

Nitric oxide promotes in vitro organogenesis in *Linum usitatissimum* L.

Charu Kalra · Shashi B. Babbar

Received: 2 October 2009 / Accepted: 19 June 2010 / Published online: 3 July 2010
© Springer Science+Business Media B.V. 2010

Abstract The effects of nitric oxide (NO) on caulogenesis, shoot organogenesis and rhizogenesis from hypocotyl explants of *Linum usitatissimum* were investigated. Exogenously supplied NO donors, 5 μ M sodium nitroprusside (SNP), 2 μ M *S*-nitroso-*N*-acetylpenicillamine (SNAP) and 2 μ M 3-morpholinosydnonimine (SIN-1), significantly promoted shoot differentiation from the hypocotyl explants of *L. usitatissimum* excised from its in vitro raised seedlings. Potassium ferrocyanide, a structural analogue of SNP, lacking NO group, did not promote shoot organogenesis. Likewise, products of NO, NO₂⁻ and NO₃⁻ supplied as 5 μ M NaNO₂ and 5 μ M NaNO₃ did not enhance shoot differentiation. Another source of NO, a mixture of sodium nitrite (SN) provided along with ascorbic acid (AsA), also caused significant promotion in the average number of shoots per responding explant. SNP also augmented the rhizogenic response of the microshoots in terms of percentage of responding explants, number of roots per responding explant and average root length. The NO scavengers, 2-(4-carboxy-phenyl)-4, 4, 5, 5-tetramethylimidazole-1-oxyl-3-oxide (cPTIO) or methylene blue (MB), provided along with SNP, SNAP, SIN-1 or SN + AsA, at concentrations equimolar to the optimum concentration of the donors, reversed the promotory influence, thereby, confirming the role of NO in promotion of in vitro morphogenesis. However, NO scavengers individually did not affect the observed morphogenic processes. Morphological and histological studies of hypocotyl segments cultured on BM or BM + SNP for 4, 8 and 12 days demonstrated that SNP enhanced shoot differentiation by

inducing a higher number of shoot primordia, each of which develops into a single shoot.

Keywords Caulogenesis · *Linum* · Nitric oxide · NO donors · NO scavengers · Organogenesis · Promotory · Rhizogenesis

Introduction

Nitric oxide (NO) is a ubiquitous, versatile and highly diffusible bioactive gaseous free radical. It influences a plethora of biological processes in both animals and plants (Lamattina et al. 2003; Wilson et al. 2008). The plant responses that have been shown to be modulated or mediated by NO are seed germination, hypocotyl elongation (Beligni and Lamattina 2000; Bethke et al. 2006), rhizogenesis (Pagnussat et al. 2003), stomatal movement (Desikan et al. 2004), senescence (Leshem and Haramaty 1996), flowering (He et al. 2004) and xylem differentiation (Gabaldón et al. 2005). NO also alleviates biotic stress by inducing host resistance (Delledonne et al. 1998) and abiotic stresses by serving as an antioxidant (Beligni and Lamattina 1999). The experiments, results of which are presented here, were undertaken to investigate the effect of NO on in vitro morphogenesis, more specifically caulogenesis (shoot differentiation), utilizing hypocotyl explants of *Linum usitatissimum*, an important fiber- and oil-yielding plant. The effect of NO on rhizogenesis of the in vitro developed shoots was also studied. A number of studies have demonstrated flax hypocotyl to be a highly responsive explant for in vitro regeneration through caulogenesis or embryogenesis (Link and Eggers 1946; Gamburg and Shyluk 1976; Kaul and Williams 1987; Dedičová et al. 2000; Salaj et al. 2005). Because of its well-characterized

C. Kalra · S. B. Babbar (✉)
Department of Botany, University of Delhi, Delhi 110007, India
e-mail: babbars@rediffmail.com

regenerative potential and capability of forming shoots on basal media, *L. usitatissimum* was chosen as experimental material for the present study.

