J Plant Growth Regul (2011) 30:378–385
DOI 10.1007/s00344-011-9199-7

SHORT COMMUNICATION

Nitric Oxide Induces Flowering in the Duckweed *Lemna aequinoctialis* Welw. (Syn. *L. paucicostata* Hegelm.) Under Noninductive Conditions

Ashima Khurana · Jitendra P. Khurana · Shashi B. Babbar

Received: 30 June 2010 / Accepted: 31 January 2011 / Published online: 25 February 2011
© Springer Science+Business Media, LLC 2011

Abstract Nitric oxide (NO) plays diverse roles in the growth and development of plants and in their responses to various abiotic and biotic stresses. It has also been reported to repress flowering in *Arabidopsis thaliana*. In the present study, NO donors sodium nitroprusside (SNP), *S*-nitroso-*N*-acetyl penicillamine (SNAP), and 3-morpholinosydnonimine (SIN-1) induced flowering in *Lemna aequinoctialis* 6746 (a short-day strain) and in *L. aequinoctialis* LP_6 (a photoperiod-insensitive strain) under noninductive conditions. Nitrate and nitrite, two stable metabolites of NO, did not induce flowering. On the other hand, cyanide donors potassium ferricyanide $\{K_3[Fe(CN)_6]\}$ and potassium cyanide (KCN) induced flowering in both strains under noninductive conditions. The flowering induced under a 8-h daily photoperiod regime in the short-day strain *L. aequinoctialis* 6746 was inhibited by NO and cyanide donors. Vegetative multiplication of both strains was adversely affected by NO and cyanide donors, irrespective of the photoperiod conditions. The observed effects of NO donors on flowering were substantially negated by NO scavengers c-PTIO [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide] and methylene blue. This confirmed the role of NO in induction of flowering. The inductive effect of CN^- also appeared to be partly mediated through NO as NO scavengers partially negated the effect of CN^-.

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

J. P. Khurana
Department of Plant Molecular Biology, University of Delhi, South Campus, New Delhi, India

Keywords Cyanide · Flowering · *Lemna* · Nitric oxide · NO donors · NO scavengers

Introduction

Nitric oxide (NO), once considered a harmful pollutant, became an important regulatory molecule when an unidentified endothelium-derived relaxation factor in mammals was recognized to be NO (Ignarro and others 1987). Despite its small and simple structure, NO plays an important regulatory role in a number of biological processes in both animals and plants (Moncada and Higgs 2006; Krasylenko and others 2010). It plays a critical role in many processes that affect plant growth and development and their responses to abiotic and biotic stresses (Arasimowicz and Floryszak-Wieczorek 2007; Besson-Bard and others 2008). It has been shown to be involved in seed germination, rhizogenesis, hypocotyl elongation, de-etiolation, stomatal movement, xylem differentiation, mitochondrial activity, oxidative stress, disease resistance, senescence, and programmed cell death (Besson-Bard and others 2008; Krasylenko and others 2010). Recently, the stimulatory effect of NO on shoot differentiation has also been reported (Kalra and Babbar 2010).

The transition from the vegetative to the reproductive stage has also been reported to be influenced by NO in *Arabidopsis thaliana*. Its flowering was delayed by an exogenous supply of NO, and also in a mutant overproducing NO (He and others 2004). Before *Arabidopsis* (a dicot) became a system of choice for molecular genetic analyses of floral induction and development, members of the family Lemnaceae (monocots, commonly called duckweeds) were used routinely as model systems for physiological studies related to flowering (Kandeler 1984).

Therefore, for the present study, *Lemna* was chosen to study the effect of NO on its flowering. The observations presented in this communication demonstrate the inductive effect of NO (provided exogenously as its donors) on the flowering of two duckweed strains, *Lemna aequinoctialis* 6746 (a short-day plant) (Kandeler 1984) and *L. aequinoctialis* LP_6 (a photoperiod-insensitive strain) (Khurana and Maheshwari 1983) under noninductive conditions.

Materials and Methods

The aseptic stock cultures of *Lemna aequinoctialis* Welw. (synonyms: *L. paucicostata* Hegelm. and *L. perpusilla* Torrey), strains 6746 and LP_6, were raised on modified Bonner and Devirian's (1939) medium, supplemented with 1% (w/v) sucrose, the pH of which was adjusted to 5.5 before autoclaving. These strains were maintained by subculturing after every 8-10 days. The cultures were maintained under a photoperiodic regimen of 16-h light (18 μE m^{-2} s^{-1}) and 8-h dark. For subculturing, single three-frond colonies were individually transferred to 100-ml Erlenmeyer flasks (Borosil, India), each containing 40 ml of autoclaved nutrient medium, in a laminar flow cabinet. The flasks were plugged with cotton plugs (non-adsorbent cotton wrapped in a layer of muslin cloth). Like stock cultures, each experimental culture was also initiated with a single three-frond colony in a 100-ml flask containing 40 ml of the medium. For one experiment, plants from one stock culture were used to minimize the variation due to the "aging" or "stale" medium effect (Khurana and Cleland 1992). Three replicates were used for individual treatments of all experiments and each experiment was repeated at least twice.

