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Nitric oxide is involved in light-specific responses of tomato during germination under normal and osmotic stress conditions

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• *Background and Aims* Nitric oxide (NO) is involved in the signalling and regulation of plant growth and development and responses to biotic and abiotic stresses. The photoperiod-sensitive mutant *7B-1* in tomato (*Solanum lycopersicum*) showing abscisic acid (ABA) overproduction and blue light (BL)-specific tolerance to osmotic stress represents a valuable model to study the interaction between light, hormones and stress signalling. The role of NO as a regulator of seed germination and ABA-dependent responses to osmotic stress was explored in wild-type and *7B-1* tomato under white light (WL) and BL.
• *Methods* Germination data were obtained from the incubation of seeds on germinating media of different composition. Histochemical analysis of NO production in germinating seeds was performed by fluorescence microscopy using a cell-permeable NO probe, and endogenous ABA was analysed by mass spectrometry.
• *Key Results* The NO donor *S*-nitrosoglutathione stimulated seed germination, whereas the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) had an inhibitory effect. Under WL in both genotypes, PTIO strongly suppressed germination stimulated by fluridone, an ABA inhibitor. The stimulatory effect of the NO donor was also observed under osmotic stress for *7B-1* seeds under WL and BL. Seed germination inhibited by osmotic stress was restored by fluridone under WL, but less so under BL, in both genotypes. This effect of fluridone was further modulated by the NO donor and NO scavenger, but only to a minor extent. Fluorescence microscopy using the cell-permeable NO probe DAF-FM DA (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate) revealed a higher level of NO in stressed *7B-1* compared with wild-type seeds.
• *Conclusions* As well as defective BL signalling, the differential NO-dependent responses of the *7B-1* mutant are probably associated with its high endogenous ABA concentration and related impact on hormonal crosstalk in germinating seeds. These data confirm that light-controlled seed germination and stress responses include NO-dependent signalling.

Key words: Abscisic acid, blue light, germination, nitric oxide, osmotic stress, *Solanum lycopersicum*, tomato, *7B-1* mutant.

INTRODUCTION

Nitric oxide (NO) is a ubiquitous signalling molecule across all the kingdoms of life. NO has been shown to be involved in signalling pathways in plant growth, development and responses to environmental stimuli (reviewed by Lamotte *et al.*, 2005; Arasimowicz and Floryszak-Wieczorek, 2007; Beson-Bard *et al.*, 2008; Neill *et al.*, 2008; Wilson *et al.*, 2008; Šírová *et al.*, 2011). NO also participates in plant responses to biotic stress induced by infection challenge of viral, bacterial and fungal pathogens (Delledonne, 2005; Mur *et al.*, 2006; Zago *et al.*, 2006). As in animal cells, under certain circumstances NO can act as an antioxidant and protect plants from increased levels of reactive oxygen species (Shi *et al.*, 2005). However, NO excess in stress conditions can lead to increased nitrosative stress that can compromise the structure and function of cellular components such as proteins, lipids and nucleic acids (Valderrama *et al.*, 2007). Despite considerable advances in NO plant research in recent years, as a consequence of numerous conflicting reports, the exact sources of NO, its sites of action and its role in plants remain largely uncertain (reviewed by Planchet and Kaiser, 2006; Arasimowicz and Floryszak-Wieczorek, 2007; Wilson *et al.*, 2008).

Research on many plant species has revealed NO as a crucial component of the signalling network in the regulation of seed dormancy and germination (Šírová *et al.*, 2011). The application of a range of NO donors has been shown to break seed dormancy and stimulate germination in *Arabidopsis thaliana* (Batak *et al.*, 2002; Bethke *et al.*, 2004*b*, 2006), *Hordeum vulgare* (Bethke *et al.*, 2004*b*), *Malus domestica* (Gniazdowska *et al.*, 2006), *Lactuca sativa* (Beligni and Lamattina, 2000), *Lupinus luteus* (Kopyra and Gwóźdź, 2003) and *Paulownia tomentosa* (Giba *et al.*, 1998; Jovanovich *et al.*, 2005). The

direct involvement of NO in the stimulation of germination of dormant *Arabidopsis* seeds was demonstrated by exogenous application of purified NO gas (Libourel *et al.*, 2006). A recent report has revealed that transcription of genes for aquaporins was stimulated by exogenous NO in germinating rice seeds (Liu *et al.*, 2007).

Recent results also suggest that NO is involved in the signalling pathways of light perception in plants and specifically in the germination of light-requiring seeds. A genome-wide analysis of the expression profiles of wild-type and photoreceptor mutants concluded that cryptochromes were probably the major photoreceptors for blue light (BL) regulation during early seedling development in *Arabidopsis*, whereas phytochrome A and phototropins could play rather limited roles (Jiao *et al.*, 2003, 2007). Using *Arabidopsis* phytochrome mutants, it was demonstrated that the exogenous application of potassium nitrate or NO-releasing donors stimulated phytochrome A-specific germination, whereas phytochrome B-specific germination was affected to a much lesser extent (Batak *et al.*, 2002).

For decades, plant hormones have been known to control seed dormancy and germination: abscisic acid (ABA) acting generally as the inhibitor and gibberellic acid (GA) as the promoter of seed germination (Gubler *et al.*, 2005). Recent findings indicate that during the breaking of seed dormancy, the ABA level is, for the most part, controlled by ABA catabolism. In *Arabidopsis*, mutation in the *cyp707a2* gene, which encodes the key enzyme of ABA catabolism, abscisic acid 8′-hydroxylase, results in a high level of ABA and a strengthened dormancy (Okamoto *et al.*, 2006). Several recent reports have reported an interesting link between the effect of NO on seed dormancy and ABA catabolism (Liu *et al.*, 2009, 2010; Matakiadis *et al.*, 2009). The rapid decrease of ABA induced by NO in the early stage of seed imbibition is mediated by increased expression of *cyp707a2* (Liu *et al.*, 2009). In this way, NO produced in the endosperm layer during imbibition regulates *cyp707a2* expression, a step preceding increased ABA catabolism required for seed germination. Transcription of the *cyp707a2* gene in imbibed *Arabidopsis* seeds is also regulated by nitrate (Matakiadis *et al.*, 2009). Moreover, increased germination of *Arabidopsis* seeds by H_2O_2 treatment during imbibition is mediated by NO, and is associated with the upregulation of ABA catabolism genes, as well as the upregulation of GA biosynthesis genes (Liu *et al.*, 2010). Together, these results highlight the important function of NO in the regulation of the *cyp707a2* gene and endogenous ABA levels, and in the control of dormancy in response to external stimuli.

