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Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants

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Abstract. Seed germination, greening of etiolated plants and inhibition of hypocotyl elongation are stimulated by light, which is sensed by various types of photoreceptor. Nitric oxide (NO) has proven to be a bioactive molecule, especially in mammalian cells and, most recently, in plants. Like some phytochrome-dependent processes, many NO-mediated ones are accomplished through increases in cGMP levels. Given these similarities, we proposed that NO could take part in light-mediated events in plants. Here we show that NO promotes seed germination and de-etiolation, and inhibits hypocotyl and internode elongation, processes mediated by light. Two NO donors, sodium nitroprusside (SNP) and *S*-nitroso-*N*-acetylpenicillamine induced germination of lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds in conditions in which this process is dependent on light (e.g. 26 °C). This was a dose-dependent response and was arrested by addition of an NO scavenger, carboxy-PTIO. In addition, nitrite and nitrate, two NO-decomposition products were ineffective in stimulating germination. Wheat seedlings sprayed with SNP and grown in darkness contained 30–40% more chlorophyll than control seedlings. Nitric-oxide-mediated partial greening was increased by light pulses, wounding and biotic stress. *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia) and lettuce seedlings grown in the dark had 20%-shorter hypocotyls in NO treatments than in control ones. On the other hand, internode lengths of potato plants growing under low light intensity and sprayed with 100 μM SNP were also 20% shorter than control ones. These results implicate NO as a stimulator molecule in plant photomorphogenesis, either dependent on or independent of plant photoreceptors.

Key words: De-etiolation – Hypocotyl elongation – Light response – Nitric oxide – Photoreceptor – Seed germination

Introduction

Plants perceive ambient light as a way to optimise photosynthetic reactions and to regulate their growth and development (Chory 1997). Many physiological processes such as germination, inhibition of hypocotyl growth, chloroplast differentiation, plant greening and expression of many nuclear- and chloroplast-encoded genes are stimulated by light (Bertram and Lercari 1997; Mustili and Bowler 1997). Stimulation of many biological processes by the red/far-red-absorbing photoreceptor phytochrome, is performed via heterotrimeric G proteins, leading to at least two main pathways: (i) dependent on calcium/calmodulin and (ii) mediated by increases in cGMP levels (Bowler et al. 1994). However, the complete molecular mechanisms by which light regulates development are largely unknown. Except for phytochrome, little is known about the biochemistry of signal perception and transduction. In many cases, plant hormones cause similar effects in developing plants. These overlapping roles raise the interesting question of whether light and hormones act independently to affect developmental responses or whether plant hormones are involved in the sequence of events initiated by physiologically active photoreceptors (Chory et al. 1994).

Nitric oxide (NO) is a bioactive molecule that in animals takes part in neurotransmission, vasorelaxation, smooth-muscle relaxation and immunoregulation of pathophysiological processes, among others (Ignarro 1990; Anbar 1995; Moilanen and Vapaatalo 1995). Many of its effects are accomplished through stimulation of soluble guanylate cyclase and consequent increase in cGMP levels. In addition, calcium and calmodulin have been described as alternative components in NO-mediated signaling (Stamler 1994). Recent

Abbreviations: ANOVA = analysis of variance; carboxy-PTIO = 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; cv. = cultivar; GA_3 = giberellic acid; NO = nitric oxide; PAR = photosynthetically active radiation; SNAP = *S*-nitroso-*N*-acetylpenicillamine; SNP = sodium nitroprusside

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reports have demonstrated the presence of NO in plants and its involvement in many biological processes. It acts both as a signal molecule in plant defense mechanisms (Noritake et al. 1996; Beligni et al. 1997; Laxalt et al. 1997; Delledonne et al. 1998; Durner et al. 1998; Beligni and Lamattina 1999a) or as a compound with hormonal properties, affecting growth and development functions (Leshem and Haramaty 1996). Moreover, Pfeiffer et al. (1995) and Durner et al. (1998) gave evidence for the presence of NO-sensitive guanylyl cyclase in plants and suggested the existence of an NO/cGMP pathway.