The stock solution of c-PTIO [2-(4-carboxyphenyl)-4, 4,5,5-tetramethylimidazoline-1-oxyl-3-oxide] (Sigma, USA), made in distilled water, was stored at −20°C. The solutions of SNP (sodium nitroprusside, Sigma, USA), SNAP (*S*-nitroso-*N*-acetyl penicillamine, Sigma, USA), SIN-1 (3-morpholinosydnonimine, Sigma, USA), KCN (potassium cyanide, Hi Media, India), $K_3[Fe(CN)_6]$ (potassium ferricyanide, Ranbaxy, India), and methylene blue (Hi Media, India) were made fresh for each experiment. These chemicals were added to the autoclaved medium after filter sterilization.

The stock and experimental cultures were kept under 16-h daily illumination (6 a.m.–10 p.m.) and 8 h of darkness (10 p.m.–6 a.m.). Short-day treatment consisted of 8 h of light (10 a.m.–6 p.m.) and 16 h of dark (6 p.m.–10 a.m.). Light was provided by cool daylight fluorescent tubes (40 W, Philips) at 18 μE m^{-2} s^{-1}. The temperature of the culture room (in both light and dark conditions) was maintained at 25 ± 2°C. Plants were either exposed to a 16-h daily photoperiod throughout the duration of experiment or were initially exposed to a 16-h photoperiod for 2 days, followed by an 8-h photoperiod for 4 days, and then transferred to a 16-h photoperiod for additional 3-6 days. These two regimens are hereafter referred to as the 16-h daily photoperiod and the 8-h daily photoperiod, respectively. Experiments conducted with *L. aequinoctialis* LP_6 strain were terminated 11-12 days after culture initiation and those with *L. aequinoctialis* 6746 were evaluated after 9-10 days of culture. On termination of the experiments, plants from each replicate flask were fixed separately in 90% alcohol. The bleached fronds were spread on a glass slide and examined under a microscope to count the number of flowering fronds (all those showing characteristic bilobed anthers, Fig. 1 A-D). For comparing the vegetative multiplication under different treatments, the total number of fronds in each flask was counted.

Statistical analysis of the data and the preparation of graphs were done by using Microsoft Excel 2007. The bars in the figures represent mean ± standard error.

Results and Discussion

The switch to flowering is a major developmental step and an inherently complex process as it depends on quantitative integration of multiple environmental and endogenous signals (Bäurle and Dean 2006). The physiological studies on floral transition have led to the identification of several putative floral signals, including growth regulators, which play important roles in chemical control of flowering (Corbesier and Coupland 2006). The involvement of NO in flowering of *A. thaliana* was revealed as one of its mutants (*nox*-1), which overproduces NO, flowered late, whereas the other mutant (*nos*-1), which produces less NO, flowered early. Moreover, an exogenously provided NO donor repressed flowering (He and others 2004). Subsequent to this report, there has been no other communication that reports the involvement of NO in the flowering of any plant. Therefore, in the present study, the role of NO in the flowering of aseptically grown plants of *L. aequinoctialis* was investigated. NO donors that mimic a NO effect are generally employed for physiological studies as the treatment of biological samples directly with NO gas is difficult (Neill and others 2003; Floryszak-Weiczorek and others 2006). Moreover, use of more than one donor is advocated to ensure that the observed effect is due to the NO released and not due to the NO donor per se (Neill and others 2003). Therefore, in the present study, the effect of three NO donors, SNP, SNAP, and SIN-1, was studied.

Under noninductive conditions of the 16-h daily photoperiod, none of the two strains of *L. aequinoctialis* flowered in the basal medium (Fig. 2a, b). However, NO

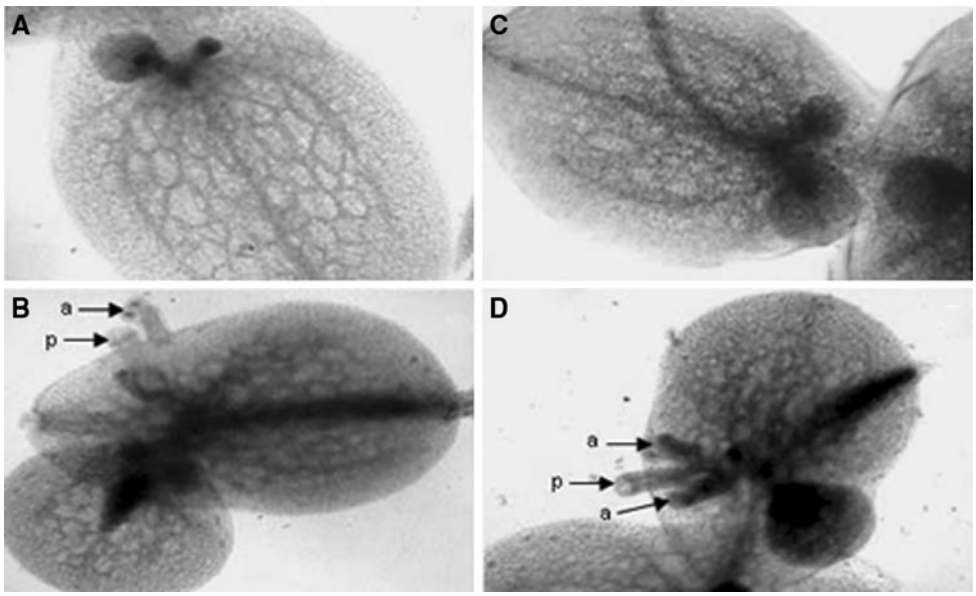

Fig. 1 **A**, **B** Vegetative and reproductive fronds of the strain 6746, respectively. **C**, **D** Vegetative and reproductive fronds of the strain LP_6, respectively. *a* anther, *p* pistil