The link between ABA and NO has also been reported in plants suffering from drought and osmotic stress. During these stress conditions, decreasing vacuolar turgor induces the synthesis of ABA, which subsequently stimulates stomal closure via NO-dependent signalling pathways (Neill *et al.*, 2008). ABA-dependent signalling in stomata includes cross-talk of NO with H_2O_2 and a close relationship between ABA, reactive oxygen species and NO synthesis in stomatal closure, as demonstrated in *Vicia* and *Arabidopsis*. In *Vicia* guard cells, NO is involved in the ABA-dependent pathway of BL-specific stomata control by inhibiting an unknown signalling component between phototropins and plasma membrane H^+-ATPase (Zhang *et al.*, 2007).

The recessive single gene mutant *7B-1* in tomato (*Solanum lycopersicum*) was originally selected for its photoperiod-dependent male sterility (Sawhney, 1997, 2004). The *7B-1* mutant shows resistance to abiotic stresses including high osmoticum, high salt and low temperatures, as revealed by seed germination tests (Fellner and Sawhney, 2001). The inhibitory effects of abiotic stresses on the germination of wild-type tomato seeds can be reversed by treatment with fluridone, an inhibitor of the biosynthesis of ABA, carotenoids and chlorophyll (Fellner and Sawhney, 2001). An elevated level of endogenous ABA in *7B-1* has been suggested to confer resistance of the mutant to abiotic stresses (Fellner *et al.*, 2001), probably in relation to an elevated endogenous level of ethylene (Fellner *et al.*, 2005). Interestingly, *7B-1* mutant seeds are hypersensitive to the inhibitory effects of exogenous ABA, whereas in continuous light, *7B-1* seed germination is resistant to ABA (Fellner and Sawhney, 2002). The inhibitory effect of osmotic stress on wild-type seed germination is specifically amplified by BL, while the *7B-1* mutant shows BL-specific resistance to osmotic stress, possibly mediated by a defect in BL perception or signal transduction (Fellner and Sawhney, 2002). It was recently demonstrated that increased resistance of the *7B-1* mutant to the bacterial toxin coronatine in BL was associated with greater accumulation of salicylic acid and ABA (Bergougnoux *et al.*, 2009). Therefore, *7B-1* is a valuable tool to study light-specific plant responses to abiotic and biotic stresses.

In relation to previous reports on the multiple involvement of NO in plant seed germination, light perception, de-etiolation, hormonal signal transduction and responses to abiotic stresses, the main focus of this study was to investigate the possible involvement of NO in light-specific processes during the germination of wild-type and *7B-1* tomato seeds under normal and osmotic stress conditions.

MATERIALS AND METHODS

Plant material

The recessive *7B-1* tomato mutant (*Solanum lycopersicum* L. background 'Rutgers') was isolated as a photoperiod-sensitive male-sterile mutant (Sawhney, 1997, 2004). For all experiments, *7B-1* and corresponding wild-type (WT, 'Rutgers') seeds were obtained from plants grown in the greenhouse. Seedlings grew in pots (100 × 150 mm) filled with soil (Potgrond H; Klasmann-Deilmann, Geeste, Germany) and were watered daily and fertilized with Osmocote. In summer, plants were cultured under natural light conditions at 20°C and higher temperatures. In winter, plants grew under additional artificial light provided by white high-pressure sodium vapour lamps (SHC (L) 400 W; Tesla, Czech Republic) for approx. 16-h photoperiods. The greenhouse temperature regime was regulated from 15°C at night to 27°C during the day.

Seed germination and plant growth

Seeds were surface-sterilized by soaking in 3 % (v/v) commercial bleach solution for 20 min and then rinsed extensively

with sterile distilled water. Sterilized seeds were arranged on MS culture medium (Murashige and Skoog, 1962) in round Petri dishes (100 × 15 mm). The basal medium (BM) contained Murashige and Skoog salts (Sigma-Aldrich, Germany), 1 % (w/v) sucrose and 1 mM 2-(*N*-morpholino)-ethanesulphonic acid (pH adjusted to 6·1 by KOH before autoclaving). Corresponding amounts of freshly prepared stock solutions of mannitol, ABA [(±)-*cis*, *trans*-ABA], fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1H)-pyridinone), PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) and SNG (*S*-nitrosoglutathione) were added after sterile filtration through a 0·22-μm Millex-GS filter (Millipore Co., Billerica, MA, USA) to cooling media after autoclaving. Thirty seeds of *7B-1* or the wild-type were germinated on Petri dishes in growth chambers (Microclima 1000; Snijders Scientific B.V., The Netherlands) at a temperature of 23°C in continuous darkness, or under continuous white light (WL) or BL. BL and WL were provided by Philips TLD-36W/18-Blue and Philips TL-D 36W/54 white fluorescent tubes, respectively. Maximum irradiance of BL was at 440 nm (10 μmol m^{-2} s^{-1} total photon flow rate). The total photon flow rate of WL was 120 μmol m^{-2} s^{-1}. The photon fluence rate was measured with a portable spectroradiometer (Model LI-1800; Li-Cor, the Netherlands) calibrated by the Department of Biophysics at Palacky University in Olomouc. Seed germination, defined as radicle protrusion, was scored from 2 to 10 d after sowing.

