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Interaction Between Polyamine and Nitric Oxide Signaling in Adaptive Responses to Drought in Cucumber

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Abstract The effect of polyamines (PAs) on nitric oxide (NO) generation was investigated in cucumber (*Cucumis sativus* cv. Dar) primary leaves using bio-imaging with an NO-selective fluorophor, DAF-2DA. Seedlings pretreated with PAs and subjected to water deficit showed early (after 5 h) and transient NO production. The amplitude of the response depended on the form of the applied polyamine. Spermine (1.0 mM) and spermidine (1.0 mM) induced higher NO-dependent fluorescence compared with putrescine (1.0 mM) and the control. The NO production was blocked by tungstate, an inhibitor of nitrate reductase, and partially by an inhibitor of nitric oxide synthase (NOS-like) enzymes. NO donor administration preceding drought had no effect on endogenous PA levels but was positively correlated with an alleviation of water deficit-induced membrane permeability and lipid peroxidation. Application of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), a membrane-permeable NO scavenger, markedly reversed the NO donor effects. Similarly, pretreating seedlings with PAs resulted in lower ion leakage from the membrane and modified lipid peroxidation. The results indicate that NO may act downstream of PAs in cucumber seedlings under water stress.

Keywords Polyamines · Nitric oxide · Cucumber · Injury index · Lipid peroxidation

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Introduction

Nitric oxide (NO) is a highly reactive gaseous molecule that plays a key role as a signal in plant responses to biotic and abiotic stresses (Delledonne and others 1998; Durner and others 1998; Garcia-Mata and Lamattina 2001; Delledonne and others 2001; Hung and others 2002). NO was also shown to be involved in plant growth and developmental processes, starting from germination up to flowering, ripening of fruit, and senescence of plant organs (Leshem and Haramaty 1996; Beligni and Lamattina 2000; Hung and Kao 2003; Pagnussat and others 2004; Prado and others 2004).

Drought stress is an especially important factor that limits crop productivity. There is evidence that plants treated with NO are more tolerant of drought and other abiotic stresses such as salt and heat (Garcia-Mata and Lamattina 2001, 2002; Uchida and others 2002). In addition, NO plays a central role in the guard cell abscisic acid (ABA) signaling network in various species, including *Arabidopsis thaliana*, *Vicia faba*, and *Triticum aestivum* (Desikan and others 2002; Garcia-Mata and Lamattina 2001, 2002). Exogenous NO induces dose-dependent stomatal closure in a manner similar to that of hydrogen peroxide (Neill and others 2002). Moreover, in wheat roots subjected to water deficit, ABA synthesis was much higher in the presence of NO donors and reactive oxygen species (ROS), which suggests synergistic action of these compounds (Zhao and others 2001). Recent reports showed that in ABA-controlled stomatal closure, NO is downstream of ROS (Neill 2007; Yan and others 2007), and in water stress signaling, NO is synthesized mainly by a NOS-like enzyme (Hao and others 2008; Sang and others 2008).

Drought, like cold, salt, and heat stresses, leads to enhanced synthesis of polyamines (PAs), small aliphatic amines found in all eukaryotic cells (Bouchereau and others 1999). Putrescine (Put), spermidine (Spd), and spermine (Spm) are the most abundant PAs in plants. Due to their polycationic nature at physiologic pH, PAs can bind to negatively charged groups in the cell membrane and thereby suppress the phase change following stress (Liu and others 2007). Moreover, PAs can act indirectly as ROS scavengers and affect the activity of antioxidant enzymes (Bouchereau and others 1999; Tang and Newton 2005; Verma and Mishra 2005). In addition, through the enhancement of proline and betaine accumulation, PAs function to promote osmoprotection of plant cells (Öztürk and Demir 2003). A protective role for spermine against drought stress has been found in *Arabidopsis*, where spermine modulates the activities of certain ion channels, raises cytoplasmic Ca^{2+} concentration, and as a consequence induces stomatal closure (Yamaguchi and others 2007). It is well documented that PA accumulation takes place at the beginning of exposure to stress, whereas under prolongation of unfavorable conditions only slight changes in the PA contents were found (Aziz and Larher 1995; Tonon and others 2004). Legocka and Kluk (2005) found that both osmotic and salt stresses trigger organ-specific changes in PA levels. Moreover, data recorded by those authors suggested that Put synthesized in roots was carried through lupine hypocotyls to leaves (Legocka and Kluk 2005).

Recently, Tun and others (2006) observed that PAs induced NO biosynthesis in specific tissues in *Arabidopsis thaliana* seedlings. Spermine and spermidine increased NO generation in *Arabidopsis* mainly in the elongation zone of root tips and in primary leaves, especially in the veins and trichomes.

Another effect was reported by Silveira and others (2006) working on an embryogenic culture of *Araucaria angustifolia*. Spermidine and spermine inhibited NO biosynthesis in both embryonic and suspensor cells of *A. augustifolia*. In turn, putrescine treatment induced NO biosynthesis in embryonic cells of the proembryogenic masses (PEMs), whereas suspensor cells presented no or little NO-dependent fluorescence. The authors concluded that the observed gradient of NO concentration could be related to the maintenance of structure polarity (Silveira and others 2006).