Given the similarities between NO and phytochrome pathways in animals and plants, respectively, we proposed that NO could take part in light-mediated signals, dependently and/or independently of photoreceptors. We were interested in studying the effect of NO on some characteristic light-stimulated responses. We report that, at least three of them, seed germination, de-etiolation and inhibition of hypocotyl and internode elongation can be triggered by NO in conditions of complete darkness or in which light fluences are not high enough to trigger these processes.

Materials and methods

Seed germination. Lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds were used for germination experiments. Lettuce seeds were harvested at the end of summer, maintained at 20 °C in the dark and used for experiments during autumn. Three replicates of 50 seeds each were placed under green light in Petri dishes containing filter paper imbibed in a solution of 100 μM sodium nitroprusside (SNP; Merck, Darmstadt, Germany), an NO donor, and kept at 26 °C in complete darkness. Control lettuce seeds were imbibed in H_2O or in a mixture containing 100 μM $NaNO_2$ and 100 μM $NaNO_3$ (normal products of NO decomposition). Different sets of dishes for each treatment were scored for radicle emergence at 12, 24, 36 and 48 h after imbibition. Analysis was performed by taking the dishes from the dark and counting the number of seeds that showed radicle emergence. Other sets of samples were given 5-min pulses of photosynthetically active radiation (50 μmol s^{-1} m^{-2}) provided by fluorescent tubes (osram; 36 W) at 12, 24 or 36 h after imbibition before returning to continuous darkness. 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, potassium salt (carboxy-PTIO) was used as an NO scavenger. The NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) was also used. Both carboxy-PTIO and SNAP were from Molecular Probes (Eugene, Ore., USA).

De-etiolation. Wheat seeds (*Triticum aestivum* L. cv. Buck Patacon) were sown under green light in pots containing a sterile mixture of soil:vermiculite (3:1), and placed at 18 °C in the dark. They were watered every 2 d under green light until emergence. For the rest of the experiment, seedlings were sprayed under green light with 100 μM SNP or with H_2O or 100 μM $NaNO_2$ and 100 μM $NaNO_3$ (controls). Some pots were kept in continuous darkness, while others received a 2-h-pulse of white light [photosynthetically active radiation (PAR) = 200 μmol photons s^{-1} m^{-2}] on the seventh day. For the analysis of wounding effect, seedlings were grown for 7 d in darkness, leaves were wounded above the meristematic region under green light (5 μmol m^{-1} s^{-2}) and seedlings kept in continuous darkness for an additional 3 d. Other seedlings were wounded as described, given a 2-h-pulse of white light, and kept for three additional days in the dark. Some seedlings received one 2-h-pulse of white light per day for 7 d, were wounded as described above and kept for three additional days under the same dark/light cycle. Seedlings that received the same pulse without wounding were also analyzed. To study the effects of biotic stress on de-etiolation, 5-d-old seedlings were sprayed with a suspension of *Septoria tritici* zoospores (10^5 zoospore ml^{-1}) under green light and kept for five additional days in continuous darkness. After that, one set of seedlings was subjected to wounding as described and grown for three additional days in the dark. Unless specified, chlorophyll content was determined on 10-d-old plants. Chlorophyll was extracted in 80% acetone and quantified considering that 1 $Abs_{652\ nm}$ = 27.7 μg chlorophyll ml^{-1}. At least three replicates were done for each condition.

Hypocotyl and internode elongation. Lettuce (*Lactuca sativa* L. cv. Grand Rapids) seeds were germinated in Petri dishes as described above at 20 °C in the dark. To avoid NO effects on germination, all seeds were first imbibed in H_2O for 24 h. They were then transferred under green light to three different dishes containing filter paper imbibed in 100 μM SNP, H_2O or 100 μM $NaNO_2$/100 μM $NaNO_3$ and kept for three additional days at 20 °C in the dark. Three replicates, each consisting of 50 seeds per condition, were used for determination of hypocotyl length.

Arabidopsis thaliana (L.) Heynh. (ecotypes Columbia and Landsberg *erecta*) seeds were imbibed in H_2O under green light as described and kept for 5 d at 4 °C in the dark. Germination was then promoted by giving a 30-min pulse of red light (20 μmol photons s^{-1} m^{-2}). After 24 h at 25 °C in the dark, seeds were transferred under green light to dishes with filter paper imbibed in 100 μM SNP, H_2O or 100 μM $NaNO_2$/100 μM $NaNO_3$ and kept in the dark. Hypocotyl length was measured 3 d after treatments.