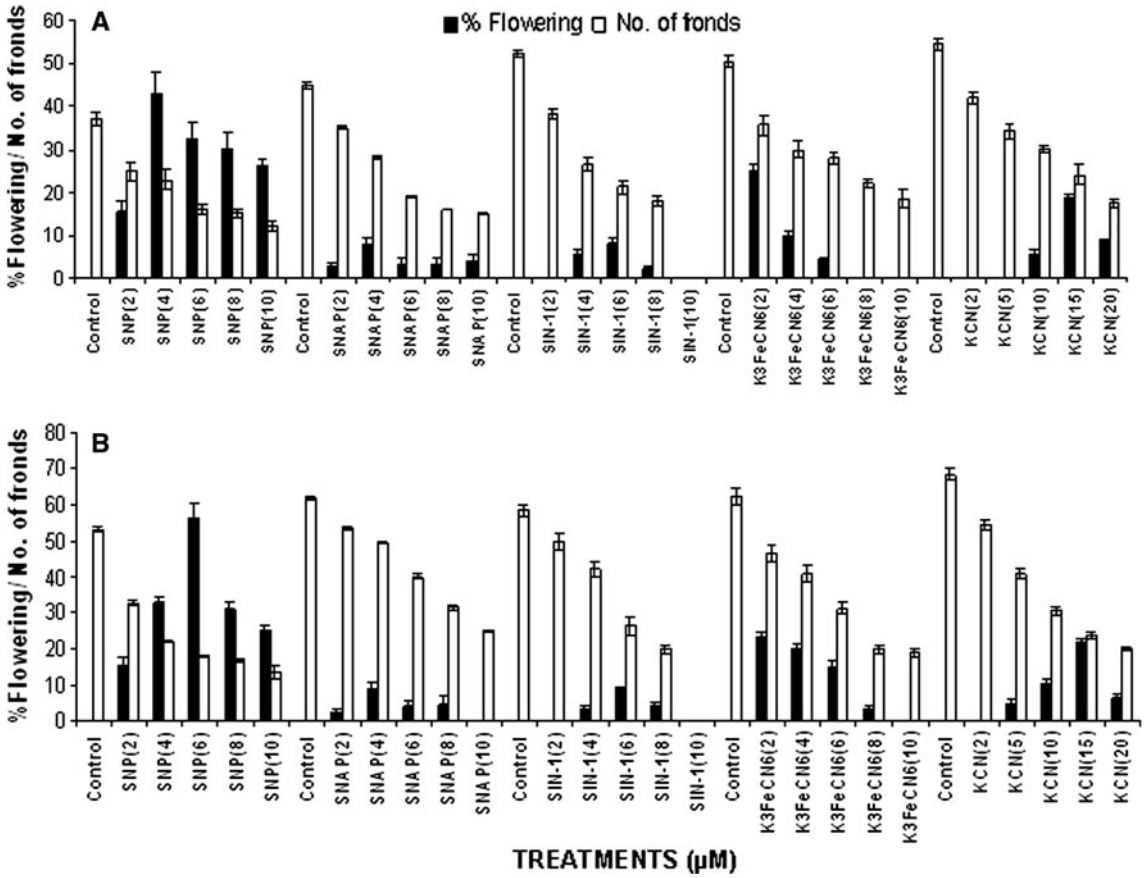

Fig. 2 Effect of different concentrations of SNP, SNAP, SIN-1, $K_3[Fe(CN)_6]$, and KCN on the flowering and vegetative growth of *L. aequinoctialis* 6746 (**a**) and LP_6 (**b**) under 16-h daily photoperiods

donors induced flowering in both strains (Fig. 2a, b). Among the different concentrations of the three donors, maximal flowering in strain 6746 was observed at 4 μM of both SNP and SNAP, whereas SIN-1 was most effective at a concentration of 6 μM. The strain LP_6 exhibited maximal flowering at 6 μM of SNP and 4 μM of both SNAP and

Table 1 Flowering and vegetative growth of *L. acquinoctialis* 6747 and LP_6 as affected by potassium ferricyanide (0–2 μM)

Concentration (μM)	6746		LP_6	
	% Flowering	No. of fronds	% Flowering	No. of fronds
0	0	56.0 ± 2.0	0	66.0 ± 2.50
0.5	0	50.30 ± 1.15	0	56.0 ± 1.60
1.0	0	45.0 ± 1.50	0	47.0 ± 2.60
1.5	2.0 ± 0.60	40.0 ± 2.25	4.50 ± 1.0	39.0 ± 1.20
2.0	14.40 ± 1.70	33.30 ± 1.60	22.30 ± 1.50	35.0 ± 1.80