Environmental stresses, especially water deficit, are reported to increase both PAs (Yang and others 2007) and NO biosynthesis in plants (Garcia-Mata and Lamattina 2001; Hao and others 2008; Sang and others 2008). However, functional cross-talk between these molecules under water stress, to the best of our present knowledge, has not been demonstrated so far.

Groppa and others (2008) studied the possible role of NO and polyamines in plant responses to heavy-metal stress, demonstrating that cadmium and PAs, especially Spm, elevated endogenous NO content and reduced wheat root growth rates.

In this study, the roles of PAs and NO in regulating water stress-induced metabolism were assessed in cucumber seedlings. Pharmacologic and biochemical analyses were used to determine whether PAs, produced in response to water deficit, regulate the accumulation of NO in young leaves and to investigate the role that NO plays during the early stage of drought stress.

Materials and Methods

Plant Material

Seedlings of cucumber plants (*Cucumis sativus* cv. Dar) were sown in perlite (15 1.5-l pots) and allowed to germinate and develop in a growth chamber with a photoperiod of 16 h (light = 250 µmol m^{-2} s^{-1}) at a temperature of 24°C (day) and 20°C (night), and 60–70% humidity. At the stage of the third fully expanded leaf, seedlings were taken out of perlite for further treatments.

PA Treatment and Stress Conditions

Roots of seedlings were immersed in 1 mM potassium phosphate buffer (pH 5.8) (control) or in buffer solutions containing Spm, Spd, and Put, and maintained for 24 h under controlled conditions (22°C, humidity 65%, continuous light of 150 µmol m^{-2} s^{-1} photosynthetically active radiation [PAR]). The solutions were continuously aerated. Plants were transferred into empty beakers and subjected to dehydration (stressed plants). Control plants were maintained in 1 mM potassium phosphate buffer (pH 5.8) and continuously aerated. Beakers with seedlings were placed in a growth chamber under controlled conditions (22°C, 65% humidity, continuous light of 150 µmol m^{-2} s^{-1} PAR). The third leaves were collected at 0, 5, 10, and 15 h after withdrawal of water and 24 h after rewatering of stressed plants. Each sample included 15 seedlings.

NO Treatment and Stress Conditions

To evaluate the effect of exogenous NO, seedling roots were immersed in 50, 100, and 500 µM sodium nitroprusside (SNP) or in 100 µM SNP + 200 µM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) or in H_2O (control plants), then incubated for 12 h under continuous light conditions (light intensity = 120 µmol m^{-2} s^{-1}), according to Floryszak-Wieczorek and others (2006). Plants were then subjected to drought stress as described above.

Relative Water Content (RWC)

RWC, indicating the level of water deficit in leaves, was estimated according to Weatherley (1950) and calculated using the formula: RWC = [(fresh weight – dry weight)/(fresh weight at full turgor – dry weight)] × 100%.

Extraction and Determination of Free Polyamines

Free PA content was estimated using the method of Flores and Galston (1982). Plant material was homogenized in 5% $HClO_4$ (0.25 g/ml w/v) and was allowed to stand in an ice bath for 1 h. Samples were then centrifuged at 48,000*g* for 1 h and PAs contained in the supernatant were subjected to benzoylation under alkaline conditions. The benzoyl PA derivates were extracted by diethyl ether. The last was dissolved in methanol. The derivatives were separated by high-performance liquid chromatography using a Supelco Ultropac ODS (4.6 mm × 250 mm × 5 μM) column eluted with 64% (v/v) methanol/water and monitored at 254 nm. The applied standards were Put, Spd, and Spm in the form of hydrochlorides (Sigma). Results are given in nmol g^{-1} dry weight.

Lipid Peroxidation

The level of lipid peroxidation was measured as the amount of malondialdehyde (MDA) in nmol g^{-1} dry weight, determined as thiobarbituric acid reactive substance (TBARS) as described by Heath and Packer (1968). Tissue (300 mg) was homogenized in 2 ml 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The mixture was heated for 15 min in a boiling water bath and then cooled and centrifuged for 10 min at 10,000*g*. The absorbance of supernatant was determined at 532 and 600 nm. A reagent blank was run simultaneously and consisted of the extract treated only with 10% TCA. Results are given in nmol g^{-1} dry weight.

Injury Index

Electrolyte leakage from leaves, determined by the conductivity method, was used as a measure of injury. The amounts of electrolytes released from the stressed or control tissues were compared to total electrolyte amounts released after boiling. The injury index was calculated according to a formula of Flint and others (1967):

$$I_D = (L_D - L_0)/(100 - L_0) \times 100\%$$

where I_D is the injury index, L_0 is the electrolyte leakage from the control tissue as a percentage of the total electrolyte content, and L_D is the electrolyte leakage from the desiccated tissue in percent of the total electrolyte content. Determinations were performed using ten replicates with one 2.0 × 3.0-cm leaf blade fragment.

