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SHORT COMMUNICATION

Stimulatory and period-specific effect of nitric oxide on in vitro caulogenesis in *Albizzia lebbeck* (L.) Benth.

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Abstract The paper reports stimulatory effect of nitric oxide (NO) on in vitro caulogenesis in *Albizzia lebbeck*, a tree legume. Exogenously supplied NO donor, sodium nitroprusside (SNP) stimulated shoot differentiation from hypocotyl explants of *Albizzia lebbeck,* excised from its in vitro seedlings. Potassium ferrocyanide, a structural analog of SNP incapable of releasing NO, did not promote shoot organogenesis. Likewise, metabolic products of NO, NO_2^- and NO_3^-, provided as $NaNO_2$ and $NaNO_3$ did not enhance shoot differentiation. The NO scavenger, 2-(4-carboxy-phenyl)-4, 4, 5, 5-tetramethylimideazoline-1-oxyl-3-oxide (cPTIO), supplemented along with SNP, at equimolar concentration, reversed the stimulatory effect of the latter, thus, confirming the role of NO in promotion of in vitro caulogenesis. The transfer of explants cultured on the basal medium (BM) to the same containing SNP and vice versa after different time intervals revealed that for its enhancing effect, SNP was required only during the initial phase (5 days) of culture. Its presence or administration beyond 5 days neither promoted nor inhibited the caulogenic response.

Keywords *Albizzia* · In vitro caulogenesis · Nitric oxide · NO donors and scavengers · Stimulation

Abbreviations

cPTIO	2-(4-carboxy-phenyl)-4, 4, 5, 5 Tetramethylimideazoline-1-oxyl-3-oxide
MB	Methylene blue
NO	Nitric oxide
SNP	Sodium nitroprusside
SPSS	Statistical package for social sciences

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Introduction

In 1987, a new gaseous signaling molecule was introduced to the biological world when endothelium derived relaxation factor (EDRF) in mammals was identified as nitric oxide (NO), a gas until than considered obnoxious (Ignarro et al. 1987). NO is a small, highly diffusible and ubiquitous bioactive molecule. Its chemical properties make NO a versatile signaling molecule that functions through interactions with cellular targets via either redox or additive chemistry (Stamler et al. 1992). It plays important role in a number of biological processes in both plants and animals (Wilson et al. 2008). In mammals, it is involved in the central nervous, cardiovascular and immune systems; platelet inhibition, programmed cell death and host responses to infection (Crawford 2006). It has turned out to be an important regulatory molecule in growth and development of plants and their responses to biotic and abiotic stresses (Delledonne 2005; Arasimowicz and Floryszak-Wieczorek 2007). Its involvement has been shown in seed germination, hypocotyl elongation (Beligni and Lamatina 2000), stomatal movements (Desikan et al. 2004; Distéfano et al. 2008), flowering (He et al. 2004; Khurana et al. 2011), xylem differentiation (Gabaldón et al. 2005), senescence (Leshem and Haramaty 1996), and rhizogenesis (Pagnussat et al. 2002; Lanteri et al. 2008). Recently, we have reported the promotory effect of NO on in vitro caulogenesis in *Linum usitatissimum*, an herbaceous plant (Kalra and Babbar 2010). The present work reports

stimulatory effect of NO on in vitro caulogenic response of *Albizzia lebbeck*, a leguminous tree. The hypocotyl explants of this plant have been well characterized for their morphogenic potential (Gharyal and Maheshwari 1981, 1982, 1990). Because of the ease to regenerate shoots on basal media, *A. lebbeck* hypocotyls explants have been used as experimental material for various in vitro morphogenic studies (Baweja et al. 1995; Khurana and Khurana 2000; Jain and Babbar 2002, 2006).