SIN-1. SNAP and SIN-1 were not as effective as SNP in inducing flowering in the two strains (Fig. 2a, b). The multiplication of fronds of both strains was adversely affected by the three NO donors, with the degree of inhibition being directly proportional to their concentration (Fig. 2c, d). Even in inhibiting vegetative multiplication, SNAP and SIN-1 were not as potent as SNP (Fig. 2c, d). To rule out the possibility of the observed effects due to NO_2^- and NO_3^-, stable metabolites of NO (Ullrich and others 1997), the effect of sodium nitrite and sodium nitrate added individually to the medium at different concentrations (2, 5, and 10 μM) was also studied. None of these initiated flowering in either of the two investigated strains (data not presented). Likewise, sodium nitrite (10, 20 μM) and ascorbic acid (5, 10 μM), provided together as a source of NO (Krönke and Kolb-Bachofen 1996), did not induce flowering in either strain (data not presented).

SNP, along with NO, also generates cyanide as the first volatile breakdown product (Bethke and others 2006). Cyanide has been shown to induce flowering in *Lemna* (Tanaka and others 1983). Therefore, studying the effect of cyanide along with SNP became necessary to ascertain the cause of the observed inductive effect on flowering by the latter. In the present study, cyanide donors ($K_3[Fe(CN)_6$ and KCN) induced flowering in both strains under a 16-h daily photoperiod, and the most effective concentrations of these two cyanide donors were 2 and 15 μM, respectively (Fig. 2a, b). However, as the most effective concentration of potassium ferricyanide was the lowest of the tested concentrations, another experiment was conducted to study its effect at levels lower than 2 μM. Also in this experiment, maximal flowering in both strains was recorded at 2 μM (Table 1). Cyanide donors also affected the multiplication of the fronds adversely in a dose-dependent manner (Fig. 2c, d).

Under an 8-h daily photoperiod, about one third of the fronds of the short-day strain 6746 flowered in the basal medium (Fig. 3a), whereas fronds of strain LP_6 remained exclusively vegetative (Fig. 3b). In strain 6746, flowering induced by the inductive short-day conditions was inhibited by SNP and SNAP, at all of the tested concentrations (Fig. 3a). Even in its inhibitory effect on flowering, SNAP was not as potent as SNP (Fig. 3a). In comparison with their inhibitory effect on flowering in the strain 6746, both SNP and SNAP induced flowering in the photoperiod-insensitive strain LP_6, with maximal flowering at 6 and 4 μM, respectively (Fig. 3b). Under these photoperiodic conditions, SNP was more effective than SNAP in inducing flowering (Fig. 3b). Although SNP induced flowering in LP_6 under 8-h-photoperiod conditions, it was not as effective as under 16-h photoperiods. Thus, the flowering response observed at the most effective concentration (6 μM) under an 8-h photoperiod was almost one third of the corresponding value under 16-h photoperiods (Figs. 2b, 3b). However, the effect of SNAP was essentially similar under both photoperiodic conditions (Figs. 2b, 3b). Irrespective of their effect on flowering, both NO donors inhibited vegetative growth of the strains 6746 and LP_6 in a dose-dependent manner, as evidenced by the decrease in total frond number (Fig. 3c, d).

Of the two CN^- donors, only the effect of $K_3[Fe(CN)_6]$ was studied on the flowering response in both 6746 and LP_6 under 8-h photoperiods. $K_3[Fe(CN)_6]$ inhibited flowering in the strain 6746, whereas it induced flowering in LP_6 at all concentrations (Fig. 3a, b). However, vegetative growth of both strains was reduced as the concentration of ferricyanide increased (Fig. 3c, d).

To confirm that the observed effects were due to NO released by the NO donors, specific NO scavengers c-PTIO (Goldstein and others 2003) and methylene blue (Cragan 1999) were used alone or in combination with NO donors. c-PTIO is known to be highly specific for NO scavenging. It is used extensively to scavenge NO because it does not react with any ROS (Goldstein and others 2003). When provided alone at concentrations equimolar to the optimal concentration of SNP, NO scavengers c-PTIO and methylene blue did not induce flowering under 16-h daily photoperiodic conditions in either strain (Fig. 4a, b). However, they had a slight inhibitory effect on the vegetative growth of both strains (Fig. 4c, d). When provided along with SNP, both NO scavengers negated the inductive effect of SNP with varying degrees of efficacy (Fig. 4a, b). Of the two NO scavengers, c-PTIO was more effective in negating the floral inductive effect of SNP than methylene blue. Along with SNAP, SIN-1, $K_3[Fe(CN)_6]$, and KCN, only the effect of c-PTIO was studied. The flowering