NO Detection by Confocal Laser Scanning Microscopy

NO formation was measured using a fluorescent DAF-2DA (Calbiochem) dye. Leaf sections (3 × 5 mm) without midribs were placed in 1 ml buffer solution (10 mM Tris–HCl [pH 7.2]). They were then incubated for 1 h at room temperature with 1 ml DAF-2DA at a final concentration of 10 μM in loading buffer (10 mM Tris–HCl [pH 7.2]), added from a 5 mM stock in DMSO. Leaf sections were also incubated in inhibitors of nitrate reductase (NR)—100 μM tungstate or mammalian nitric oxide synthase (NOS)—200 μM NG-nitro-L-Arg methyl ester (L-NAME) for 1 h before DAF-2DA incubation. The incubation solutions were then pipetted off and leaf sections were washed three times with fresh loading buffer to remove excess fluorochrome. After several minutes sections were affixed with silicon grease to the coverslip bottom of a chamber slide, where they remained immersed in 250 μl fresh loading buffer.

A Zeiss Axiovert 200 M inverted microscope equipped with a confocal laser scanner (Zeiss LSM 510) was used in this study. Sections were excited with the 488-nm line of an argon laser. Dye emissions were recorded using a 505–530-nm band-pass filter and the autofluorescence of chloroplasts was captured with a 585-nm long-pass filter. Microscope, laser, and photomultiplier settings were held constant during the experiment to obtain comparable data. Images were processed and analyzed using the Zeiss LSM 510 software.

Results

Effects of Exogenous PAs on Endogenous NO Generation in Leaf Tissue Under Water Deficit

Real-time imaging of NO was performed using the specific fluorochrome DAF-2DA. Treatment of well-watered cucumber leaves with 1.0 mM Spm, 1.0 mM Spd, or 1.0 mM Put had no effect on NO generation, measured as NO-dependent green fluorescence, the level being similar to that in nontreated seedlings (data not presented).

Drought greatly induced NO release from leaf tissue (Fig. 1a–c). Initially (after 5 h), fluorescence was observed in the form of tiny spots in single epidermal cells, and subsequently NO generation spread over the entire leaf area. The highest NO accumulation was detected at 15 h of drought stress.

A sequence of PA/drought treatments effectively modified the kinetics and intensity of NO generation (Fig. 1g–x).