Materials and methods

The seeds of *Albizzia lebbeck* were procured from Pratap Nursery and Seed Stores, Dehradun, India. After surface sterilization with 0.1% (w/v) mercuric chloride for 7–8 min followed by thorough rinsing with sterile distilled water, these were implanted on to the B5 basal medium (BM; Gamborg et al. 1968), supplemented with 3% sucrose and gelled with 0.8% bacteriological grade agar (Qualigens Fine Chemicals, Mumbai, India). 1 cm long hypocotyl segments from 11-day-old in vitro seedlings, having 5.5–6.0 cm long hypocotyls, were used as the explants. BM was supplemented with different concentrations of freshly prepared NO donor, sodium nitroprusside (SNP, alone or along with NO scavengers, 2-(4-carboxy-phenyl)-4, 4, 5, 5-tetramethylimideazoline-1-oxyl-3-oxide (cPTIO) or methylene blue (MB) at concentrations equimolar to the optimal concentration of SNP. Potassium ferrocyanide $\{K_4[Fe(CN)_6]\}$, sodium nitrite ($NaNO_{2)}$ or sodium nitrate ($NaNO_3$) were supplemented to the BM individually at 4 μM. Thermolabile chemicals {SNP, cPTIO, $K_4[Fe(CN)_6]$ and MB}were filter sterilized using Millipore filters of 0.45 μm pore size (Millipore, Bangalore, India) and added to the autoclaved medium. Subsequent to adjustment of pH to 5.8, autoclaving was done at 1.06 kg cm^{-2} at 121°C for 15 min. The media were dispensed in glass test tubes (25 mm × 150 mm, Borosil India), each containing 20 ml of medium. These were closed with cotton plugs (non-adsorbent cotton covered with two layers of cheese cloth) without any sealant. Two explants were cultured in each culture tube. All cultures were exposed to 16 h photoperiod of 18 μE m^{-2} s^{-1} provided by 40 W cool daylight fluorescent tubes, and incubated at 25 ± 2°C. After 45 days, the cultures were scored for the percentage of responding explants, number of shoots per responding explant and the average length of shoots. All experiments were repeated at least twice with 30 explants per treatment each time.

To find if any morphogenic gradient existed along the *Albizzia* hypocotyls, in the very first experiment, hypocotyl segments were segregated based on their position on the seedlings. These segments, designated as I to V based on their proximity to the root-shoot junction of the seedling, were cultured on BM. To arrive at the concentration of SNP optimal for shoot differentiation, effect of its three concentration ranges (0–40, 0–10 and 0–5 μM) was studied in three independent experiments. To know whether for its promotory effect, SNP is required during the entire culture duration (45 days) or only during specific period(s) and if latter is the situation, does it influence the response adversely for the remaining period, the explants cultured on BM or BM + SNP (4 μM) for different periods (5, 10, 15 and 20 days) were transferred to the other medium for rest of the culture period (45 days).

The data were subjected to one-way analysis of variance (ANOVA, $p \leq 0.05$) using SPSS version 10 to test the significance of the observed differences and comparisons between the mean values of treatments were made by Post Hoc Tukey HSD (Honestly Significant Different) test at $p \leq 0.05$.

Results and discussion

Before initiating the experiments to study the possible role of NO in in vitro morphogenesis, it was considered necessary to ascertain the absence of any inherent variability among the selected explants. Earlier, differential morphogenic responses by the segments of the same organ have been reported. For example, hypocotyl segments of *Santalum album* and *Solanum melongena* proximal to the root-shoot junction exhibited better caulogenic response than the distal ones (Bapat and Rao 1984; Sharma and Rajam 1995). Contrary to this, hypocotyl segments from different positions along the seedling of *Liquidambar stryaciflua* did not exhibit any significant difference in adventitious shoot differentiation (Kim et al. 1997). In the present study too, no significant difference in the caulogenic response of different hypocotyl segments, in terms of percentage of responding explants, average number of shoots per responding explant and growth of shoots as evidenced by their length was recorded (data not presented). This ruled out the existence of any morphogenic gradient in the hypocotyls of *Albizzia*. Therefore, for further experiments, hypocotyl segments were randomly mixed and distributed among different treatments.

Pharmacological studies using NO donors and scavengers have generally been used to gain insight in the role of NO in plant growth and development (Besson-Bard et al. 2008). NO donors, well suited to mimic a NO response (Floryszak-Wieczorek et al. 2006; Ederli et al. 2009), are routinely used for physiological studies as treatment of biological samples directly with NO gas is difficult due to its short half-life in vivo and difficulty in maintaining a steady-state concentration of exogenously supplied NO in an experimental system (Delledonne et al. 1998, 2001;