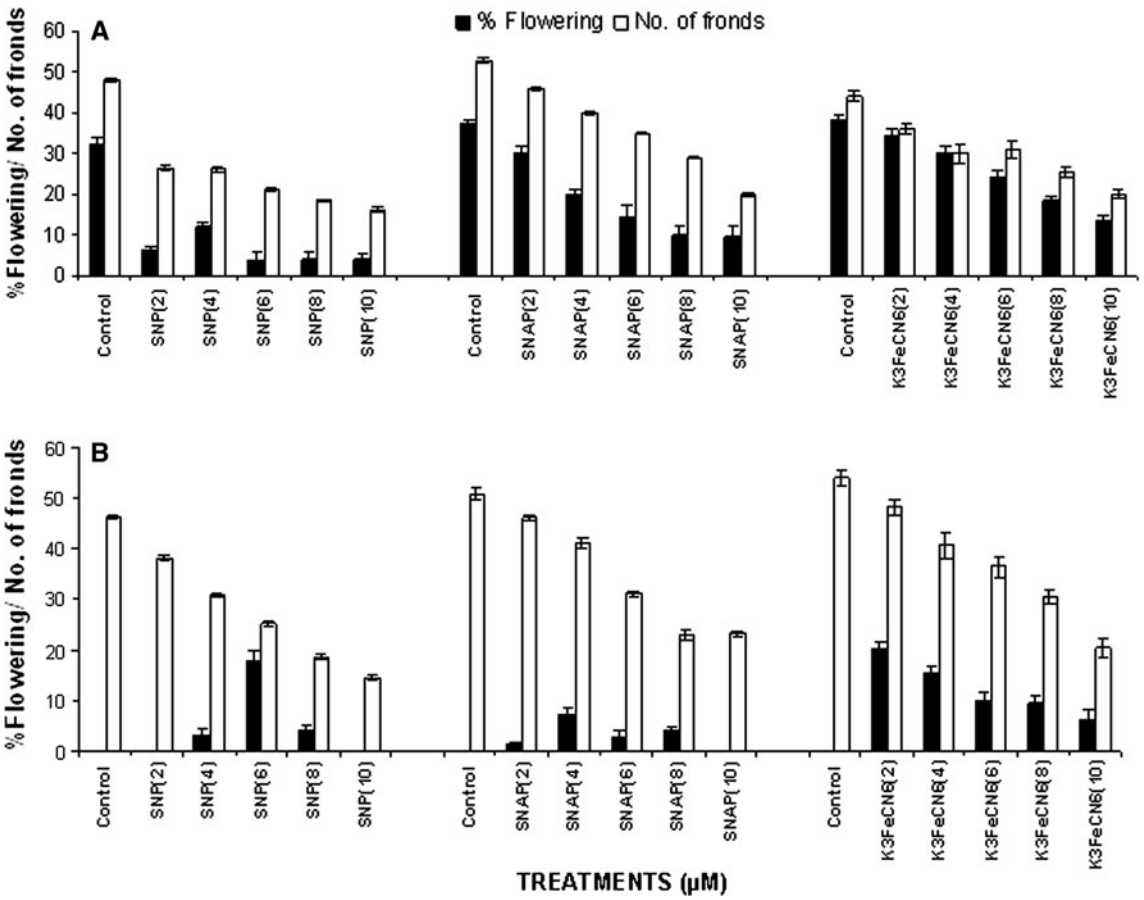

Fig. 3 Effect of different concentrations of SNP, SNAP, and $K_3[Fe(CN)_6]$ on the flowering and vegetative growth of *L. aequinoctialis* 6746 (**a**) and LP_6 (**b**) under 8-h daily photoperiods

induced by these in both strains was negated by c-PTIO (Fig. 4a, b).

Under inductive short-day conditions, the inhibitory influence of SNP and SNAP on the flowering response of strain 6746 was significantly negated by c-PTIO (Fig. 5a). The other NO scavenger, methylene blue, also was able to counteract the inhibitory effect of SNP on flowering of strain 6746 (Fig. 5a, b); however, it was not as effective as c-PTIO. In 8-h daily photoperiod conditions, SNP and SNAP induced flowering in strain LP_6. This inductive effect of SNP and SNAP was negated by c-PTIO and methylene blue to different extents, the latter being used only with SNP (Fig. 5b). When provided alone, c-PTIO did not have any significant effect on the flowering of either strain (Fig. 5a, b). The inhibitory effect of SNP or SNAP on the vegetative growth of both strains was not negated by c-PTIO or methylene blue (Fig. 5c, d).

This counteracting effect of NO scavengers strengthened the view that the observed induction or inhibition of flowering in the investigated duckweeds was due to NO. Moreover, NO scavenger c-PTIO partly negated the effect of CN^-, although it is not known to scavenge CN^- (Bethke and others 2006). These results are in agreement with an earlier report of reduction of *Arabidopsis* seed dormancy (Bethke and others 2006) by both NO and CN^-. However, c-PTIO did not negate the effect of CN^- to the same extent as that of NO donors. One intriguing possibility indicated by these observations is that the flower-inducing effect of cyanide is partly through NO. This implies that it somehow stimulates NO production, as was also indicated by the observations of Bethke and others (2006). In their study, the NO scavenger c-PTIO negated both cyanide- and SNP-stimulated *Arabidopsis* seed germination. It was suggested that cyanide could break dormancy by increasing the NO concentration. This elevation of NO concentration was ascribed to either reduced breakdown of NO or an increase in NO synthesis. Moreover, cyanide could have potentiated the sensitivity of seeds to NO and acted downstream of NO production (Bethke and others 2006). Alternatively, cyanide might have increased the NO concentration by interfering with its catabolic cycle involving hemoglobin (Bethke and others 2006). Yet another possibility is that the breakdown of NO because of its reduction by cytochrome oxidase (Borutaité and Brown 1996) might