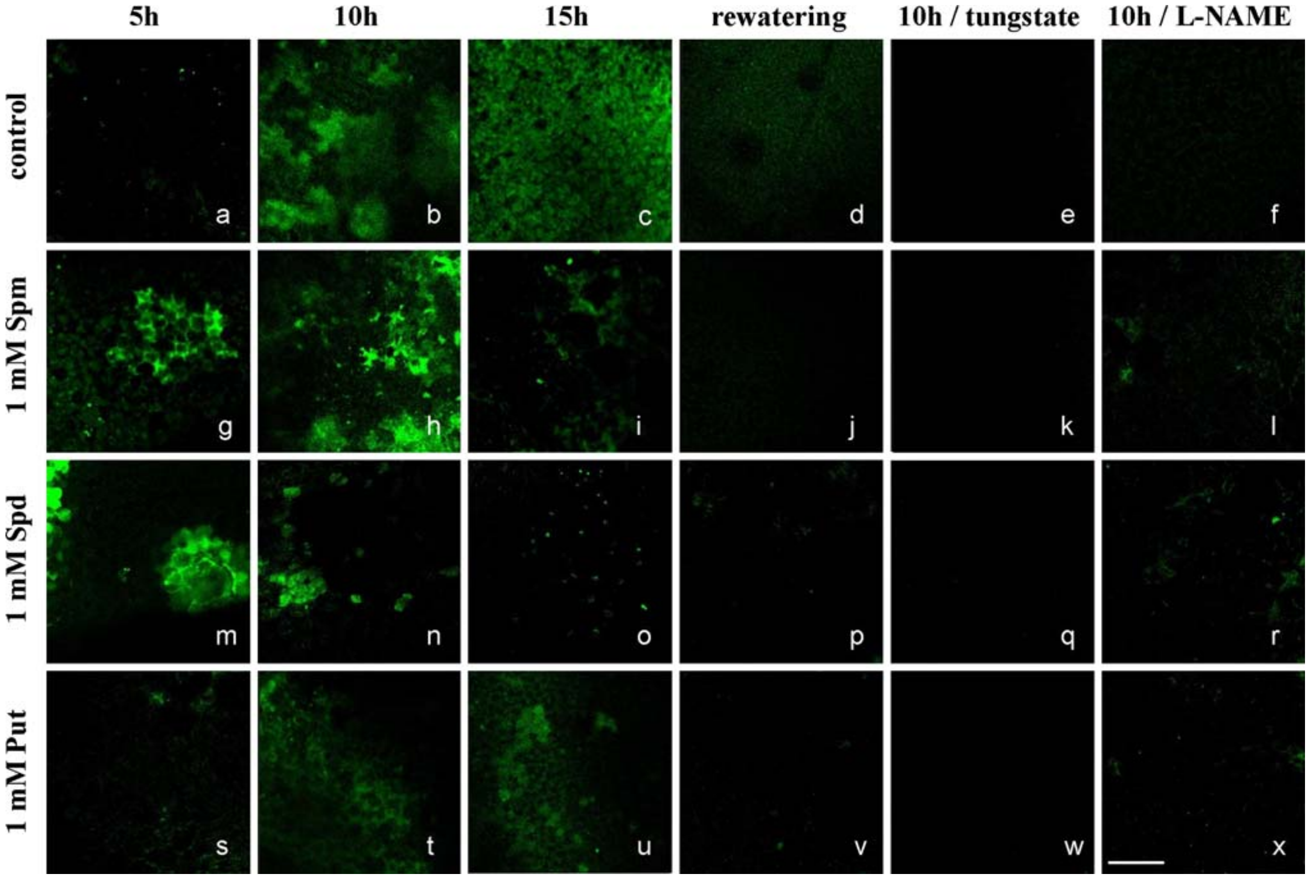

Fig. 1 Imaging of nitric oxide in cucumber leaves using the NO-selective fluorochrome DAF-2DA. NO generation was analyzed at 5, 10, and 15 h during water deficit, 24 h after rewatering, and at 10 h of drought after 100 μM tungstate and 200 μM N^G-nitro-L-Arg methyl ester (L-NAME) treatment; **a–f** control. **g–l** 1 mM Spm. **m–r** 1 mM Spd. **s–x** 1 mM Put. Scale bar: 120 μm

In Spm- and Spd-treated plants, strong fluorescence, covering a considerable area of the leaf blade, appeared at 5 h of water deficit (Fig. 1g and m). At 10 h of stress, NO accumulation was markedly enhanced (Fig. 1h and n), but at 15 h it was reduced to less than that in non-PA-treated leaves (Fig. 1i and o). Put treatment resulted in slight NO release, which was maintained up to 15 h of drought (Fig. 1s–u). Slight green fluorescence was observed 24 h after rewatering stressed seedlings (Fig. 1d), and sequential PA/drought treatments showed a more reduced intensity of DAF-2T fluorescence of cucumber leaf strips (Fig. 1j, p, and v).

The application of tungstate, an inhibitor of NR, completely eliminated green fluorescence in all samples (Fig. 1e, k, q, and w), whereas pretreatment with L-NAME, a well-known competitive inhibitor of mammalian NOS, partially but not completely reduced NO-dependent fluorescence (Fig. 1f, l, r, and x).

Effect of Exogenous NO on Endogenous PA Contents

In well-watered leaves, SNP, an NO donor, did not cause significant changes in free PA content (data not presented). Whereas drought induced an increase in endogenous Put levels, as reported recently by Kubiś (2008), seedlings pretreated with SNP, when undergoing drought showed an additional significant enhancement in the levels of Put, Spd, and Spm only at 15 h of stress conditions (Fig. 2a–c). Treatment with 50 μM SNP caused an approximately 10% increase in free Put levels, whereas treatment with 100 and 500 μM SNP at 15 h of water shortage resulted in an accumulation of Put to about 25% above the control (Fig. 2c). Moreover, when cPTIO was added, PAs dropped to the control level.

Effects of Exogenous NO on Drought Intensity and Membrane Status

Progressive water loss decreased RWC to approximately 55% during the analyzed 15 h of dehydration. To verify whether exogenous NO is capable of modifying the reaction of cucumber seedlings to water deficit, an NO donor was supplied. Plants pretreated with SNP, depending on the concentration, tended to retain more water and differences were statistically significant for 50 and 100 μM SNP at