Clarke et al. 2000; Neill et al. 2003; Miller and Megson 2007). Commonly used NO donors are SNP (sodium nitroprusside) GSNO (S-nitrosoglutathione), SNAP (S-nitroso-N-acetylpenicillamine), RBS (Roussin's Black Salts), NOR-3 (+/−)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (Neill et al. 2003). In the present study, SNP was used as a NO donor. In the initial experiment conducted to study the effect of 0–40 μM SNP on the caulogenic response of the hypocotyl explants, the lowest concentration (5 μM), did not significantly affect the caulogenic response in terms of percentage of caulogenic explants, average number of shoots per responding explants and average shoot length. At 10 μM, average number of shoots per responding explant was significantly reduced, while the other two parameters were statistically comparable to the control. However, 20 and 40 μM SNP totally inhibited the response (data not shown). When a narrow range (0–10 μM) of SNP with increments of 2 μM was used, 4 μM SNP significantly promoted the average number of shoots per explant. Though, at the higher levels (6–10 μM), average number of shoots gradually declined, the values were not significantly different from that of the control cultures. The percentages of responding explants and the average shoot length among these treatments were not significantly different (data not presented). Further narrowing of the range of concentrations of SNP to 1–5 μM, with increment of 1 μM, revealed the stimulatory effect of 3 and 4 μM on the caulogenic response. The enhancement was only in terms of the number of shoots per responding explants, with 4 μM being the optimum (Table 1) as was also observed with explants of *Linum usitatissimum* (Kalra and Babbar 2010).

Although B5 basal medium, used in the present study, contains about 27 mM of nitrate in the form of ammonium and potassium nitrate, the observed stimulatory effect of NO donor could have been due to a negligible increase in the concentrations NO_2^- and NO_3^-, two stable metabolites of NO (Ullrich et al. 1997). This remote possibility was ruled out as neither of $NaNO_2$ and $NaNO_3$, provided individually at 4 μM, had any effect on the caulogenic response (Table 2).

SNP, along with NO, generates cyanide (Meeussen et al. 1992; Yamamoto and Bing 2000; Butler and Megson 2002; Murgia et al. 2004; Bethke et al. 2006a, b; Manjunatha et al. 2008) as the first volatile breakdown product. Cyanide, also a small gaseous molecule, has been extensively studied to reveal its role in plant growth, metabolism and development. It acts as a protective molecule against herbivores (Nahrstedt 1985; Zagrobelny et al. 2004). It plays regulatory role in seed germination, nitrate assimilation, sugar and lipid metabolism and in different plant responses to some environmental stimuli (Solomonson and Barber 1990; Bogatek et al. 1991, 1999; Bethke et al. 2006a, b). It also interplays with ethylene signaling (Grossmann 1996;

Table 1 Caulogenic response of hypocotyl explants of *A. lebbeck* cultured on BM supplemented with different concentrations of SNP (1–5 μM) for 45 days

SNP (μM)	Total no. of explants	% Caulogenic explants	Av. no. of shoots per responding explant	Av. shoot length (cm)
0	92	81.5[a]	4.21[c]	1.03[a] ± 0.18*
1	92	82.6[a]	4.54[bc]	1.08[a] ± 0.20
2	90	82.2[a]	4.36[bc]	1.13[a] ± 0.16
3	90	82.2[a]	5.17[ab]	1.00[a] ± 0.29
4	90	84.4[a]	6.02[a]	1.47[a] ± 0.23
5	92	85.0[a]	4.74b[c]	0.98[a] ± 0.14

* Mean ± standard error. Values followed by the same letter in a column are not significantly different ($p \leq 0.05$)

Table 2 Caulogenic response of hypocotyl explants of *A. lebbeck* cultured on BM supplemented with 4 μM of SNP, $NaNO_2$, $NaNO_3$ or potassium ferrocyanide $\{K_4[Fe(CN)_6]\}$ for 45 days

Treatment	Total no. of explants	% Caulogenic explants	Av. no. of shoots per responding explant	Av. shoot length (cm)
BM	96	82.3[a]	4.15[b]	1.02[a] ± 0.36*
SNP	90	82.0[a]	5.93[a]	1.56[a] ± 0.24
$NaNO_2$	96	81.2[a]	4.64[b]	0.95[a] ± 0.11
$NaNO_3$	96	80.0[a]	3.81[b]	0.98[a] ± 0.18
$K_4[Fe(CN)_6]$	92	76.0[a]	4.52[b]	1.07[a] ± 0.29

* Mean ± standard error. Values followed by the same letter in a column are not significantly different ($p \leq 0.05$)

Smith and Arteca 2000) and is mostly correlated with the inhibition of the terminal cytochrome oxidase in mitochondria (Siegień and Bogatek 2006). Therefore, to rule out the possibility of the observed effect of SNP due to cyanide, effect of 4 μM K_4 [$Fe(CN)_6$] (a structural analog of SNP that releases cyanide but does not possess NO moiety, was also studied. $K_4[Fe(CN)_6]$ had no effect on the caulogenic response (Table 2), thus ruling out the role of CN^- in observed promotion of caulogenic response due to SNP.