Potato *Solanum tuberosum* (L. cv. Pampeana) tubers were sown in pots containing a sterile mixture of soil:vermiculite (3:1, v:v) at a 3-cm depth from the soil surface and kept at 25 °C under low-intensity white light (PAR = 5 μmol photons s^{-1} m^{-2}). They were watered for 5 d until buds emerged over the surface. Then, the pots were divided into three groups that were sprayed with either 100 μM SNP, H_2O or 100 μM $NaNO_2$/100 μM $NaNO_3$ three times a week. Twenty days after beginning the experiment, internode lengths were measured. Average values of a total of 50 internodes per condition were determined.

Results

Nitric oxide induces seed germination. At temperatures above 25 °C, germination of Grand Rapids lettuce seed occurs only in the light, becoming a phytochrome-dependent process (Bewley and Black 1982). To test whether NO could promote germination in the dark, lettuce seeds were imbibed in 100 μM SNP, an NO donor, and kept at 26 °C under complete darkness. Control lettuce seeds were imbibed in H_2O or in 100 μM $NaNO_2$ and 100 μM $NaNO_3$. Nitrite and NO_3^- are normal products of NO decomposition. We had previously demonstrated that 100 μM SNP was effective in maintaining chlorophyll levels in potato leaves infected by *Phytophthora infestans* or treated with reactive oxygen species (Laxalt et al. 1997; Beligni and Lamattina 1999a). Nanomolar amounts of NO are released from an aqueous solution of 100 μM SNP (Poderoso J., Buenos Aires University, personal communication). Almost all seeds from a total of 50 per experiment had germinated in NO treatments by 48 h after imbibition (Fig. 1a, Table 1). In contrast, neither H_2O- nor NO_3^-/NO_2^--imbibed lettuce seeds germinated during the whole experimental period (Fig. 1a). When analysing the time course of germination, visible radicle emergence was initiated between 12 and 24 h after imbibition in