Determination of ABA content

Emerged radicles after testa and endosperm rupture (approx. 1–2 mm long) were used. The plant material (approx. 100 mg of sample fresh weight) was extracted using an MM 301 vibration mill (Retsch GmbH & Co. KG, Haan, Germany) at a frequency of 27·5 Hz for 3 min using 3-mm tungsten carbide beads (Retsch). Internal standard [50 pmol (+)-3′,5′,5′,7′,7′,7′-$^2H_6$-ABA] and 750 μL of a cold mixture of methanol/water/acetic acid (80 : 19 : 1) were added to the samples. After 1 h of shaking in the dark at 4°C, the homogenates were centrifuged (16 000 *g*, 5 min, 4°C) and the pellets were re-extracted in 0·5 mL extraction solvent for 60 min. The supernatants were transferred to fresh glass tubes and dried under vacuum. Extracts were dissolved in 1 ml of 99 % water/1 % acetic acid (v/v) and purified by solid-phase extraction on Oasis® HLB cartridges (60 mg, 3 mL, Waters, USA). The fraction containing ABA was eluted with 3 ml methanol/water/acetic acid (80 : 19 : 1) and evaporated to dryness in a Speed-Vac (UniEquip, Planegg, Germany). Evaporated samples were methylated, purified by ABA-specific immunoaffinity extraction (Hradecká *et al.*, 2007) and analysed by UPLC-ESI(+)-MS/MS (Turečková *et al.*, 2009).

Histochemical analysis of NO production

Seeds of *7B-1* and WT germinating in BL or WL were used for analyses. Emerged radicles after testa and endosperm rupture (approx. 1–2 mm long) were incubated in a solution of the cell-permeable NO probe DAF-FM DA (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate; 10 μM) for 30 min, washed extensively with phosphate buffer and mounted on microscope slides. Detection was carried out using a fluorescence microscope (Model BX50, Olympus Optical Co., Tokyo, Japan; fluorescence mirror unit U-MWB2) equipped with a digital camera system connected to a PC. Control samples of radicles were treated for 30 min with 0·1 mM NO scavenger carboxyPTIO (cPTIO) prior to DAF-FM DA staining (negative control).

Data analysis

Germination, growth and treatment of seeds and seedlings were conducted in three independent experiments. Data presented in the graphs represent the mean ± s.d. of three independent measurements.

RESULTS

Influence of NO donor and NO scavenger on the germination of tomato seeds under WL or BL in normal conditions

The germination of seeds of *7B-1* and WT tomato plants under WL or BL on BM or media supplemented with 3 μM ABA or 10 μM fluridone is shown in Figs 1–3. As previously reported (Fellner and Sawhney, 2002), higher germination of WT seeds on BM was observed under WL than under BL (Fig. 1A, C), whereas *7B-1* seeds germinated to a similar extent under both light conditions (Fig. 1B, D). Under BL, the NO donor SNG considerably increased the speed and percentage of germination in both genotypes (Fig. 1C, D). SNG also increased the germination rate in both genotypes under WL, but it did not markedly stimulate the percentage of germination (Fig. 1A, B). In contrast, the NO scavenger PTIO strongly inhibited seed germination in both genotypes under WL (Fig. 1A, B). Under BL conditions, PTIO inhibited the germination of WT seeds, but had no effect on seed germination in the *7B-1* mutant (Fig. 1C, D).

Addition of ABA to BM markedly suppressed the germination of both tomato genotypes under BL and WL (Fig. 2), and almost inhibited *7B-1* germination under WL (Fig. 2B). In both genotypes, application of SNG to the medium with ABA resulted in a very slight increase of germination, which was more apparent in WT under BL and WL (Fig. 2).

Addition of fluridone to the culture medium markedly increased the ability of WT seeds to germinate under WL as well as under BL (Fig. 3A, C). This positive effect of fluridone was not as striking on the germination of *7B-1* seeds (Fig. 3B, D). Under WL, SNG applied to the medium along with fluridone increased the germination rate in both genotypes (Fig. 3A, B). Under BL conditions, SNG slightly increased the germination rate of WT seeds 2–5 d after imbibition, whereas the germination of *7B-1* seeds was markedly increased during the entire time period (Fig. 3C, D). PTIO strongly reduced germination in both genotypes under WL (Fig. 3A, B), whereas under BL conditions the inhibitory effect of PTIO on seed germination was strongly reduced (Fig. 3C, D).

Influence of NO donor and NO scavenger on the germination of tomato seeds under osmotic stress

Osmotic stress induced by the addition of 100 mM mannitol to the growth medium almost fully suppressed seed germination

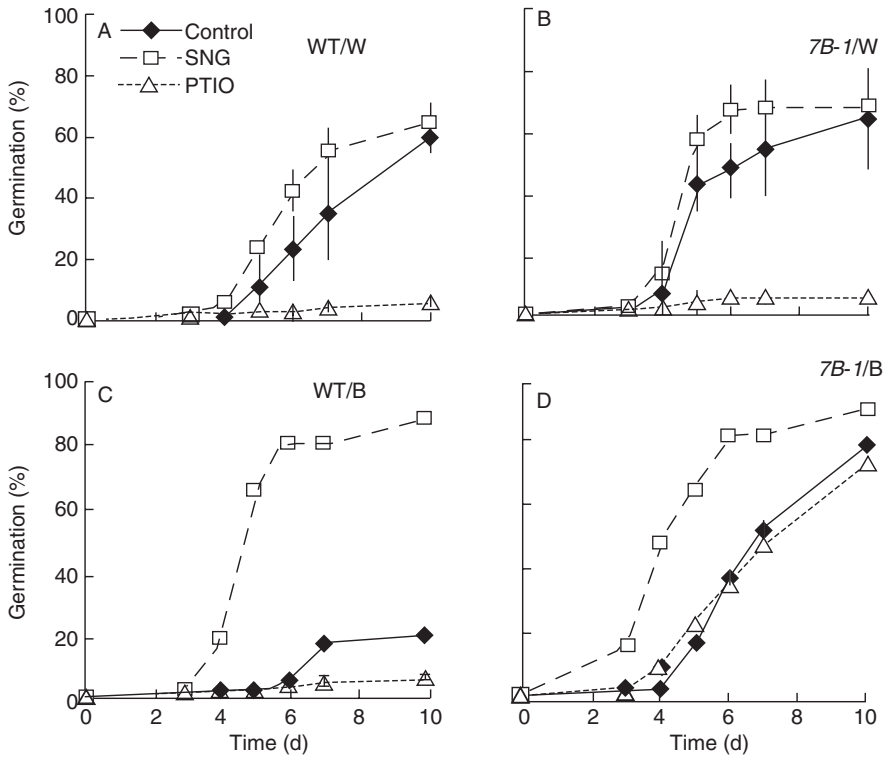

FIG. 1. Kinetics of seed germination in WT and the *7B-1* mutant in the presence of NO donor (SNG, 0·2 mM) or NO scavenger (PTIO, 0·1 mM) in basal medium. Control seeds were germinated on the basal medium. (A) WT, white light; (B) *7B-1*, white light; (C) WT, blue light; (D) *7B-1*, blue light.

