in a; $n=12$ in b. Asterisks indicate

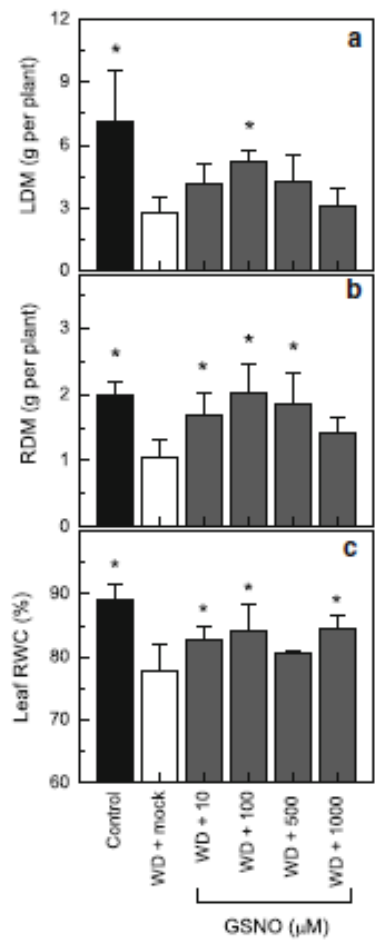
645 significant differences between a specific condition and the mock treatment (Student's t-
646 test, $P < 0.05$).

647

648 **Fig. 5.** The potential quantum efficiency of PSII (F_V/F_M) in leaf discs of sugarcane plants
649 under dehydration. In a, plants were sprayed with water (mock), 100 μM GSNO and 100
650 μM GSH. In b, plants were fumigated with gaseous NO or commercial air (Reference).
651 The data represents the mean value \pm standard deviation. The number of replications
652 varied as follows: $n=8$ in a; and $n=3$ in b. Asterisks indicate significant differences
653 (Student's t-test, $P < 0.05$) between a specific condition and the mock (in a) or between a
654 specific condition and the reference (in b).