To further ascertain that the observed promotion in the caulogenic response was due to NO released by its donor, specific NO scavenger, cPTIO (Goldstein et al. 2003; Beligni and Lamattina 2000; París et al. 2007), was added alone or along with SNP. cPTIO reacts stoichiometrically with NO (Kasprowicz et al. 2009) and is known to be specific for NO scavenging (Cragon 1999). The NO scavenger added along with the donor brought down the average number of shoots per responding explant statistically equivalent to the control level (Table 3). cPTIO, when added alone, did not affect the caulogenic response. This observation seemingly suggests that there is no role of endogenous NO in in vitro caulogenesis of *A. lebbeck,* similar to what was observed with the caulogenic response of *Linum* (Kalra and Babbar 2010).

The results of the experiment involving transfer of the explants from BM to BM + SNP and vice-versa revealed that (a) SNP stimulated caulogenesis even if present only during the initial phase (5 days) of culture, (b) continued presence of SNP for period beyond 5 days neither promoted nor inhibited the response and (c) SNP if provided after initial 5 days of culture had no effect on the caulogenic response (Table 4). SNP is known to release NO for about 12 h (Floryszak-Wieczorek et al. 2006). Therefore, by extrapolation one can say that NO stimulated the caulogenesis by acting during the first 12 h of culture, during which determination and differentiation leading to shoot differentiation at cell level might have taken place. Moreover, no response of SNP beyond 5 days is quite expected as by this time SNP is completely spent as far its capability of releasing NO is concerned. As administration of SNP after 5 or more days of culture did not have affect caulogenic response, it can be concluded that NO influences only the initial stages of shoot differentiation and development.

To conclude, the present paper on *A. lebbeck*, a tree legume, affirms the role of NO in in vitro caulogenesis

Table 3 Caulogenic response of hypocotyl explants of *A. lebbeck* cultured on BM supplemented with cPTIO (4 μM) alone or in combination with SNP (4 μM) for 45 days

Treatment (μM)	Total no. of explants	% Caulogenic explants	Av. no. of shoots per responding explant	Av. shoot length (cm)
BM	92	73.8[a]	3.95[b]	1.06[a] ± 0.25*
SNP	90	79.9[a]	5.84[a]	1.42[a] ± 0.34
SNP + cPTIO	90	75.4[a]	4.57[b]	1.01[a] ± 0.22
cPTIO	92	76.0[a]	3.85[b]	1.04[a] ± 0.16

*Mean ± standard error. Values followed by the same letter in a column are not significantly different ($p \leq 0.05$)

Table 4 Caulogenic response of hypocotyl explants of *A. lebbeck* cultured on BM or BM + SNP (4 μM) for 45 days and those cultured on either of these media for 5, 10,.15, 20 days before being transferred to the other medium for rest of the culture period of 45 days

Treatment (μM)	Total no. of explants	% Caulogenic explants	Av. no. of shoots per responding explant	Av. shoot length (cm)
BM (45 days)	94	89.1[a]	4.00[b]	1.00[b] ± 0.11*
SNP (45 days)	90	91.1[a]	5.62[a]	1.51[a] ± 0.31
BM (5 days) → SNP (40 days)	94	84.0[a]	3.98[b]	0.99[b] ± 0.14
SNP (5 days) → BM (40 days)	92	91.3[a]	5.87[a]	1.49[a] ± 0.33
BM (10 days) → SNP (35 days)	92	89.1a	4.27[b]	1.07[b] ± 0.12
SNP (10 days) → BM (35 days)	94	85.1[a]	6.29[a]	1.57[a] ± 0.39
BM (15 days) → SNP (30 days)	90	90.0[a]	3.81[b]	0.96[b] ± 0.24
SNP (15 days) → BM (30 days)	94	89.3[a]	5.58[a]	1.44[a] ± 0.38
BM (20 days) → SNP (25 days)	94	85.1[a]	4.10[b]	1.09[a] ± 0.17
SNP (20 days) → BM (25 days)	92	83.6[a]	5.94[a]	1.61[a] ± 0.28

* Mean ± standard error. Values followed by the same letter in a column are not significantly different ($p \leq 0.05$)

reported recently in *Linum usitatissimum*, an herbaceous plant (Kalra and Babbar 2010). This extends the versatility of NO molecule by confirming its effect on caulogenesis of two taxonomically divergent taxa. The ease with which *A. lebbeck* hypocotyls explants develop shoots on various basal media makes them a good experimental material for further biochemical and molecular analyses for the elucidation of signaling cascade downstream NO leading to its stimulatory effect on caulogenesis.