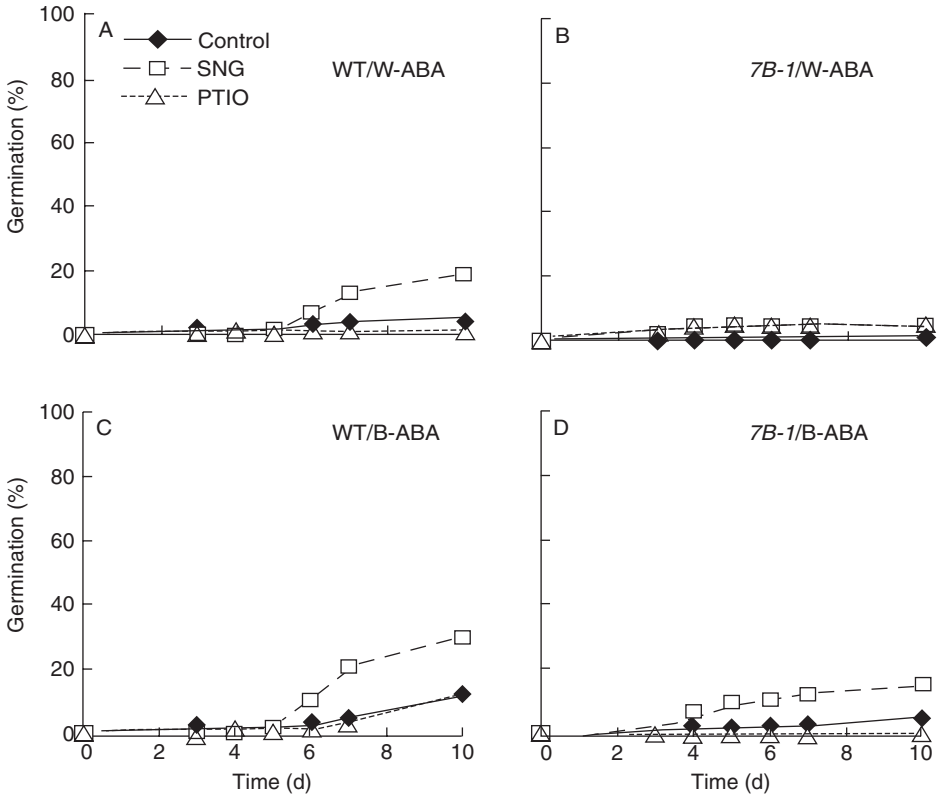

FIG. 2. Kinetics of seed germination in WT and the *7B-1* mutant in the presence of NO donor (SNG, 0·2 mM) or NO scavenger (PTIO, 0·1 mM) in basal medium with addition of 3 μM ABA. Control seeds were germinated on BM with ABA only. (A) WT, white light; (B) *7B-1*, white light; (C) WT, blue light; (D) *7B-1*, blue light.

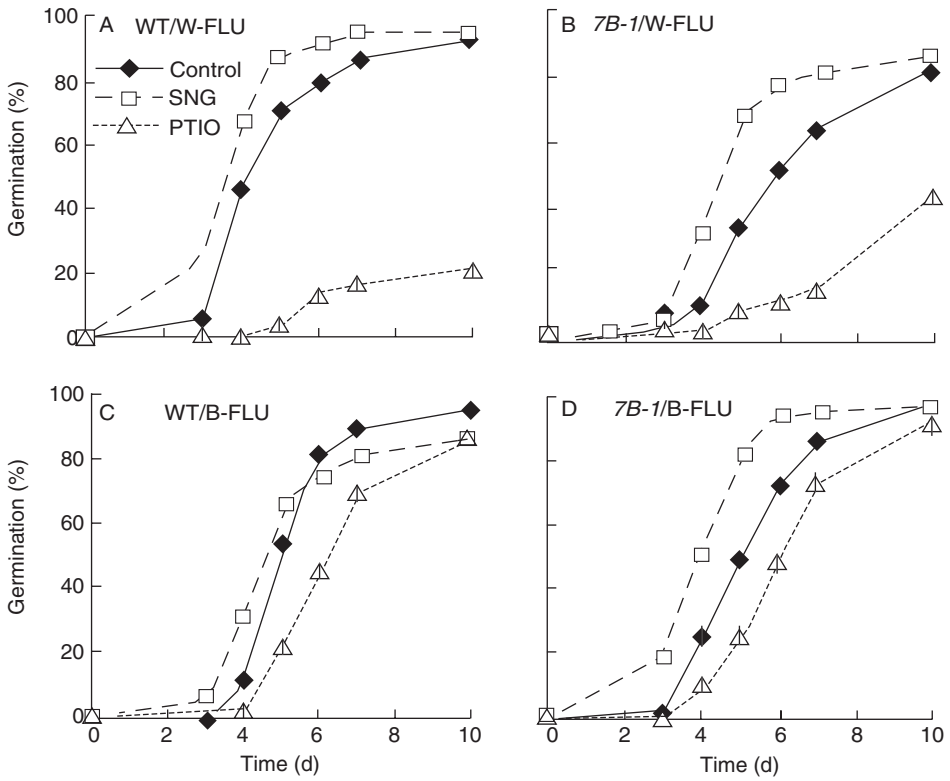

FIG. 3. Kinetics of seed germination in WT and the *7B-1* mutant in the presence of NO donor (SNG, 0·2 mM) or NO scavenger (PTIO, 0·1 mM) in basal medium supplemented with 10 μM fluridone. Control seeds were germinated on BM with fluridone only. (A) WT, white light; (B) *7B-1*, white light; (C) WT, blue light; (D) *7B-1*, blue light.