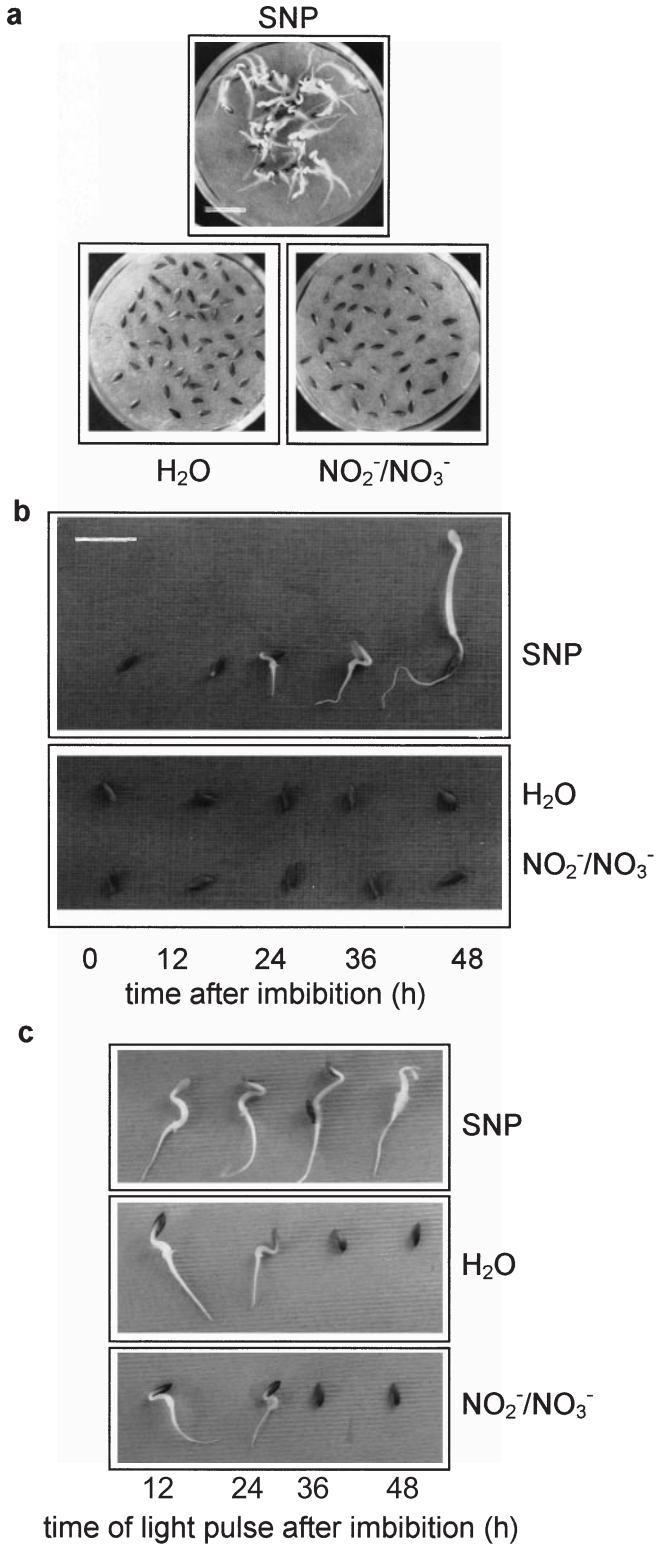


Fig. 1a–c. Nitric-oxide-stimulated germination of lettuce seeds. Grand Rapids lettuce seeds were imbibed in 100 μM SNP at 26 °C in the dark. Control seeds were imbibed in H_2O or in $NaNO_2/NaNO_3$ (100 μM each). **a** Photograph corresponding to 48 h after imbibition. **b** One representative seed was chosen to illustrate the kinetics of seed germination in SNP, H_2O and NO_2^-/NO_3^- treatments at 12, 24, 36 and 48 h after imbibition. **c** Other sets of samples were given 5-min pulses of white light at 12, 24 or 36 h after imbibition before returning to continuous darkness. Samples were photographed 48 h after imbibition. The kinetics of seed germination are depicted. Three independent experiments, each consisting of 50 seeds per sample, were performed. Bar = 1 cm

NO-treated lettuce seeds, whereas the hypocotyl started to become visible 36 h after imbibition (Fig. 1b). Twenty- to 30-mm seedlings were observed 48 h after imbibition. As was expected, germination was not observed either in control seeds analysed at 12, 24, 36 and 48 h after imbibition (Fig. 1b), or when an NO scavenger (carboxy-PTIO) was added together with 100 μM SNP (Table 1). On the other hand, germination occurred when lettuce seeds were imbibed in 100 μM SNAP, another NO donor (Table 1).

As light at low fluences is able to promote germination, we analyzed the effect of a light pulse on the germination of lettuce seeds kept at 26 °C in the dark. Figure 1c shows the effect of 5-min pulses of white light given at 12, 24 and 36 h after imbibition (Fig. 1c, 12, 24 and 36, respectively) and scored for radicle emergence at 48 h post imbibition. Figure 1c shows that the earlier the pulse was given, the longer the seedlings were at 48 h, indicating that germination was effectively stimulated by the light pulse in the absence of NO. In contrast, germination was independent of light in NO-imbibed seeds. The same results were obtained when red light (20 μmol s^{-1} m^{-2}) was used instead of white light (not shown).

As expected (Bewley and Black 1982), germination occurred in control, SNP and SNP+ scavenger treatments at temperatures below 25 °C, both in the dark and in the light (data not shown), showing the temperature-dependence of the process. On the other hand, no germination occurred at 34 °C, the temperature at which seeds suffer secondary dormancy not revertible by light (Bewley and Black 1982).

In experiments performed under the same conditions (26 °C and darkness) 100 μM gibberellic acid (GA_3) was able to induce lettuce germination similarly to 100 μM SNP. However, dormancy could not be broken by 10 μM GA_3 (not shown), whereas 50% germination occurred with 10 μM SNP (Table 1).