Materials and methods

The seeds of *L. usitatissimum* cv. Neelam were procured from the botanical garden of the Department of Botany, University of Delhi, India. The seeds were surface sterilized with 0.1% mercuric chloride (w/v) for 7–8 min, and were then implanted on to the basal medium (BM), comprising Murashige and Skoog (1962) medium, supplemented with 3% sucrose and gelled with 0.8% agar. Hypocotyl explants (1 cm long), excised from 11-day-old in vitro raised seedlings were used as caulogenic explants. In vitro regenerated microshoots (5.5–6 cm) were used for the experiments on rhizogenesis. BM was supplemented with different concentrations of freshly prepared solutions of NO donors, sodium nitroprusside (SNP, 0–40 and 0–6 μM), 3- morpholinosydnonimine (SIN-1, 2–16 and 0–5 μM) and *S*-nitroso-*N*-acetylpenicillamine (SNAP, 2–16 and 0–5 μM), alone or along with a NO scavenger, 2-(4-carboxy-phenyl)-4, 4, 5, 5-tetramethylimidazole-1-oxyl-3-oxide (c-PTIO) or methylene blue (MB), at concentrations equimolar to the concentration of NO donor. The effect of potassium ferrocyanide, a structural analogue of SNP but not a NO donor, was tested at 5 μM . Thermolabile chemicals (SNP, SNAP, SIN-1, cPTIO and MB) were filter sterilized and were added to other constituents of the medium which were sterilized by autoclaving. Prior to autoclaving, the pH of the media was adjusted to 5.8. All cultures were exposed to continuous light of 70 $\mu\text{Mm}^{-2}\text{s}^{-1}$ and 16 h photoperiod, provided by 40-W cool day fluorescent tubes, and incubated at $25 \pm 2^\circ\text{C}$ with 50–60% relative humidity. For all but one experiment, the cultures were evaluated for the number of caulogenic or rhizogenic explants after 21 days of culture. The results of these experiments have been expressed as the percentage of responding explants, average number of roots or shoots per responding explant and average shoot or root length. In one experiment, explants were scored for the number of shoot domes (protuberances), shoot buds and shoots after 4, 8 and 12 days of culture. All experiments were repeated at least twice with 30 explants per treatment each time.

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, pairwise comparisons were made by using Post Hoc Tukey HSD test at $P \leq 0.05$. Graphs were generated by using Microsoft Excel 2007. The bars in the figures represent mean \pm standard deviation. Different letters on the bars represent significantly different values.

The origin of shoots was traced by carrying out histological studies of hypocotyl segments cultured on BM or BM + SNP for different time periods (4, 8 and 12 days). The explants were fixed in FAA (100% formalin : glacial acetic acid : 50% ethanol, 1:1:18). These were dehydrated by passing through tertiary butyl alcohol dehydration series (50, 70, 85, 95 and 100%), followed by infiltration and embedding in paraffin wax as described by Johansen (1940). The paraffin blocks with hypocotyl segments were mounted on wooden blocks and 6- μm -thick sections were cut using a rotary microtome. The sections stained with 1% safranin and 0.05% astra blue were mounted in DPX on glass slides (Johansen 1940). The sections were observed under a compound microscope (Nikon microscope E600) and photographed with a Nikon digital camera (DXM 1,200) fitted over it. For each of the two treatments for which histological study was carried out, sections from 12 explants were observed.

Results and discussion

For studying the effect of NO, generally its donors are used as exogenous sources rather than the gas directly because direct exposure to NO is technically difficult and cumbersome. Commonly used NO donors are SIN-1 (Hung and Kao 2004), SNP, *S*-nitrosoglutathione, SNAP, Roussin's Black Salts, NOR-3 (+/-)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (Neill et al. 2003). Sodium nitrite was provided along with an antioxidant as ascorbic acid releases NO at acidic pH (Hung and Kao 2004). In the present study, initially the effect of 0–40 μM SNP was studied on the caulogenic response of *L. usitatissimum* hypocotyl explants. At 5 μM , SNP increased the average number of shoots per explant, whereas the percentage of responding explants remained unaffected. At the next higher concentration (10 μM), the caulogenic response was lower than even that of the control. At 20 and 40 μM , explants bleached without showing any morphogenic development (data not presented). Therefore, the next experiment was conducted using a narrow range (0–6 μM). All the concentrations of SNP augmented the caulogenic response by increasing the average number of shoots per explant with significant increase being only at 4 and 5 μM (Fig. 1). Although the percentages of responding explant and average shoot length also varied among the treatments, the differences were not significant, and therefore these data have not been presented in the figures.