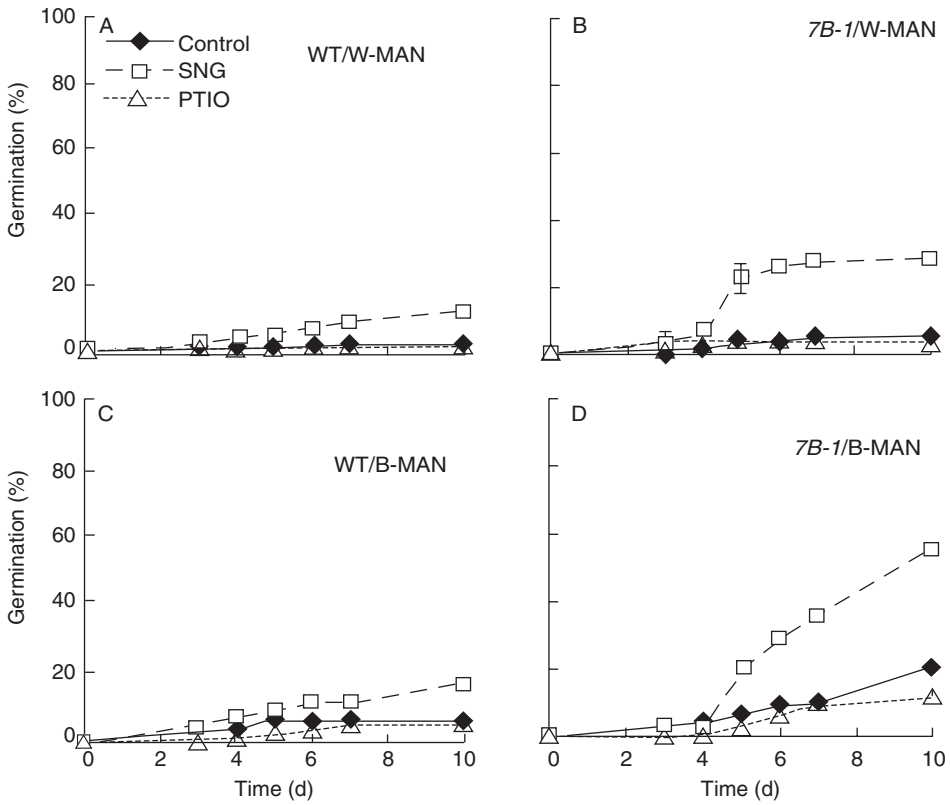

FIG. 4. Kinetics of seed germination in WT and *7B-1* mutant in the presence of NO donor (SNG, 0·2 mM) or NO scavenger (PTIO, 0·1 mM) in basal medium (BM) supplemented with 100 mM mannitol. Control seeds were germinated on BM with mannitol only. (A) WT, white light; (B) *7B-1*, white light; (C) WT, blue light; (D) *7B-1*, blue light.

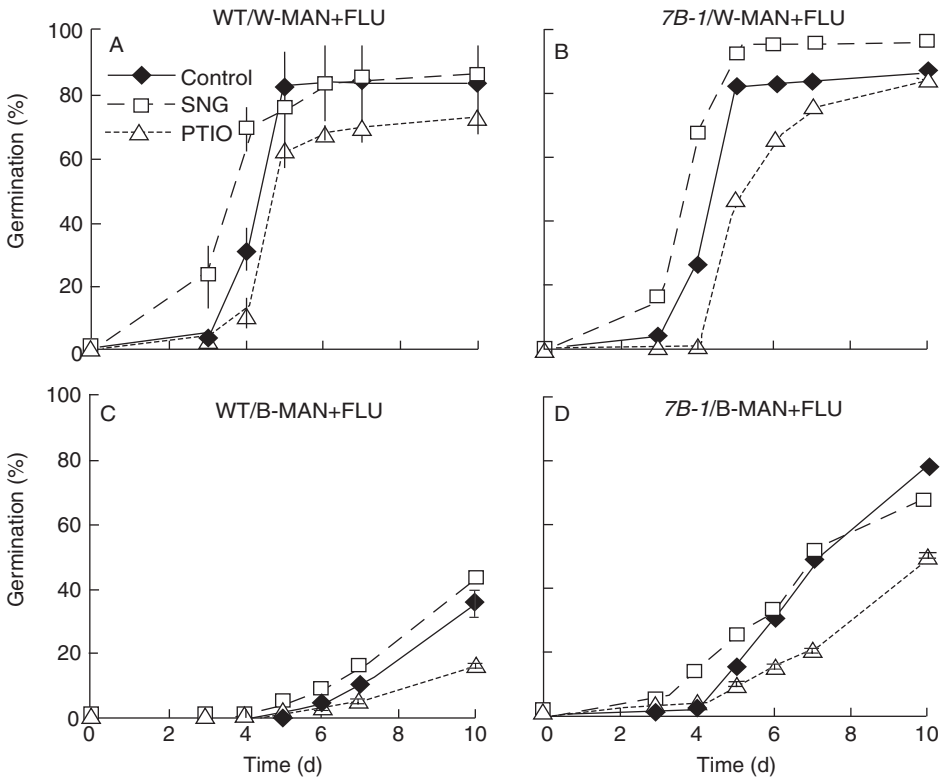

FIG. 5. Kinetics of seed germination in WT and *7B-1* mutant in the presence of NO donor (SNG, 0·2 mM) or NO scavenger (PTIO, 0·1 mM) in basal medium (BM) supplemented with 100 mM mannitol and 10 μM fluridone. Control seeds were germinated on BM with mannitol and fluridone only. (A) WT, white light; (B) *7B-1*, white light; (C) WT, blue light; (D) *7B-1*, blue light.

in both genotypes under WL conditions (Fig. 4A, B). Under BL, the germination of *7B-1* seeds was inhibited by mannitol to a lesser extent than WT seed germination (Fig. 4C, D). SNG supplemented to mannitol-containing medium had only a minor effect on WT seed germination (Fig. 4A, C), whereas it distinctly stimulated the germination of *7B-1* seeds, especially under BL conditions (Fig. 4B, D). A very low rate of germination of seeds of both genotypes under osmotic conditions prevented us from quantifying the additional inhibitory effect of PTIO (Fig. 4).