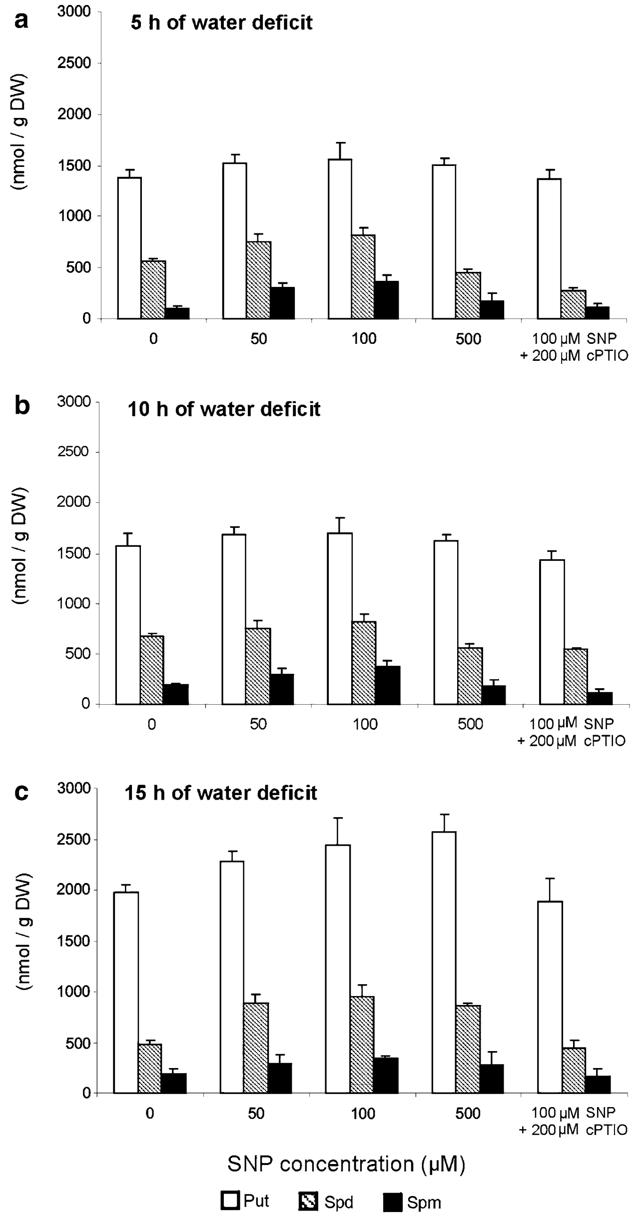

Fig. 2 The effect of NO on the accumulations of the free PAs Put, Spd, and Spm during progressive dehydration of cucumber leaves: **a** 5 h. **b** 10 h. **c** 15 h of drought

15 h of drought (Fig. 3a). The application of a NO scavenger together with SNP prevented the NO effect, reversing the RWC to the control level (data presented for 100 µM SNP). Similarly, after rewatering the water content returned to the control level, and only in plants pretreated with 50 µM SNP did it remain slightly lower.

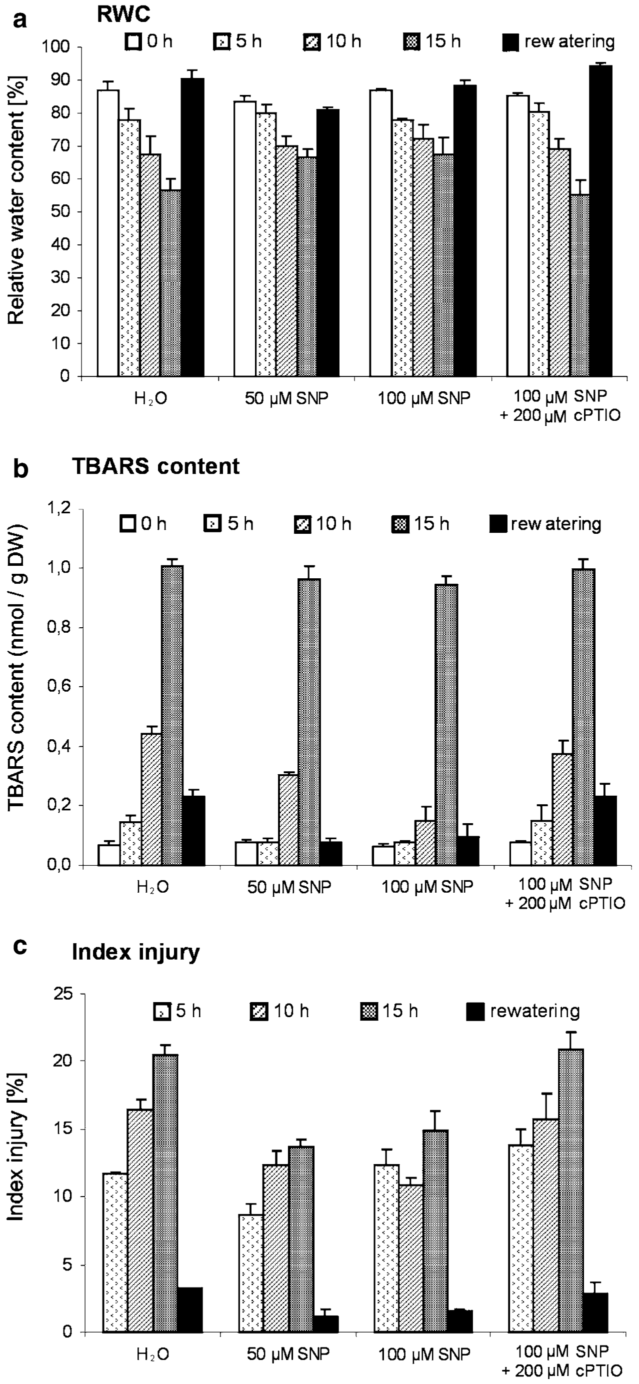

Fig. 3 The effect of exogenous NO (50 µM SNP, 100 µM SNP, and 100 µM SNP + 200 µM cPTIO) on RWC (**a**), TBARS content (**b**), and injury index (**c**) during progressive dehydration of cucumber leaves (0, 5, 10, and 15 h) and after rewatering of stressed plants

Exogenous NO was able to reduce membrane damage under water deficit, expressed as the TBARS content and ion leakage. The largest reduction in TBARS content was observed in leaves supplied with 100 µM SNP at 10 h of water stress (Fig. 3b). Injury index, measured as ion leakage, increased from 12% at 5 h to 21% at 15 h of drought (Fig. 3c), whereas treatment with 50 and 100 µM SNP resulted in a significant decrease of electrolyte leakage from approximately 9% at 5 h to 15% at 15 h. A considerable increase in the injury index was recorded only for 500 µM SNP (data not presented) at all analyzed time points. After elimination of NO, TBARS and electrolyte leakage returned to control levels. Furthermore, membrane damage (TBARS content and ion leakage), recorded after rewatering, for plants subjected to SNP before dehydration was about 50% lower compared to nontreated plants.