The other two NO donors, SNAP and SIN-1, were also tested first at concentrations ranging from 2 to 16 μM . Among the tested levels, only 2 μM of both donors increased the average number of shoots significantly with response at the higher concentrations either comparable to

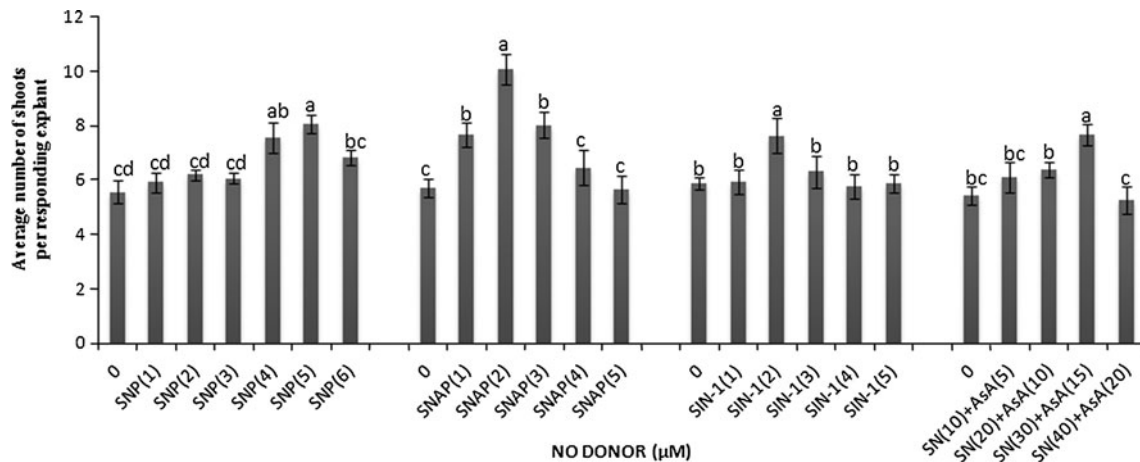


Fig. 1 Caulogenic response of hypocotyl explants of *Linum usitatissimum* cultured for 21 days on MS medium supplemented with different concentrations of SNP (0–6 μM), SNAP (0–5 μM) SIN-1 (0–5 μM), and a combination of sodium nitrite and ascorbic acid.

Bars represent mean ± SD. Different letters on the individual bars of each of the four experiments presented signify significantly different values among the treatments of the same experiment ($P \leq 0.05$)

or less than the control (data not presented). However, unlike SNP, even at the highest concentration of these donors, explants were visibly healthy and some developed shoots. As 2 μM was the only concentration of both that promoted caulogenic response, in the next set of experiments the effect of these donors was tested at 1–5 μM. Both SNAP and SIN-1 optimally raised the average number of shoots per explant at 2 μM (Fig. 1). The other source of NO, that is SN at different concentrations along with AsA in the ratio of 2:1 (Kröncke and Kolb-Bachofen 1996), also promoted the caulogenic response with the optimal combination being 30 μM SN + 15 μM ASA (Fig. 1). This combination is not commonly used as a NO source because of the possibility of the formation of other

radicals. However, it has been shown to counteract toxicity due to reactive oxygen species (Beligni and Lamattina 1999; Hung and Kao 2004). An average of 10 shoots were produced from the explants cultured at the optimal concentration of SNAP compared to about 7–8 from the explants cultured on the optimum concentrations of SNP, SIN-1 or SN + AsA. Whether these differences are statistically significant cannot be ascertained as all these four experiments were conducted at different times maintaining BM control each time.

To rule out the possibility that the observed promotory effects of NO donors were due to NO_2^- and NO_3^- , stable metabolites of NO (Ullrich et al. 1997; Beligni and Lamattina 1999), the effect of 5 μM NaNO_2 and 5 μM