The presence of ABA in the medium with mannitol had a strong inhibitory effect on seed germination, which could not be reversed significantly by the addition of SNG (data not shown). ABA-treated WT seeds showed a slightly increased germination in the presence of SNG under WL, whereas *7B-1* seeds germinated only slightly in the presence of exogenous ABA under BL (data not shown).

Adding fluridone to the medium containing mannitol increased seed germination. In both genotypes, fluridone fully restored germination under WL conditions (Fig. 5A, B). Under BL, fluridone increased seed germination to approx. 40 % in WT and to approx. 80 % in *7B-1* (Fig. 5C, D). The addition of SNG or PTIO to the medium had a minor promoting or inhibitory effect, respectively, on seed germination in both genotypes under WL as well as under BL (Fig. 5). The effects of SNG and PTIO in combination with mannitol and/or fluridone on the germination of tomato seeds under BL or WL are shown in Fig. 6. As previously reported (Fellner *et al.*, 2001), in contrast to WT, *7B-1* seedlings were not bleached when germinated on BM containing fluridone.

Endogenous level of ABA in radicles of germinating seeds under WL and BL

ABA content was determined in radicles of approx. 1–2 mm length after testa and endosperm rupture. A higher content of ABA was detected in *7B-1* than in WT seeds germinated on BM, while *7B-1* seeds germinating under BL contained a lower ABA level compared with *7B-1* seeds germinating under WL (Fig. 7). Under osmotic stress in the presence of mannitol, *7B-1* radicles contained a substantially higher ABA level under WL than under BL, while under BL the ABA content was similar in both genotypes.

Influence of osmotic stress on NO production in radicles of germinating seeds

NO production in the radicles of germinating seeds was monitored by fluorescence microscopy using the cell-permeable NO-specific fluorescent probe DAF-FM DA (Fig. 8). A significant increase in NO production was observed only in the radicles of *7B-1* seeds exposed to mannitol. The specificity of the NO probe was confirmed by pre-incubation of samples with cPTIO, which completely abolished the NO fluorescence observed in the radicles of osmotically stressed *7B-1* seeds.

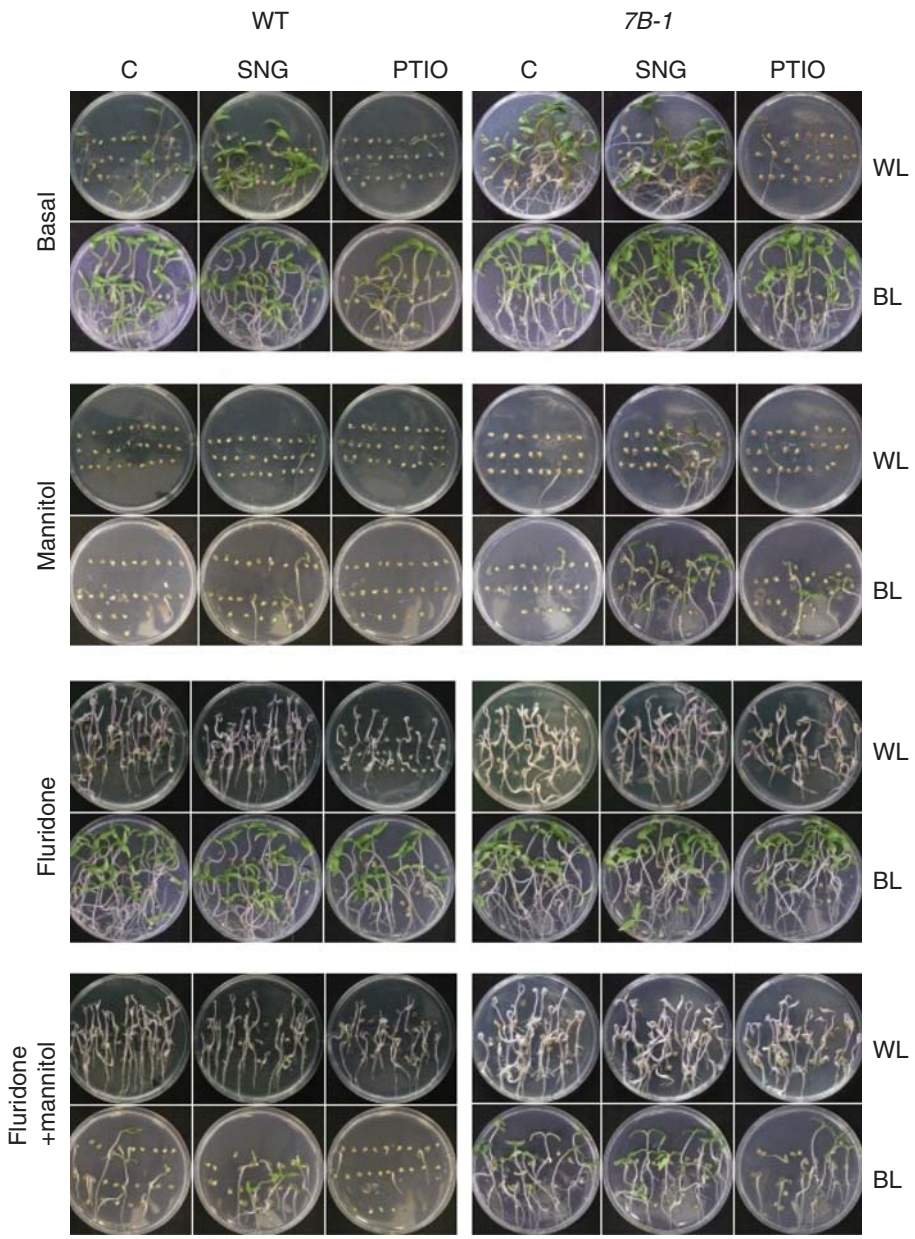

FIG. 6. Representative samples of WT and *7B-1* seeds germinated for 7 d on the growth media under white (WL) or blue (BL) light.