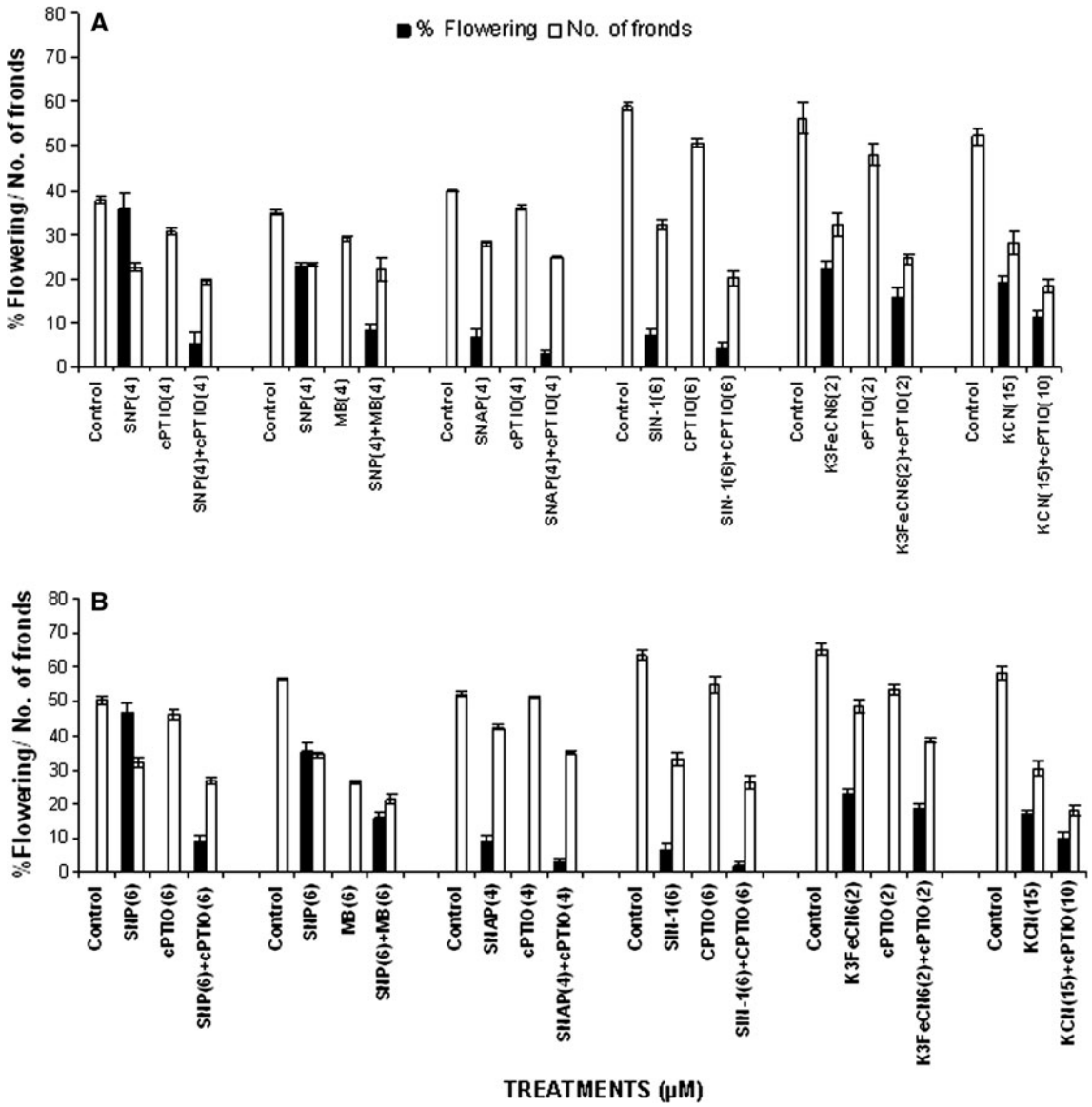

Fig. 4 Effect of c-PTIO and methylene blue, provided alone or along with SNP, SNAP, SIN-1, $K_3[Fe(CN)_6]$, or KCN on the flowering and vegetative growth of *L. aequinoctialis* 6746 (**a**) and LP_6 (**b**) under 16-h daily photoperiods

be prevented by the inhibition of cytochrome oxidase by cyanide, a well-known effect of the latter (Siegień and Bogatek 2006).