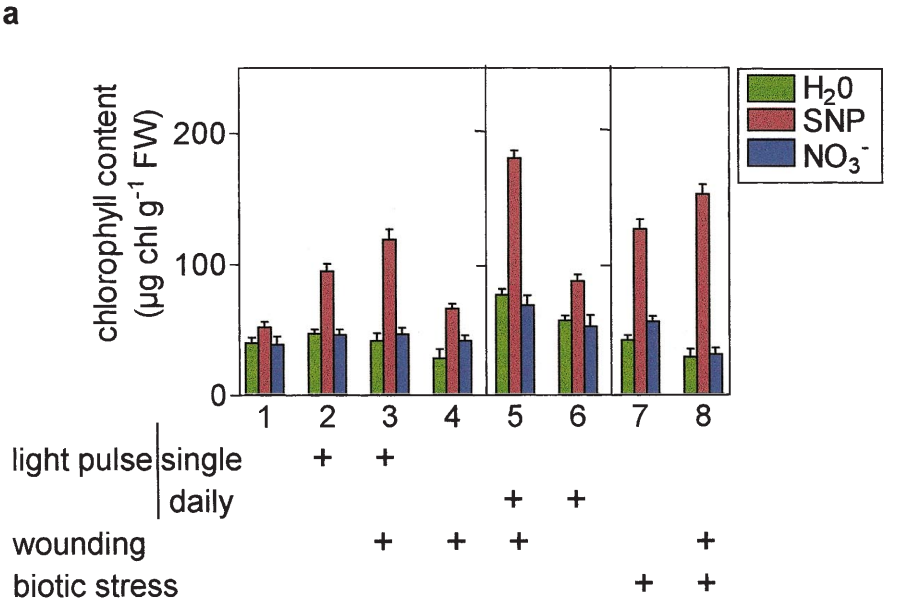
Table 1. Nitric oxide stimulates light-dependent germination of lettuce seeds in the dark[a]

Treatment	% germination[b]
100 μM SNP	98.2 ± 2.4
10 μM SNP	50.1 ± 4.3
100 μM SNP + 100 μM carboxy-PTIO	3.6 ± 1.8
100 μM SNAP	96.7 ± 2.3
100 μM SNAP + 100 μM carboxy-PTIO	4.4 ± 2.1

[a]The experiment was performed at 26 °C, at which germination of lettuce seeds is dependent on light
[b]The germination percentages are the mean values ± SD of three independent experiments consisting of 50 seeds per replicate

Nitric oxide allows partial de-etiolation. In the dark, or even under short-day photoperiods, wheat seedlings become etiolated. To unravel whether NO was able to prevent etiolation, wheat seedlings (cv. Buck Patacon) were sprayed with 100 μM SNP at 18 °C in the dark (see *Materials and methods*). Control seedlings were sprayed with H_2O or with $NaNO_2/NaNO_3$ (100 μM each). Figure 2a shows that the chlorophyll content was 30% higher in NO-treated seedlings than in control ones (Fig. 2a, 1). Nitric-oxide-stimulated wheat de-etiolation was also dose-dependent. An SNP concentration of 1 μM was not effective in stimulating greening, whereas 10 and 500 μM SNP were as effective as 100 μM SNP (not shown). A 2-h pulse of white light (200 μmol photons s^{-1} m^{-2}) produced a 2-fold increase in the chlorophyll content of NO-treated seedlings. In contrast, a very little increase was observed in control treatments (Fig. 2a, 2).

We had previously demonstrated that NO protects chlorophyll levels in stressed potato leaves (Laxalt et al. 1997; Beligni and Lamattina 1999a). This gave us the idea that NO-mediated partial greening could be enhanced upon abiotic or biotic stress. For that purpose, wheat seedlings were grown in the dark, subjected to wounding under green light (see *Materials and methods*) and returned to darkness. Other pots were given a