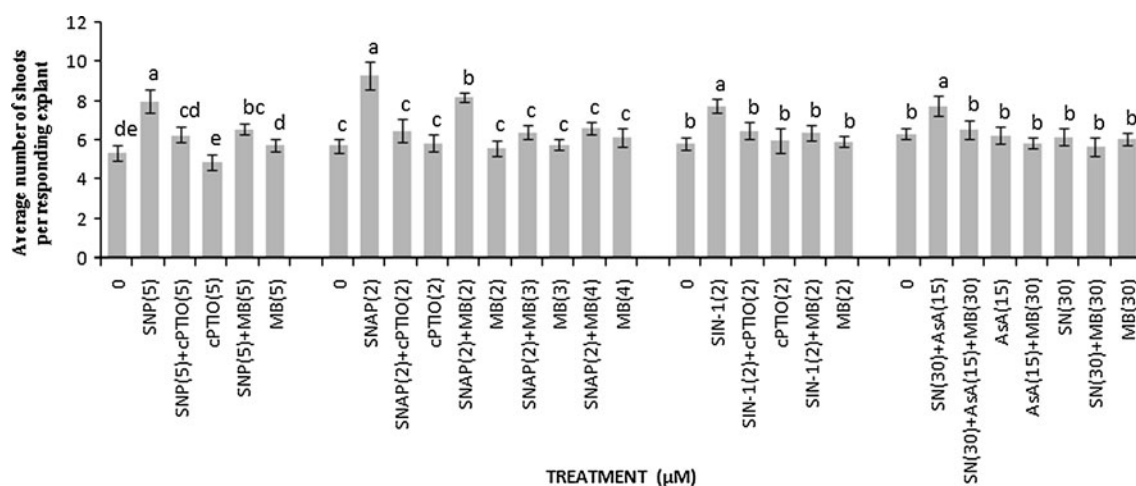


Fig. 2 Caulogenic response of hypocotyl explants of *Linum usitatissimum* cultured for 21 days on MS medium supplemented with optimum concentration of SNP (5 μM), SNAP (2 μM), SIN-1 (2 μM) and sodium nitrite (30 μM) and ascorbic acid (15 μM) alone or along

with equimolar concentrations of cPTIO or MB. Bars represent mean ± SD. Different letters on the individual bars of each of the four experiments presented signify significantly different values among the treatments of the same experiment ($P \leq 0.05$)

NaNO_3 was also studied. Although MS basal medium, used in the present study, contains about 40 mM (40,000 μM) of nitrate, an additional 5 μM of NO_2^- or NO_3^- , was supplemented to rule out the remote possibility of the observed increase in response due to this negligible rise in nitrate or nitrite concentration. As expected, neither of these had any effect on the caulogenic response (data not presented). Likewise, potassium ferrocyanide, a structural analog of SNP which lacks NO moiety, did not affect the caulogenic response. This also ruled out the possibility of the observed effect of SNP being due to cyanide (CN^-), known to be released by SNP (Manjunatha et al. 2008; Murgia et al. 2004).

To further ascertain that the observed promotion in the caulogenic response was due to NO released by its donors, specific NO scavengers cPTIO (Beligni and Lamattina 2000; París et al. 2007) and MB (Gouvêa et al. 1997; Giba et al. 1998; Zhao et al. 2007) were used alone or along with NO donors at concentrations equimolar to the optimum concentration of the respective NO donor. Both scavengers when provided along with a NO donor partially or

completely negated their promotory effect (Fig. 2). Of the two, cPTIO was more effective than MB. However, when provided alone, these scavengers did not affect either the morphogenic response or the onset of differentiation of hypocotyl explants. This apparently rules out the involvement of endogenous NO. However, the existence of endogenous NO in the hypocotyl explants and its effect on their morphogenic response can not be conclusively ruled out as cPTIO is supposed to be cell impermeable, and therefore can react only with NO or its derivatives which diffuse out of the cells (Planchet and Kaiser 2006).

In order to decipher whether SNP promotes a caulogenic response by increasing the number of shoot primordia or by promoting the development of more than one shoot from each primordium, ontogenic observations were made at both morphological and histological levels. In addition, this study also aimed to reveal whether SNP advances the onset of differentiation. Morphological observations made at intervals of 1 day revealed that, by the fourth day of culture, dome-like protuberances developed on the surface of both the control and treated explants. These protuberances

Fig. 3 a–f Development of shoots from the hypocotyl explants of *Linum usitatissimum* cultured on basal medium (BM; a–c) or the same adjuvated with SNP (5 μM ; d–f) for 4 (a,d), 8 (b,e) and 12 days (c,e)