655

656 Figure 1

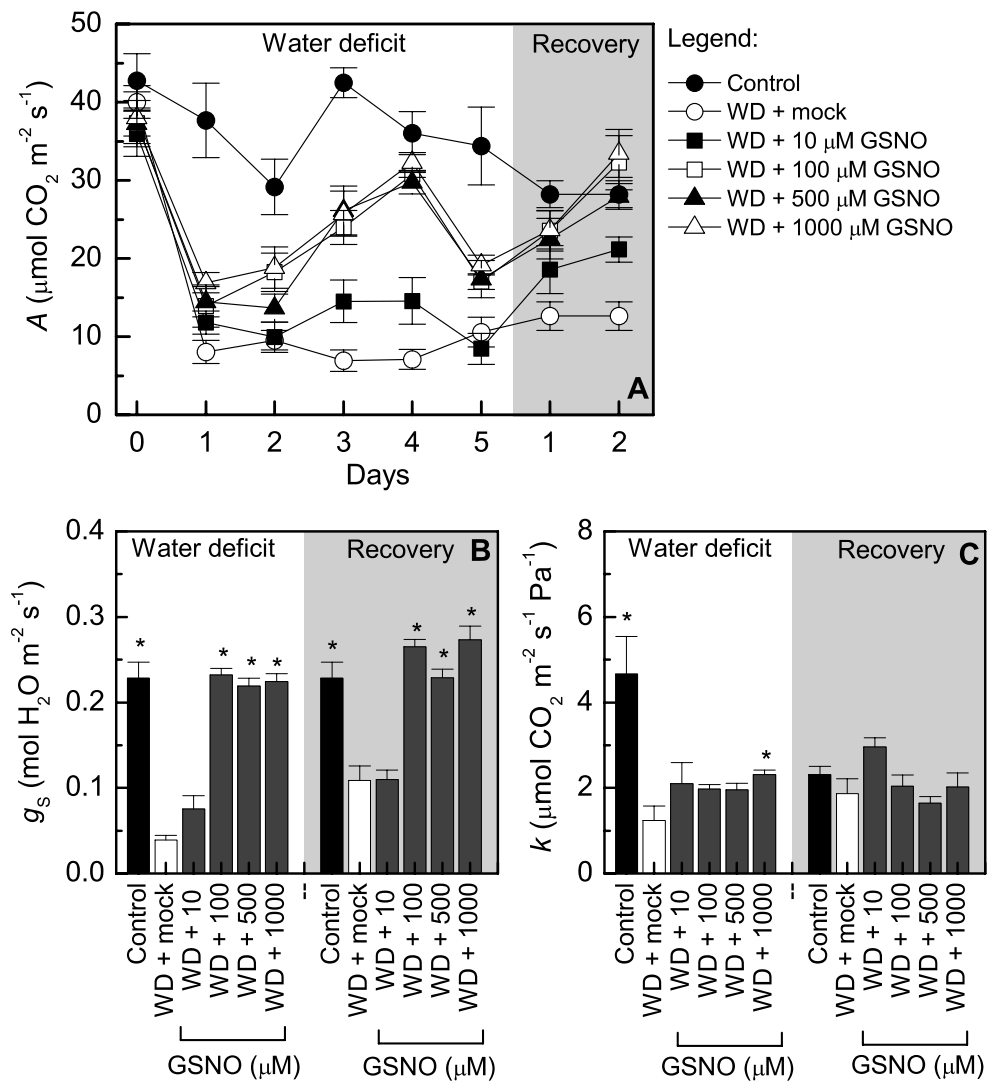


657

658

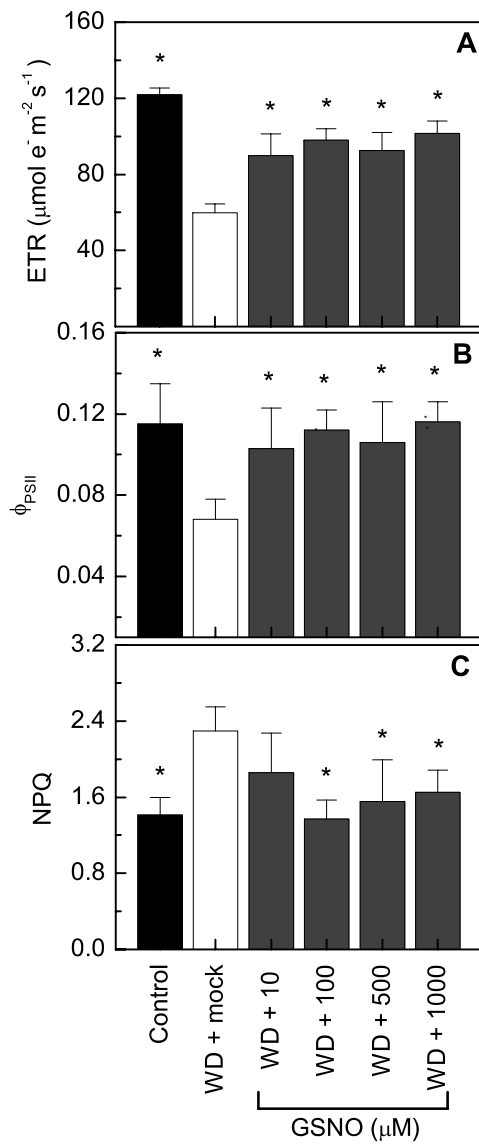
659 Figure 2

660



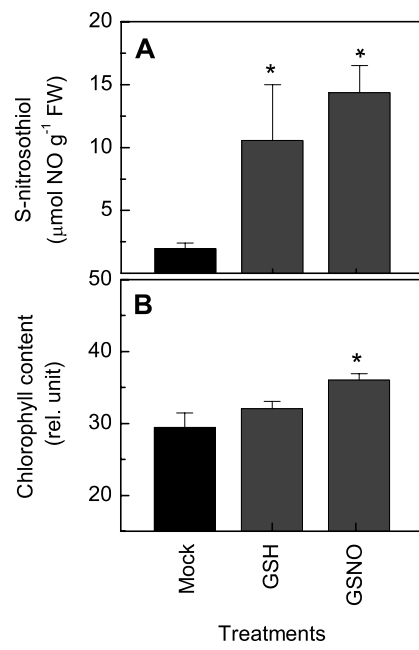
661

662



667 Figure 4

668



669

670

671

672 Figure 5

673