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References

Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signaling molecule in plant stress responses. Plant Sci 172:876–887

Bapat VA, Rao PS (1984) Regulatory factors for in vitro multiplication of sandalwood tree (*Santalum album* Linn.). I. Shoot bud regeneration and somatic embryogenesis in hypocotyl cultures. Proc Indian Acad Sci 93:19–27

Baweja K, Khurana JP, Gharyal-Khurana P (1995) Influence of light on somatic embryogenesis in hypocotyls of *Albizzia lebbeck*. Curr Sci 68:544–546

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210:215–221

Besson-Bard A, Courtois C, Gauthier A, Dahan J, Dobrowolska G, Jeandroz S, Pugin A, Wendehenne D (2008) Nitric oxide in plants: production and cross-talk with Ca^{2+} signaling. Mol Plant 1:218–228

Bethke PC, Libourel IG, Reinohl V, Jones RL (2006a) Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. Planta 223:805–812

Bethke PC, Libourel IG, Jones RL (2006b) Nitric oxide reduces seed dormancy in *Arabidopsis*. J Exp Bot 57:517–526

Bogatek R, Dziewanowska K, Lewak S (1991) Hydrogen cyanide and embryonal dormancy in apple seeds. Physiol Plant 83:417–421

Bogatek R, Côme D, Corbineau F, Picard MA, Zarska-Maciejewska B, Lewak S (1999) Sugar metabolism as related to the cyanide-mediated elimination of dormancy in apple embryos. Plant Physiol Biochem 37:577–585

Butler AR, Megson IL (2002) Non-heme iron nitrosyls in biology. Chem Rev 102:1155–1165

Clarke A, Desikan R, Hurst RD, Hancock JT, Neill SJ (2000) NO way back**:** nitric oxide and programmed cell death in *Arabidopsis th**al**iana* suspension cultures. Plant J 24:667–677

Cragon JD (1999) Teratogen update: methylene blue. Teratology 60:42–48

Crawford NM (2006) Mechanisms for nitric oxide synthesis in plants. J Exp Bot 57:471–478

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 98:13454–13459

Delledonne M (2005) NO news is good news for plants. Curr Opin Plant Biol 8:390–396

Desikan R, Cheung MK, Bright J, Henson D, Hancock T, Neill SJ (2004) ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. J Exp Bot 55:205–212

Distéfano AM, García-Mata C, Lamattina L, Laxalt AM (2008) Nitric oxide-induced phosphatidic acid accumulation: a role for phospholipases C and D in stomatal closure. Plant Cell Environ 31:187–194

Ederli L, Reale L, Madeo L, Ferranti F, Gehring C, Fornaciari M, Romano B, Pasqualini S (2009) NO release by nitric oxide donors in vitro and in planta. Plant Physiol Biochem 47:42–48

Floryszak-Wieczorek J, Milczarek G, Arasimowicz M, Ciszewski A (2006) Do nitric oxide donors mimic endogenous NO-related responses in plants? Planta 224:1363–1372

Gabaldón C, Ros LVG, Pedreño MA, Barceló AR (2005) Nitric oxide production by the differentiating xylem of *Zinnia elegans*. New Phytol 165:121–130

Gamborg OL, Miller RA, Ojima K et al (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–168

Gharyal P, Maheshwari SC (1981) *In vitro* differentiation of somatic embryoids in a leguminous tree-*Albizzia lebbeck* L. Naturwissenschaften 68:379–380

Gharyal P, Maheshwari SC (1982) In vitro differentiation of plantlets from tissue cultures of Albizzia lebbeck L. Plant Cell Tiss Org Cult 2:49–53

Gharyal P, Maheshwari SC (1990) Differentiation in explants from mature leguminous trees. Plant Cell Rep 8:550–553

Goldstein S, Russo A, Samuni A (2003) Reactions of PTIO and carboxy-PTIO with *NO, $*NO_2$, and O_2*. J Biol Chem 278:7757–7763

Grossmann K (1996) A role for cyanide, derived from ethylene biosynthesis, in the development of stress symptoms. Physiol Plant 97:772–775

He Y, Tang RH, Hao Y, Stevens RD, Cook CW, Ahn SM, Jing L, Yang Z, Chen L, Guo F, Fiorani F, Jackson RB, Crawford NM, Pei ZM (2004) Nitric oxide represses the *Arabidopsis* floral transition. Science 305:1968–1971