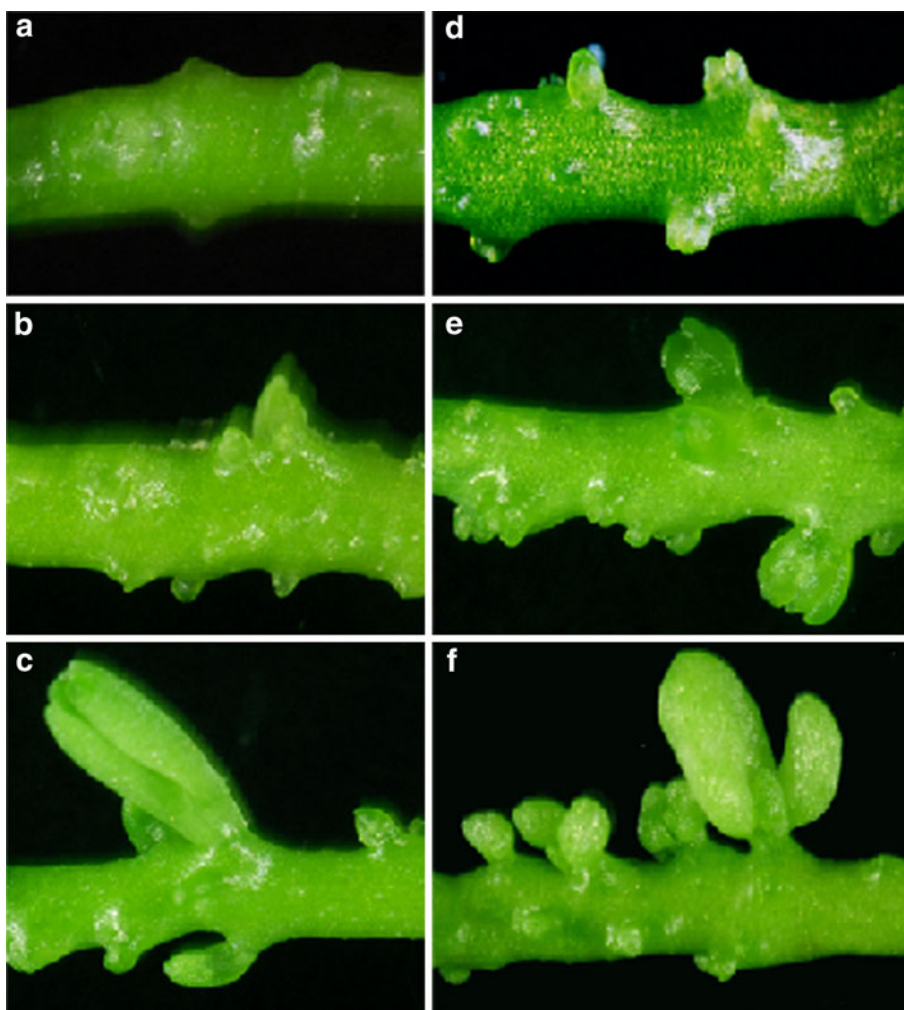


Table 1 Ontogeny of caulogenesis at morphological level in hypocotyl explants cultured on MS basal media alone or supplemented with SNP for 21 days

Treatment (μM)	Total explants	Average number of meristematic domes/shootbuds/shoots per responding explant			
		4 days	8 days	12 days	21 days
BM	96	4.60 \pm 0.67 b	5.67 \pm 0.54 b	5.90 \pm 0.33 b	5.76 \pm 0.46 b
SNP (5)	96	6.28 \pm 0.38 a	7.63 \pm 0.80 a	7.99 \pm 0.49 a	7.78 \pm 0.89 a

Values represent means \pm standard deviation

Values in a column followed by different letters are significantly different at $P \leq 0.05$

increase in size during the next 4 days and get converted to shoot buds. After 4 days of culture, the dome-like structures, developed on the surface of hypocotyl explants cultured on medium adjuvated with SNP, were at advanced stages of development as compared with explants on BM. By the eighth day, shoot buds along with newly developed shoot domes (protuberances) could be observed on the explants. By the end of 12 days, most of the shoot buds had developed into elongated shoots (Fig. 3a–f). As new shoot domes continue to develop even after 4 days of culture, at each time interval, shoot primordia and shoot buds as well as open shoots were counted. At any given time (4, 8 and

12 days), the total number of shoot primordia/shoot buds/shoots was significantly higher on SNP-adjuvated medium than on BM (Table 1).