In the present study, although the possible qualitative differences in the actions of SNP, SNAP, and SIN-1 were not detected, SNP proved to be more potent than SNAP and SIN-1. This could not have been due to the amount of NO produced by each donor as SNAP generates higher amounts of NO than SNP (Wink and others 1996). In addition, SNAP is much more effective in releasing NO, both in vitro and in vivo (Floryszak-Weiczorek and others 2006). The major reason that there is more flowering with SNP appears to be that along with NO it also produces cyanide, which too induces flowering when provided alone through CN^- donors. On the other hand, both SNAP and SIN-1 decompose to produce only NO. This explanation gains more credence because the quantitative flowering response (~30%) due to SNP was almost equal to the sum total of the flowering induced by SNAP/SIN-1 (~7-9%) and CN^- (~20%). The observed higher efficacy of c-PTIO than of methylene blue was probably because of differences in their modes of action. c-PTIO reacts specifically with NO (Graziano and others 2002), whereas methylene blue inhibits NO production and/or action (Cragan 1999). $K_3[Fe(CN)_6]$ shares structural features with SNP but lacks a nitroso group and thus the ability to generate NO. Dissociation of $K_3[Fe(CN)_6]$ and KCN results in the production of free cyanides (Meeussen and others 1992).

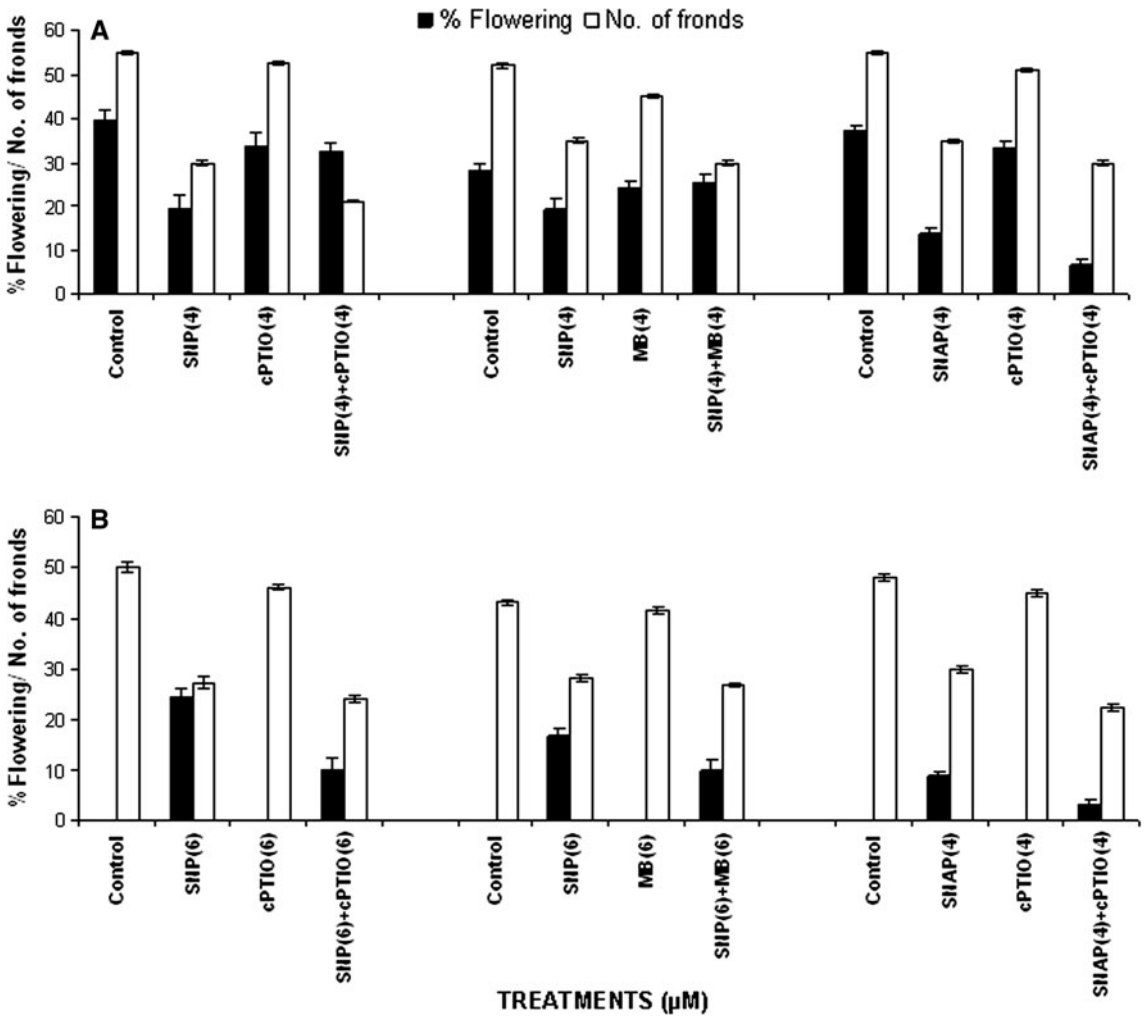

Fig. 5 Effect of c-PTIO and methylene blue, provided alone or along with SNP or SNAP, on the flowering and vegetative growth of *L. aequinoctialis* 6746 (**a**) and LP_6 (**b**) under 8-h daily photoperiods

$K_3[Fe(CN)_6]$ was much more effective than KCN in inducing flowering at their most effective concentrations. This could be because ferricyanide has six times more cyanide on a molar basis than KCN and thus generates approximately six times more HCN than KCN.