DISCUSSION

NO has been shown to be a crucial component of signalling pathways in the release of plant seed dormancy and seed germination (Beligni and Lamattina, 2000; Bethke *et al.*, 2004*a*, *b*, 2006; Libourel *et al.*, 2006), and to play an important role in plant responses to abiotic and biotic stresses (Garcia-Mata and Lamattina, 2001; Gould *et al.*, 2003; Corpas *et al.*, 2011). The aim of this study was to expand the current knowledge on the involvement of NO in light-specific responses of germinating seeds under normal and osmotic stress conditions in relation to ABA. We used a specific plant model, namely the *Solanum lycopersicum* L. recessive single gene mutant *7B-1*, which exhibits BL-specific tolerance to abiotic stresses (Fellner and Sawhney, 2001, 2002). The effects of substances known to modulate endogenous NO content in plant cells on the germination of tomato WT and *7B-1* seeds were investigated under WL and BL illumination.

In agreement with previously published data on other plant species, our results confirmed the stimulatory effects of NO donor SNG on the germination of tomato seeds. Supplemented to the BM, SNG was a more potent inducer of germination in both WT and *7B-1* under BL than under WL. Conversely, the NO scavenger PTIO partially or almost completely inhibited seed germination. The data indicate that in tomato seeds, NO plays a positive role in seed germination and that BL-induced inhibition of seed germination in tomato (Fellner and Sawhney, 2002) is mediated at least in part by the absence of NO in incubated seeds (Fig. 1). In *Arabidopsis* seeds, potassium nitrate or

organic nitrites as NO donors presumably affect seed germination mainly through phytochrome A-dependent pathways (Batak *et al.*, 2002). In lettuce, PTIO treatment inhibits seed germination (Beligni and Lamattina, 2000).

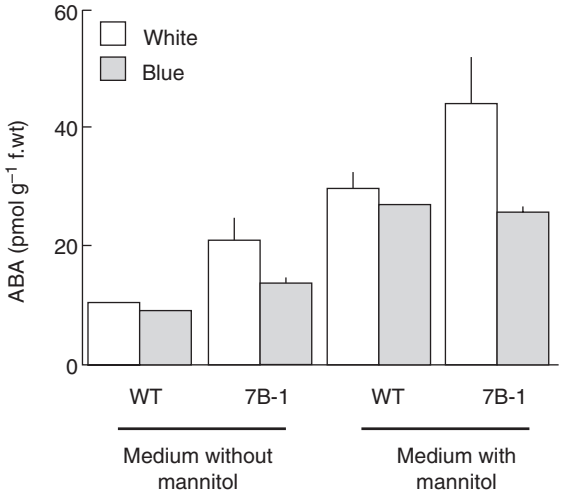

FIG. 7. ABA content in emerged radicles of seeds germinated on basal medium with and without mannitol (100 mM) under WL or BL. ABA content was determined in radicles 1–2 mm long.

It was previously reported that the level of endogenous ABA in hypocotyls of *7B-1* mutant 7-d seedlings was doubled compared with the level in WT seedlings, and that increased endogenous ABA might be related to the increased resistance of the *7B-1* mutant to different abiotic stresses during seed germination (Fellner *et al.*, 2001). We confirmed substantial differences in ABA content in the radicles of seeds germinated in the absence or presence of osmotic stress and controlled by BL or WL illumination. The fact that SNG was able to partially restore seed germination inhibited by ABA indicates that ABA-induced inhibition of seed germination is associated with a lowered level of NO in tomato seeds.

The observation that SNG partially reversed the inhibitory effect of mannitol indicates that the inhibitory effect of osmotic stress on seed germination might be at least partially due to a reduced level of NO in tomato seeds. The fact that the restoration of germination inhibited by mannitol was much more efficient in *7B-1* than in WT seeds suggests that the accumulation of NO in *7B-1* is greater than in WT seeds. This is also supported by the histochemical localization of NO production using the fluorescent probe DAF-FM DA, which showed that radicles of germinated *7B-1* seeds exposed to osmotic stress produced more NO than WT seeds (Fig. 8), although we were not able to bring direct experimental