Histological analysis of the longitudinal sections of hypocotyl explant cultured on BM or BM + SNP revealed that domes observed at morphological levels after 4 days of culture are, in fact, meristematic tissue arising from epidermal or sub epidermal regions of the explant. The latter could be distinguished from the adjoining tissue because of their deep staining and small sized cells (Fig. 4a, d). During the course of culture, domes get converted into shoot buds which later elongate to form shoots (Fig. 4b, c, e, f). The shoot buds originated from the control as well as treated explants without any intervening callus phase (Fig. 4a–f). In an earlier published study on *L. usitatissimum* (Link and Eggers 1946), direct shoot bud development from the epidermal cells of the hypocotyl explants was reported. Another noteworthy observation was that, at any time interval, shoot bud primordia, shoot buds or shoots were at advanced stages of development on SNP-adjuvated medium compared with BM. However, SNP or other donors did not affect the morphology of the shoots.

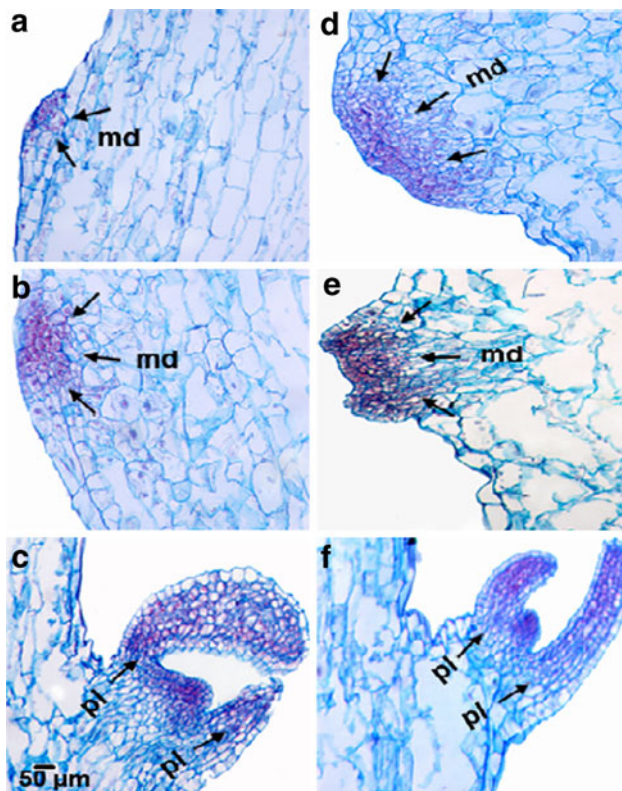


Fig. 4 a–f Longitudinal sections of hypocotyl explant of *Linum usitatissimum* cultured on basal medium (BM; a–c) or the same adjuvated with SNP (5 μM ; d–f) for 4 (a,d), 8 (b,e) and 12 days (c,f) showing meristematic dome (md) and shoot buds with leaf primordia (pl). Scale bar pertains to 50 μm

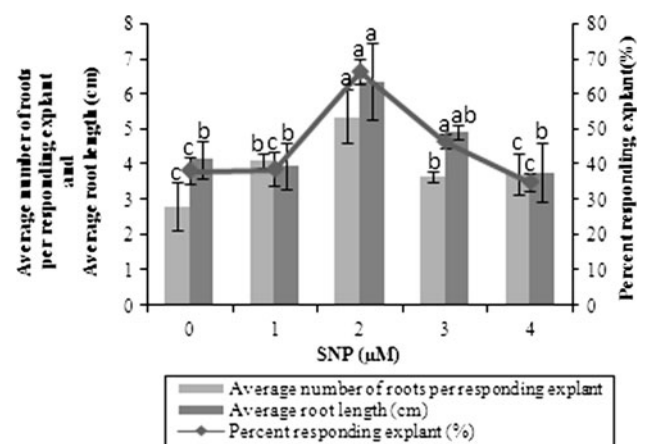


Fig. 5 Rhizogenic response of in vitro developed shoots of *Linum usitatissimum* cultured on MS medium for 21 days supplemented with different concentrations of SNP (0–4 μM). Bars represent mean \pm SD. Different letters on the individual bars signify significantly different values ($P \leq 0.05$)