Effects of Exogenous PAs on Drought Intensity and Membrane Status

Seedling pretreatment with exogenous PAs showed a similar effect, in relation to drought tolerance, compared with the NO donor treatment. The application to cucumber roots of 1 mM Spm, Spd, or Put before water deficit resulted in retention of up to 10% more water in leaves during the entire stress period (Fig. 4a).

PA treatment prior to dehydration stress caused a significant amelioration of drought-induced membrane damage (Fig. 4b and c). The lipid peroxidation level, estimated as TBARS content, increased in the water-stressed Spm- and Spd-pretreated plants by approximately 20–40%, depending on the PA applied (Fig. 4b). The lowest TBARS level was observed in Put-treated plants, whereas seedlings treated with Spm and Spd showed a decrease of stress-induced electrolyte leakage compared with untreated plants (Fig. 4c). In plants supplied with 1 mM Spm and Spd, the injury index dropped by about 50% at 5 and 10 h, whereas at 15 h of stress it showed a less marked decrease of leakage. In contrast, in Put-treated seedlings the injury index was comparable to that of the stressed control.

In the rehydration phase, Spm- and Spd-pretreated cucumber seedlings showed reduced membrane permeability and lipid peroxidation compared with control and Put-treated plants (Fig. 4b and c).

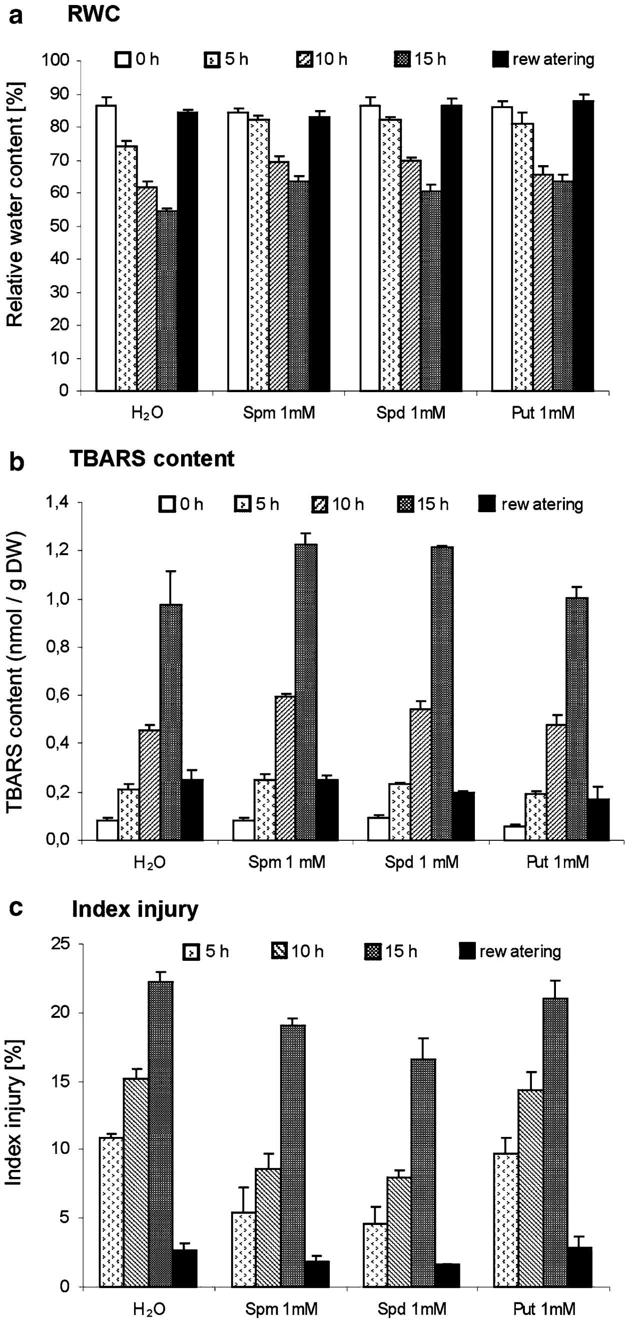

Fig. 4 The effect of exogenous PAs (1 mM Spm, 1 mM Spd, and 1 mM Put) on RWC (**a**), TBARS content (**b**), and injury index (**c**) during progressive dehydration of cucumber leaves (0, 5, 10, and 15 h) and after rewatering of stressed plants

Discussion

One response that helps plants become more tolerant of unfavorable environmental conditions, for example, salt, drought, or hypoxia stress, is the accumulation of polyamines (Flores 1991). Recent reports have suggested that NO is also an integral component of stress-induced signaling networks. Nitric oxide was identified as a key regulatory signal in ABA-mediated stomatal closure (Bright and others 2006; Garcia-Mata and Lamattina 2007) and in the acquisition of plant adaptation to drought stress (Garcia-Mata and Lamattina 2002; Neill 2007).