Ignarro JS, Buga GM, Wood KS, Byrns RE, Chaudhuri G (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84:9265–9269

Jain N, Babbar SB (2002) Gum katira—a cheap gelling agent for plant tissue culture media. Plant Cell Tiss Org Cult 71:223–229

Jain R, Babbar SB (2006) Xanthan gum: an economical substitute for agar in plant tissue culture media. Plant Cell Rep 25:81–84

Kalra C, Babbar SB (2010) Nitric oxide promotes in vitro organogenesis in *Linum usitatissimum* L. Plant Cell Tiss Org Cult 103:353–359

Kasprowicz A, Szubaz A, Volkmann D, Baluška F, Wojtaszek P (2009) Nitric oxide modulates dynamic actin cytoskeleton and vesicle trafficking in a cell type-specific manner in root apices. J Exp Bot 60:1605–1617

Kim MK, Sommer HE, Bongarten BC, Merkle SA (1997) High-frequency induction of adventitious shoots from hypocotyl segments of *Liquidambar styraciflua* L by thidiazuron. Plant Cell Rep 16:536–540

Khurana J, Khurana P (2000) Biolistic-mediated DNA delivery into hypocotyls of a leguminous tree—*Albizzia lebbeck* L.: influence of biological and physical parameters. J Plant Biochem Biotechnol 9:31–34

Khurana A, Khurana JP, Babbar SB (2011) Nitric oxide induces flowering in the duckweed *Lemna aequinoctialis* Welw. (Syn. *L. paucicostata* Hegelm.) under noninductive conditions. J Plant Growth Reg. doi:10.1007/s00344-011-9199-7

Lanteri ML, Laxalt AM, Lamattina L (2008) Nitric oxide triggers phosphatidic acid accumulation via phospholipase D during auxin-induced adventitious root formation in cucumber. Plant Physiol 147:188–198

Leshem YY, Haramaty E (1996) The characterization and contrasting effects of the nitric oxide free radicals in vegetative stress and senescence of *Pisum sativum* Linn foliage. J Plant Physiol 148:258–263

Manjunatha G, Raj SN, Shetty NP, Shetty HS (2008) Nitric oxide donor seed priming enhances defense responses and induces resistance against pearl millet downy mildew disease. Pesticide Biochem Physiol 91:1–11

Meeussen JL, Keizer MG, van Riemsdijk WH (1992) Dissolution behavior of iron cyanide (Prussian blue) in contaminated soils. Environ Sci Toxicol 26:1832–1838

Miller MR, Megson IL (2007) Recent developments in nitric oxide donor drugs. Brit J Pharmacol 151:305–321

Murgia I, Tarantino D, Vannini C, Bracale M, Carravieri S, Soave C (2004) *Arabidopsis thaliana* plants over expressing thylakoidal ascorbate peroxidase show increased resistance to paraquat-induced photo-oxidative stress and to nitric oxide-induced cell death. Plant J 38:940–953

Nahrstedt A (1985) Cyanogenic compounds as protective agents for organisms. Plant Syst Evol 150:35–47

Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signalling in plants. New Phytol 159:11–35

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

París R, Lamattina L, Casalongué CA (2007) Nitric oxide promotes the wound-healing response of potato leaflets. Plant Physiol Biochem 45:80–86

Sharma P, Rajam MV (1995) Genotype explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). J Exp Bot 46:135–141

Siegień I, Bogatek R (2006) Cyanide action in plants—from toxic to regulatory. Acta Physiol Plant 15:483–498

Solomonson LP, Barber MJ (1990) Assimilatory nitrate reductase: functional properties and regulation. Annu Rev Plant Physiol Plant Mol Biol 41:225–253

Smith JM, Arteca RN (2000) Molecular control of ethylene production by cyanide in *Arabidopsis thaliana*. Physiol Plant 109:180–187

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

Ullrich T, Oberle S, Abate A, Schröder H (1997) Photoactivation of the nitric oxide donor SIN-1. FEBS Lett 406:66–68

Wilson ID, Neill SJ, Hancock JT (2008) Nitric oxide synthesis and signalling in plants. Plant Cell Environ 31:622–631

Yamamoto T, Bing R (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for NR-dependent formation of active nitrogen species. Proc Exp Biol Med 225:200–206

Zagrobelny M, Bak S, Rasmussen AV, Jorgensen B Naumann CM, Moller BL (2004) Cyanogenic glycosides and plant-insect interactions. Phytochemistry 65:293–330