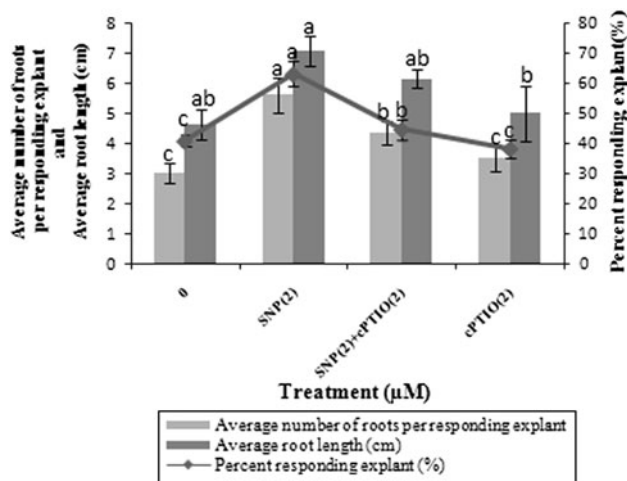


Fig. 6 Rhizogenic response of in vitro developed shoots of *Linum usitatissimum* cultured on MS medium for 21 days supplemented with optimum concentration of SNP (2 μM) alone or along with equimolar concentration of cPTIO. Bars represent mean ± SD. Different letters on the individual bars signify significantly different values ($P \leq 0.05$)

On the basis of both morphological and histological observations, it is concluded that SNP augments the caulogenic response by increasing the number of meristems. Enhanced differentiation of meristems in SNP-adjuvated medium could possibly be explained on the basis of earlier reports (Ötovös et al. 2005; Pagnussat et al. 2004) showing regulation of cell cycle-regulatory genes and mitogen-activated protein kinases by NO. In addition, NO might be modulating expression of genes associated with shoot meristem differentiation.

The promotion of adventitious roots by NO has been one of the widely reported and analyzed NO effects on plants (Pagnussat et al. 2002, 2003; Stöhr and Stremlau 2006). In the present study, too, NO provided as SNP promoted rooting of microshoots regenerated from the hypocotyl explants. However, unlike its effect on shoot differentiation, NO promoted rhizogenesis in terms of all the following parameters observed in this study: the percentage of responding explants, average number of roots per shoot, and average root length (Fig. 5). This promotory effect of SNP on rhizogenesis was also reversed by the NO scavenger, cPTIO (Fig. 6). cPTIO alone did not affect the rhizogenic response. This observation is at variance with the previous reports by Pagnussat et al. (2002, 2003) who observed a significant reduction in rhizogenic response of cucumber hypocotyl explants by cPTIO alone. However, Gouvêa et al. (1997) reported that MB, though negating the elongation of maize roots induced by NO donors, had no effect when provided alone.

Along with its well-known effect on rhizogenesis, the present study reports the promotory influence of NO on

shoot differentiation under in vitro conditions, an unknown effect of NO until recently. However, very recently, Han et al. (2009) reported promotion of in vitro regeneration and multiplication of *Malus hupehensis* by SNP. In this report, although the promotory effect of SNP was attributed to NO, no additional experiments involving the use of NO scavengers alone or along with SNP were conducted. The present observations conclusively demonstrate the effect of NO on de novo differentiation of shoots, thus adding one more dimension to already known effects of this small, simple and versatile signaling molecule. This opens up new vistas of research for the elucidation of components of the signal cascade downstream NO leading to its effect on caulogenesis.

Acknowledgments C.K. gratefully acknowledges the awards of Junior and Senior Research Fellowships by the Council of Scientific and Industrial Research (New Delhi). This work was partially financed by R & D miscellaneous grant provided to S.B.B. by the University of Delhi.