In this study, functional cross-talk was demonstrated between PAs and NO in cucumber leaves under drought stress. Although exogenous PAs (1.0 mM Spd, Spm, and Put) did not affect NO production in well-watered cucumber seedlings, treatment of seedlings with PAs prior to imposition of water deficit yielded early and transient NO production, as shown by bio-imaging with an NO-specific fluorophor. The intensity of green fluorescence of the triazole molecule (DAF-2T) formed from DAF-2DA and NO (Arita and others 2007) varied depending on the nature of the applied PA. Spermine and spermidine induced higher NO-dependent fluorescence in cucumber leaves compared with the control and putrescine treatment following drought. Strong fluorescence caused by Spm and Spd was observed at the initial stages (5 and 10 h) of water deficit, whereas at 15 h markedly weaker NO production was recorded. In the control and Put-supplemented seedlings, NO synthesis appeared at the later stages of drought, with the most enhanced NO synthesis after 15 h of stress duration. In the presence of mammalian NOS and NR inhibitors, NO-dependent fluorescence intensity was visibly weaker or eliminated completely. This observation provides evidence that PAs have a regulatory effect on NO biosynthesis during water deficit. The involvement of both NOS and NR in water stress-induced NO production, of which NOS was the major source, were described for maize plants by Sang and others (2008).

A direct link between PA and NO was shown by Keefer and others (1996). Spermine NONOate has been favored as a chemical NO donor because it spontaneously releases NO in aqueous solutions (Wang and others 2005). Given that under anoxic conditions NO can react with PAs to produce NONOates (Yamasaki and Cohen 2006), this compound could be translocated through the xylem or phloem system to the entire plant, functioning as an endogenous NO donor in cells. Then again, conjugated compounds of PA-NO could scavenge and diminish the cytotoxicity of NO that is overproduced during severe stress conditions.

From our experiments we speculate that early and periodical biosynthesis of NO as a consequence of spermine and spermidine treatments would reduce tissue damage under prolonged stress conditions by triggering effective adaptation mechanisms. In turn, the maintained high NO level recorded at later stages of drought (15 h) in the control, nontreated leaf tissue might reflect nitrosative stress, as observed under salt stress conditions by Valderrama and others (2007).

Coming back to the early events that occur after water loss, Tun and others (2006) observed a rapid, within minutes, spermine-induced NO release from *Arabidopsis* seedlings, suggesting the presence of an unknown enzyme that converts PAs directly to NO and other products. Plants have several potential sources for NO synthesis, including nitrite and arginine-dependent pathways (Arasimowicz and Floryszak-Wieczorek 2007; Besson-Bard and others 2008). Our data indicate that in cucumber leaves both the NOS-like enzyme and NR were associated with PA-induced NO synthesis during drought. It is unclear whether and in what way PAs could regulate NO-generating enzymes. However, there is evidence that binding PAs to 14-3-3 proteins is responsible for the inactivation of NR as a source of NO (Athwal and Huber 2002).

In animals NO can directly affect polyamine biosynthesis through nitrosylation of ornithine decarboxylase (ODC) (Bauer and others 2001). In plants it has been demonstrated that NO inhibits ethylene biosynthesis through S-nitrosylation of methionine adenosyltransferase (MAT), leading to the reduction of the S-adenosylmethionine (SAM) pool (Lindermayr and others 2006). Because it is well known that ethylene and polyamines are linked through a common precursor, SAM, it is possible that there exists a negative feedback between NO and PA biosynthesis. A model of a potential interaction between PAs and NO under water stress conditions is presented in Fig. 5. Moreover, recently it was documented by Bright and others (2006) that ABA stimulated NO generation via NR and was required for ABA-induced stomatal closure. On the other hand, ABA modulates PA metabolism under drought stress conditions (Alcázar and others 2006; Xiong and others 2006).

Our results indicated that exogenous NO (SNP) might slightly reduce water loss under drought conditions. Cucumber seedlings supplemented with an NO donor and then exposed to stress retained more water, up to 5-12% in relation to the SNP concentration, than control seedlings. This supports the results of Tian and Lei (2006) who showed that SNP enhanced growth in wheat seedlings and maintained high relative water content. Likewise, Hao and others (2008) observed that maize leaves pretreated with SNP maintained a higher water content than did untreated controls, and in the authors' view the effect was largely due to a reduced transpiration rate and a relatively better hydration state under water stress.

Oxidative stress that occurs during tissue dehydration can cause ion leakage from the cell to intercellular compartments (Halliwel and Gutteridge 1984). As shown earlier by Kubiś (2005, 2006), Spd treatment of cucumber seedlings was accompanied by the proper structure and function of membranes being maintained during drought. In Spd-supplied plants, Kubiś (2006) observed an enhanced MDA level that correlated with an increased lipoxygenase (LOX) activity. It is possible that lipid hydroperoxides produced by LOX are used as substrates for stress-induced jasmonic acid (JA) biosynthesis (Rosahal 1996; Creelman and Mullet 1997) without modifying membrane stability. This is consistent with the results presented here, that lipid peroxidation, measured as TBARS content, increased in PA-treated (1 mM Spd, Spm, Put) plants; however, it was not reflected in enhanced ion fluxes across the membranes.