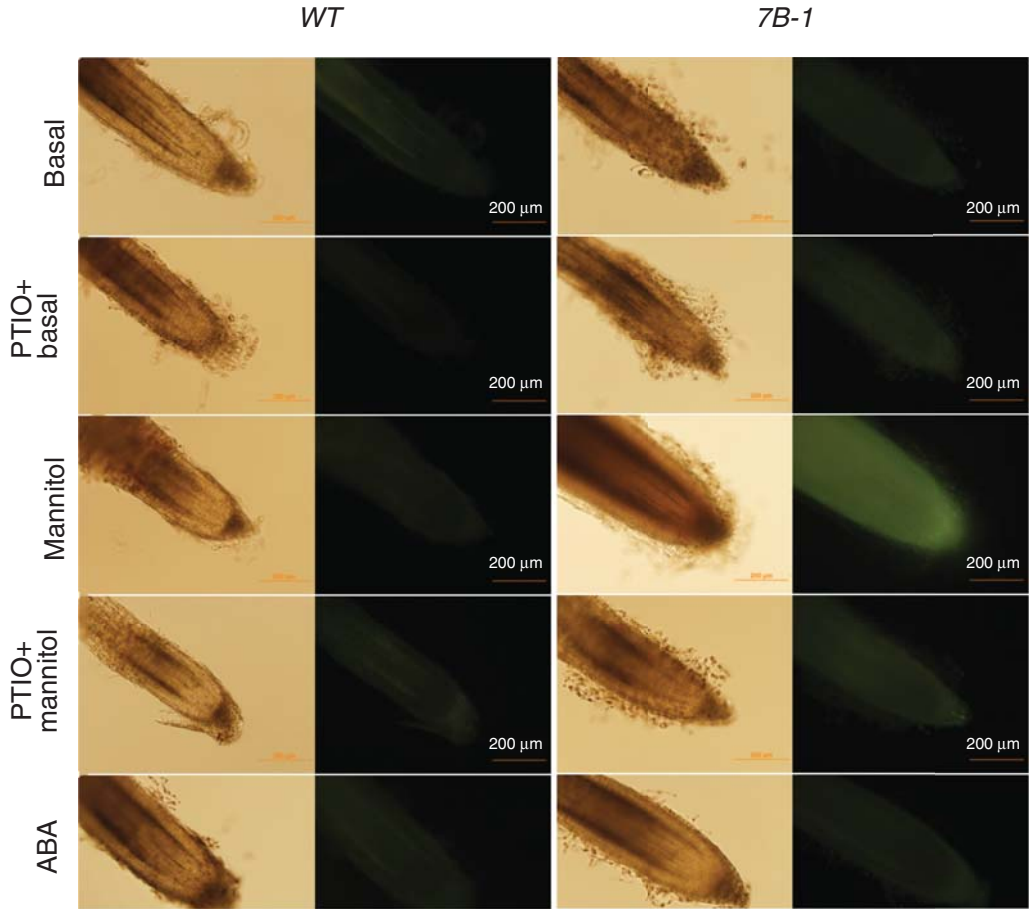

FIG. 8. Histochemical localization of NO production using the cell-permeable fluorescent NO probe DAF-FM DA in emerged radicles of WT and *7B-1* mutant seeds incubated under BL. Seeds were germinated on basal medium supplemented with and without mannitol (100 mM) or with ABA (3 µM). Incubation with cPTIO (0·1 mM) 30 min prior to the DAF-FM DA staining was included as a negative control.

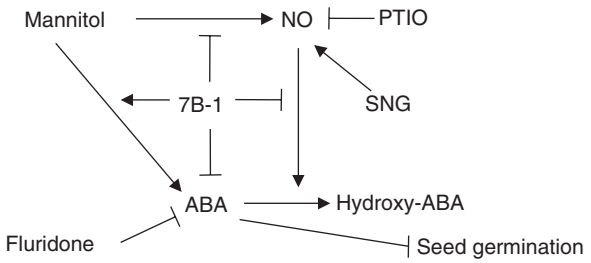

FIG. 9. Working model showing a role of the functional *7B-1* gene product in NO- and ABA-induced responses of tomato seed germination to osmotic stress under BL conditions, and the involvement of NO donor SNG and NO scavenger PTIO in this process. Arrows and T-bars represent positive and negative effects, respectively.

evidence of increased NO production in germinating seeds before or during radicle protrusion.

It has previously been proposed that mannitol at high concentrations inhibits seed germination through an increased level of endogenous ABA (Fellner and Sawhney, 2002). Our experiments with fluridone confirmed this proposal (Fig. 5). Furthermore, the differential effects of NO donor or scavenger on seeds germinating under osmotic stress conditions were substantially influenced by the addition of fluridone (Fig 4 and 5). This is in agreement with the hypothesis that the NO-dependent signalling in germinating seeds under osmotic stress includes reduced ABA accumulation.

Exogenous ABA is known to inhibit germination, retard coleorhizal elongation and essentially suppress shoot growth (Gubler *et al.*, 2005). As reported previously, contrary to the relationship between ABA and NO in stomata closure, ABA and NO act antagonistically in seed germination (Bethke *et al.*, 2004*a*; Gubler *et al.*, 2005; Sarath *et al.*, 2006). Cellular NO signalling pathways have a crucial role in triggering dormancy release and germination of switchgrass seeds (Sarath *et al.*, 2006). Moreover, the NO produced is able to reverse the inhibition of seed germination caused by exogenous ABA (Sarath *et al.*, 2007).

A high level of endogenous ABA is characteristic of the *7B-1* mutant with a defect in BL signalling (Fellner and Sawhney, 2002). The results of our experiments confirmed the role of NO in the response of both experimental genotypes to osmotic stress, but to varying degrees dependent on the level of endogenous ABA and light conditions. In Fig. 9, we present a working model which attempts to explain a role of the *7B-1* gene product in NO- and ABA-induced responses of tomato seed germination to osmotic stress under BL conditions, and the involvement of the NO donor SNG and NO scavenger PTIO in this process. The model shows that in the absence of osmotic stress, functional *7B-1* (i.e. in WT) negatively affects the ABA level in incubated seeds, whereas it promotes ABA accumulation via the influence of mannitol (Fig. 7; Fellner *et al.*, 2001; Fellner and Sawhney, 2002). Other results presented here suggest that *7B-1* is further negatively involved in mannitol-induced increase of NO level as well as possibly in NO-induced ABA catabolism required for seed germination. The recessive mutation in the *7B-1* gene then results in ABA over-production (Fig. 7; Fellner *et al.*, 2001). However, under osmotic stress, the defect in the *7B-1* gene leads to less additional accumulation of ABA than in WT, simultaneously resulting in increased accumulation of NO (Fig. 8), and thus in increased NO-mediated ABA catabolism. This model can explain the differential responses observed between *7B-1* and WT seed germination in the absence or presence of mannitol and/or fluridone and/or NO and PTIO.

In summary, the results presented here have confirmed the important role of NO in seed germination. As in other plant species, NO donor stimulated and NO scavenger inhibited the germination of tomato seeds. Using the tomato *7B-1* mutant with defective BL signalling, we have expanded existing knowledge to include the involvement of NO in the BL signalling pathway under normal and stress conditions. Moreover, we have shown that NO and ABA signalling under osmotic stress is modulated by light conditions, and that BL controls and amplifies tomato stress responses in an NO-dependent manner. Follow-up experiments concerned with the involvement of NO in the interplay of ABA, GA and ethylene signalling in the germination of WT and *7B-1* seeds are currently being conducted in our laboratory.

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