References

- Beligni MV, Lamattina L (1999) Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. *Planta* 208:337–344
- Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and deetiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta* 210:215–221
- Bethke PC, Libourel IGL, Reinöhl V, Jones RL (2006) Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. *Planta* 223:805–812
- Dedičová B, Hricová A, Šamaj J, Obert B, Bobák M, Preto'vá A (2000) Shoot and embryo-like structures regenerated from cultured flax (*Linum usitatissimum* L.) hypocotyl segments. *J Plant Physiol* 157:327–334
- Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. *Nature* 394: 585–588
- 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
- 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, Shyluk JP (1976) Tissue culture, protoplasts and morphogenesis in flax. *Bot Gaz* 137:301–306
- Giba Z, Grubišić D, Todorović S, Sajc L, Stojaković D, Konjević R (1998) Effect of nitric oxide-releasing compounds on phytochrome-controlled germination of empress tree seeds. *Plant Growth Reg* 26:175–181
- Gouvêa CMCP, Souza JF, Magalhães ACN, Martins IS (1997) NO releasing substances that induce growth elongation in maize root segments. *Plant Growth Reg* 21:183–187
- Han X, Yang H, Duan K, Zhang X, Zhao H, You S, Jiang Q (2009) Sodium nitroprusside promotes multiplication and regeneration of *Malus hupehensis* in vitro plantlets. *Plant Cell Tissue Org Cult* 96:29–34
- 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
- Hung KT, Kao CH (2004) Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. *J Plant Physiol* 161:43–52
- Johansen DA (1940) *Plant microtechnique*, 2nd edn. Tata McGraw-Hill, Bombay-New Delhi
- Kaul V, Williams EG (1987) Multiple shoot induction in vitro from the hypocotyl of germinating embryos of flax (*Linum usitatissimum* L.). *J Plant Physiol* 131:441–448
- Kröncke KD, Kolb-Bachofen V (1996) Detection of nitric oxide interaction with zinc finger proteins. *Meth Enzymol* 269: 279–284
- Lamattina L, García-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: the versatility of an extensive signal molecule. *Annu Rev Plant Biol* 54:109–136
- Leshem YY, Haramaty E (1996) The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage. *J Plant Physiol* 148: 258–263
- Link GKK, Eggers V (1946) Mode, site and time of initiation of hypocotyledonary bud primordia in *Linum usitatissimum* L. *Bot Gaz* 107:441–454
- 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. *Pest Biochem Physiol* 91:1–11
- Murashige TF, Skoog A (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Planta* 15: 473–497
- Murgia I, De Pinto MC, Delledonne M, Soave C, De Gara L (2004) Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death, and cell redox state in plant cells. *J Plant Physiol* 161:777–783
- Neill SJ, Desikan R, Hancock JT (2003) Nitric oxide signalling in plants. *New Phytol* 159:11–35
- Ótovös K, Pasternak TP, Miskolczi P, Domoki M, Dorjgotov D, Szűcs A, Bottka S, Dudits D, Fever A (2005) Nitric oxide is required for, and promotes auxin-mediated activation of, cell division and embryonic cell formation but does not influence cell cycle progression in alfalfa cell cultures. *Plant J* 43:849–860
- Pagnussat GC, Simontacchi M, Puntarulo S, Lamattina L (2002) Nitric oxide is required for root organogenesis. *Plant Physiol* 129:954–956
- Pagnussat GC, Lanteri ML, Lamattina L (2003) Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiol* 132:1241–1248
- Pagnussat GC, Lanteri ML, Lombardo MC, Lamattina L (2004) Nitric oxide mediates the indole acetic acid induction activation of a mitogen-activated protein kinase cascade involved in adventitious root development. *Plant Physiol* 135:279–286
- París R, Lamattina L, Casalagué CA (2007) Nitric oxide promotes the wound-healing response of potato leaflets. *Plant Physiol Biochem* 45:80–86
- Planchet E, Kaiser WM (2006) Nitric oxide production in plants: facts and fictions. *Plant Signal Behav* 1:46–51
- Salaj J, Petrovská B, Obert B, Preřová A (2005) Histological study of embryo-like structures initiated from hypocotyl segments of flax (*Linum usitatissimum* L.). *Plant Cell Rep* 24:590–595
- Stöhr C, Stremlau S (2006) Formation and possible roles of nitric oxide in plant roots. *J Exp Bot* 57:463–470
- 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
- Zhao DY, Tian QY, Li LH, Zhang WH (2007) Nitric oxide is involved in nitrate induced inhibition of root elongation in *Zea mays*. *Ann Bot* 100:497–503