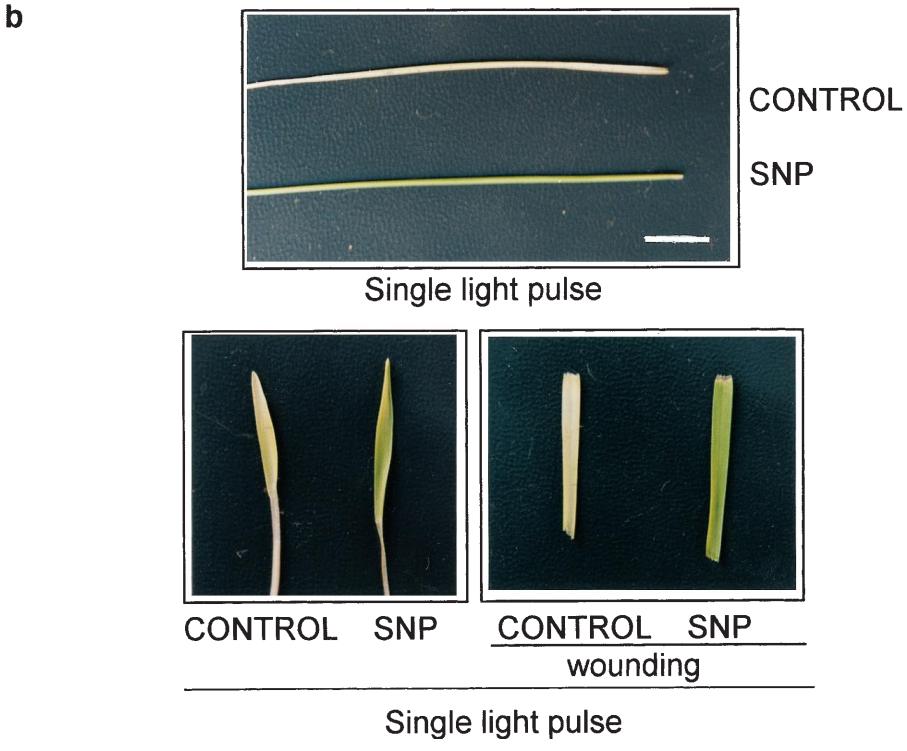


Fig. 2a,b. Nitric-oxide-mediated greening of etiolated wheat seedlings. Wheat seedlings were grown at 18 °C in the dark and sprayed with 100 μM SNP, H_2O (control) or $NaNO_2/NaNO_3$ (control). **a** Determination of chlorophyll content. Some pots were kept in darkness (*1*), while others received a 2-h pulse of white light (*2*). Alternatively, seedlings were grown for 7 d under darkness, when leaves were wounded (see *Materials and methods*). Then, they received a 2-h pulse of white light and were kept for three further days in the dark (*3*). Other seedlings were wounded and kept in continuous darkness (*4*). Some seedlings received one 2-h pulse of white light per day for 7 d, wounded and kept for three further days under the same dark/light cycle (*5*). Other seedlings received the same pulse without wounding (*6*). Three-day-old seedlings were sprayed with *Septoria tritici* and kept for 5 d in darkness (*7*). After that, seedlings were subjected to wounding and grown for three further days in the dark (*8*). With the exception of treatment 8, all chlorophyll determinations were made on 10-d-old plants. The plants in treatment 8 are 13 d old. Bars indicate mean values of at least three independent experiments ± SD. **b** Pictures showing NO-mediated greening: upper and lower-left panels correspond to 2nd and 1st leaves, respectively, of set 2 in Fig. 2a; lower-right panel corresponds to 1st leaf of set 3. Bar = 1 cm

2-h pulse of white light (PAR = 200 μmol photons s^{-1} m^{-2}) immediately after wounding and returned to darkness. Figure 2a shows that a single light pulse plus wounding produced a 3-fold increase in NO-mediated de-etiolation compared with control treatments (Fig. 2a, 3), whereas wounding alone produced a 1.6- to 2.3-fold increase (Fig. 2a, 4). Figure 2b shows a representative wheat leaf subjected to wounding plus the 2-h light pulse (Fig. 2a, 3), as well as one subjected only to the light pulse (Fig. 2a, 2). When daily light pulses were given for 7 d before wounding (see *Materials and methods*), the amount of chlorophyll in NO-treated leaves increased 2.5-fold (net) (Fig. 2a, 5). In turn, NO-treated seedlings had 70% more chlorophyll than control ones after 10 d of daily pulses without wounding (Fig. 2a, 6). Other light pulses (between 5 and 90 min) produced similar results, but longer pulses greened control seedlings to a similar extent as NO-treated ones (not shown).