A similar effect on membrane stability was recorded when NO was supplied exogenously. Stress-evoked changes in membrane permeability and lipid peroxidation in cucumber leaves were significantly lower in the presence of exogenous NO. In confirmation of these results, scavenging of NO by addition of cPTIO resulted in a marked increase of ion leakage and TBARS content. These data suggest that NO in a certain range of concentrations promotes plant fitness and adaptive responses to water deficit during the initial phases of stress. Exposure of plants to higher levels of an NO donor (500 μM SNP) was not

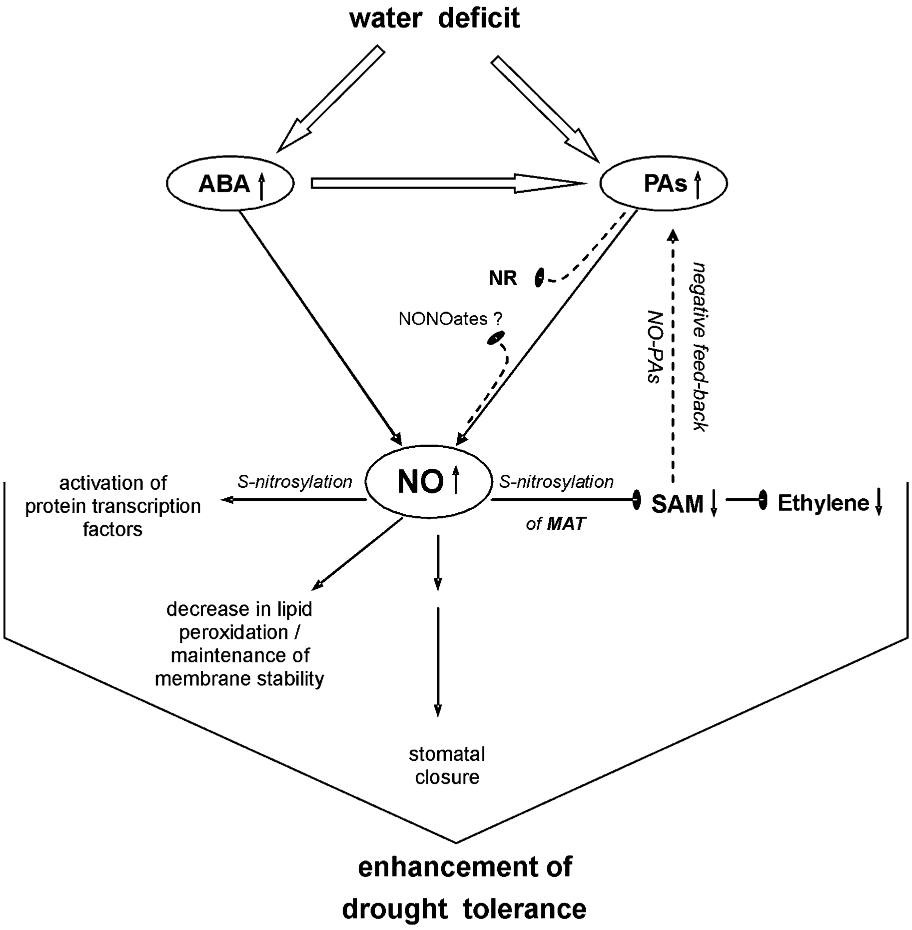

Fig. 5 A model of potential interaction between polyamines (PAs) and nitric oxide (NO) during water stress conditions, considering abscisic acid (ABA) biosynthesis. MAT, methionine adenosyltransferase; NR, nitrate reductase; SAM, S-adenosylmethionine

correlated with a beneficial effect of NO on membrane stability because an excess of NO can enhance the Fenton reaction, leading to hydroxyl radical ($\dot{\mathrm{OH}}$) production and the destruction of membranes (Fukuto and others 2000).

The protective effects of NO on lipid peroxidation might be explained by terminating the radical chain reaction by the reaction of NO with the membrane lipid peroxyl radical (O'Donnell and others 1997, 1999). However, it is also known that NO may promote lipid peroxidation, and this effect is dependent on NO concentration and the membrane environment (Hiramoto and others 2003; Stöhr 2007).

To verify the time sequence of stress-induced events in the PA-NO cross-talk, seedlings were pretreated with an NO donor prior to imposition of water deficit. This treatment did not cause significant changes in the levels of endogenous PAs (Spm, Spd, and Put) compared with those of the control (nontreated with SNP). An increase in the levels of individual PAs observed in the later stages of stress (15 h) was the result of leaf tissue dehydration rather than the action of NO. In confirmation of these observations, no changes in PA levels were found in this study in well-watered seedlings subjected to different doses of SNP (data not presented). Based on the above and other results, it may be concluded that under water deficit NO acts as a downstream signal of PAs. NO applied to cucumber roots improved leaf metabolism, especially protecting membranes against oxidative damage. An early response to water stress signals via NO could help plants adapt to drought and prevent them from being seriously damaged.

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