To study the effect of biotic stress on greening, wheat seedlings grown in the dark were sprayed with a suspension of *Septoria tritici* zoospores. Figure 2a shows that the chlorophyll content was 2.4- to 3-fold higher in NO-treated seedlings (Fig. 2a, 7). Stressed leaves were then wounded and kept in the dark. At the end of the whole treatment, chlorophyll levels were 5-fold higher in NO treatments than in control ones (Fig. 2a, 8). The growth of *S. tritici* on malt agar was not affected by 100 μM SNP (not shown).

Nitric oxide diminishes hypocotyl and internode elongation. To test whether NO was able to inhibit hypocotyl and internode elongation, three different plant systems were analyzed. Lettuce seeds (cv. Grand Rapids) were allowed to germinate in the dark (see *Materials and methods*). Germinated seeds were then transferred to dishes with 100 μM SNP, H_2O or $NaNO_2/NaNO_3$ (100 μM each) and kept in the dark. Table 2 shows that, after 3 d, hypocotyls were 20% shorter in NO-treated seedlings than in control ones. The differences were statistically significant, as determined by one-way analysis of variance (ANOVA) and Tukey-Kramer Multiple Comparison Test ($n = 150$, $P < 0.01$).

In the second plant system, *Arabidopsis thaliana* seeds (ecotypes Columbia and Landsberg *erecta*) were allowed to germinate in the dark (see *Materials and methods*). Seeds were then transferred to 100 μM SNP, H_2O or 100 μM $NaNO_2$/100 μM $NaNO_3$ and kept in complete darkness. Table 2 shows that, after 3 d, hypocotyls of Columbia seedlings were 22% shorter in NO treatments than in controls, tested again by one-way ANOVA and Tukey-Kramer Multiple Comparison Test ($n = 150$, $P < 0.001$). In contrast, there were no differences between the three treatments for the Landsberg *erecta* ecotype (Table 2, ANOVA and Tukey Test; $n = 150$, $P > 0.05$).

In a third experimental system, potato (cv. Pampeana) tubers were allowed to sprout under low-intensity white light (5 μmol photons s^{-1} m^{-2}). Three different groups were sprayed with either 100 μM SNP, H_2O or $NaNO_2/NaNO_3$ (100 μM each). Table 2 shows that, after 20 d, average values of internode lengths were 23% lower in NO-treated plants than in control ones, from a total of 50 internodes per condition (ANOVA and Tukey Test; $n = 50$, $P < 0.01$).

Discussion

In this work, we provide evidence for an involvement of NO in light responses. It stimulated the germination of lettuce seeds in light-dependent situations, being even more potent than GA_3. However, it is difficult to assess whether NO acts as an artificial inducer of germination or if it is an endogenous one. As H_2O-treated seeds do not germinate in the dark above 25 °C, we suppose that endogenous NO is not present in a sufficient amount to promote germination. Moreover, seeds germinated in the light even in the presence of carboxy-PTIO, an NO scavenger (not shown). This suggests that other components provided by the light shoot germination.

Gibberellins are very efficient inducers of germination. Some phytochrome-dependent processes are thought to act via increases in GA levels or sensitivity (Lee et al. 1998). Thus, GA is expected to break dormancy in the dark whenever germination is dependent

Table 2. Effects of NO on hypocotyl lengths of *Arabidopsis* and lettuce seedlings, and internode lengths of potato plants

	H_2O	SNP	NO_3^-/NO_2^-	Light condition
Arabidopsis, Columbia[a]	9.1 ± 1.1 (100)	7.1 ± 1.2* (78)	9.2 ± 0.9 (101)	dark
Arabidopsis, Landsberg *erecta*[a]	4.6 ± 1.2 (100)	4.3 ± 0.9 (96)	4.5 ± 1.3 (98)	dark
Lettuce cv. Grand Rapids[a]	22.1 ± 5.1 (100)	17.8 ± 3.8** (80)	21.0 ± 4.4 (95)	dark
Potato cv. Pampeana[b]	54.0 ± 9.2 (100)	41.8 ± 8.9** (77)	55.2 ± 8.7 (102)	5 μmol s^{-1} m^{-2}

[a]Data are the means ± SD of 150 seedlings per condition
[b]Data are the means ± SD of 50 internode lengths per condition
Data between parentheses are the percentages of values corresponding to H_2O-treated plants. Asterisks indicate the significance of differences between NO treatments and controls (H_2O and NO_3^-/NO_2^-) as determined by one-way ANOVA and Tukey multiple comparison tests (*$P < 0.001$; **$P < 0.01$)

on phytochrome. In turn, when dormancy is imposed even in the light (i.e. above 34 °C), GA would not probably promote germination. The same conclusions may be drawn for NO as a stimulator of light responses. However, whether GA and NO act through the same or different pathways, cannot be determined from our results. Germination stimulated by NO independently of phytochrome cannot be ruled out either. In seeds, phytochrome is basically a light detector, enabling germination when seeds are near the soil surface (Bewley and Black 1982). From this standpoint, NO emission within the soil could be of relevance in promoting germination in deeper soil layers, where light fluences are too low to allow photoperception.

In another report, dormant seeds of *Emmenanthe penduliflora* were induced to germinate with nitrogen dioxide (NO_2) (Keeley and Fotheringham 1997), a product of NO oxidation. We did not measure whether some NO_2 was formed in our experiments, but we can speculate that lettuce seed germination did not occur due to NO_2 formation. First, the reaction of NO with O_2 to give NO_2 is a slow process that critically depends on the initial concentration of NO. Because of the short biological half-life of NO, some of its other biological reactions become more important (Stamler et al. 1992). Second, the reaction of NO with carboxy-PTIO generates NO_2 (Akaike and Maeda 1996). In our experiments, carboxy-PTIO did not promote germination in combination with NO (Table 1).

Our results also suggest that NO participates in bringing about partial greening of etiolated wheat seedlings. Chory et al. (1994) reported cytokinin-mediated chlorophyll protection in *Arabidopsis* leaves subjected to dark conditions, raising again the question of whether light and plant hormones act independently or not, as well as the possibility that NO could also have hormonal properties in plants. The NO-mediated partial greening is enhanced by light pulses, wounding and biotic stress. The involvement of calmodulin and cGMP during both light and defense responses in plants, and their participation in NO-mediated events are possible explanations for the synergistic effect of NO and stress on greening experiments (Bowler et al. 1994; Pfeiffer et al, 1995; Durner et al. 1998; Stankovic and Davies 1998).

Another light response stimulated by NO was the inhibition of hypocotyl and internode elongation. In this case, the different results obtained with the two *Arabidopsis* ecotypes (see Table 2) could be of benefit to further investigations on the locus/loci involved in plant responsiveness to NO. A series of polymorphic molecular markers have been mapped on some recombinant inbred lines generated from a cross between Columbia and Landsberg *erecta* ecotypes. With this tool, hypocotyl elongation response to NO could be analyzed and its segregation compared with that of the molecular markers.

General remarks. With each investigation, NO and its chemical reactions within biological systems acquire more significance. Although the most studied mechanism for NO production in animals involves NO synthase, there are other ways of generating it in vivo (Schmidt et al. 1996; Modolell et al. 1997). In plants, no NO synthase has yet been isolated and there is some controversy about the source of NO biosynthesis (Beligni and Lamattina 1999b; Yamasaki et al. 1999). However, many reports provide firm evidence in favor of the presence of NO in plants and its emergent physiological roles. It has been proven to inhibit foliage expansion, protect plants from oxidative stress and take part in plant defense mechanisms (Leshesm and Haramaty 1996; Noritake et al. 1996; Beligni et al. 1997; Laxalt et al. 1997; Delledonne et al. 1998; Durner et al. 1998; Beligni and Lamattina 1999a). Given that NO and phytochrome effects are accomplished via heterotrimeric G proteins, cGMP, Ca^{2+} and calmodulin, it would not be surprising that they could share a common transduction pathway in plants in response to light. It would be interesting in the future to study the possible role of NO in other plant responses to light, such as flowering, circadian rhythmicity and developmentally regulated gene expression.

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