The observed differential effect of NO on flowering of *Arabidopsis* (He and others 2004) and *Lemna* (present study) is nothing new, as promotive or suppressive effects of plant hormones on flowering is known to vary among different species (Fujioka and others 1983). Thus, *Lemna*, a monocot, responding to NO in a manner different from *Arabidopsis*, a dicot, is not surprising. One possible explanation for the contrasting effect of NO on the flowering of *Arabidopsis* and *Lemna* could be that NO under inductive conditions represses flowering, whereas under noninductive conditions it induces or promotes this response. The present observations that NO induced flowering under noninductive conditions (as in *L. aequinoctialis* 6746 under long days and in *L. aequinoctialis* LP_6 under either of the photoperiods) and inhibited flowering under inductive photoperiodic conditions (as in *L. aequinoctialis* 6746 under short days) conform with this presumption. The inductive effect of NO on flowering could have been due to the initiation of a cascade of events leading to the activation of genes involved in flowering normally affected by a favorable photoperiod. It is well known that several genes affected by NO are also regulated by light (Grün and others 2006; Thomas 2006). He and others (2004) also observed that NO influences the regulation of genes responsible for photoperiod perception and the ones associated with floral transition. The inhibitory effect of NO on flowering in *L. aequinoctialis* 6746 under inductive short-day conditions could have been due to a buildup of supraoptimal levels of NO itself or component(s) of its signaling pathway. The speculation derives credence from the fact that NO is known to be a global player in the hormone signaling

pathway (Neill and others 2003) and can directly or indirectly influence signaling cascades involving Ca^{2+}, cGMP, and so forth (Durner and others 1998).

In conclusion, the present communication reports the inductive effect of NO donors on the flowering of duckweeds. The earlier reported and presently confirmed effect of CN^- on flowering of *Lemna* appears to be partly mediated through NO. However, the signaling events leading to induction of flowering of the studied plants by NO at the moment remain largely inexplicable. Therefore, it would be worthwhile to investigate the signaling pathway connecting NO and flowering. One possibility that also needs examination is that the observed flowering due to NO could be a stress response. Stress-induced flowering has been reported in many plants (Wada and Takeno 2010) and NO, besides being known to alleviate stress, causes stress in certain situations (Arasimowicz and Floryszak-Wieczorek 2007). The observed inhibitory effect of NO donors on the vegetative growth of the studied plants is also indicative of stress caused by NO donors. The present findings would necessitate a fresh outlook into relating NO with flowering.

Acknowledgments AK gratefully acknowledges the award of Junior and Senior Research Fellowships by the Council of Scientific and Industrial Research, New Delhi. The research work presented in this article was also supported by a R & D Miscellaneous Grant to SBB from the University of Delhi. We are also thankful to Iffat and Hemant, doctoral students in the laboratory, for their help in preparation of the manuscript.

References

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

Bäurle I, Dean C (2006) The timing of developmental transitions in plants. Cell 125:655–664

Besson-Bard A, Pugin A, Wendehenne D (2008) New insights into nitric oxide signaling in plants. Annu Rev Plant Biol 59:21–39

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

Bonner J, Devirian PS (1939) Growth factor requirements of four species of isolated roots. Am J Bot 26:661–665

Borutaité V, Brown GC (1996) Rapid reduction of nitric oxide by mitochondria, and reversible inhibition of mitochondrial respiration by nitric oxide. Biochem J 315:295–299

Corbesier L, Coupland G (2006) The quest for florigen: a review of recent progress. J Exp Bot 57:3395–3403

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

Durner J, Wendehenne D, Klessig DF (1998) Defence gene induction in tobacco by NO, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 95:10328–10333

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

Fujioka S, Yamaguchi I, Murofushi N, Takahashi N, Kaihara S, Takimoto A (1983) Flowering and endogenous levels of benzoic acid in *Lemna* species. Plant Cell Physiol 24:235–239

Goldstein S, Merenyi G, Russo A, Samuni A (2003) The role of oxoammonium cation in the SOD-mimic activity of cyclic nitroxides. J Am Chem Soc 125:789–795

Graziano M, Beligini MV, Lamattina L (2002) Nitric oxide improves internal iron availability in plants. Plant Physiol 130:1852–1859

Grün S, Lindermayr C, Sell S, Durner J (2006) Nitric oxide and gene regulation in plants. J Exp Bot 57:507–516

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 LJ, 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

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

Kandeler R (1984) Flowering in the *Lemna* system. Phyton 24: 113–124

Khurana JP, Cleland CF (1992) Role of salicylic acid and benzoic acid in flowering of a photoperiod-insensitive strain, *Lemna paucicostata* LP_6. Plant Physiol 100:1541–1546

Khurana JP, Maheshwari SC (1983) Effect of 8-hydroxyquinoline on flowering and endogenous levels of iron and copper in *Lemna paucicostata*, strain LP_6. Plant Cell Physiol 24:1251–1254

Krasylenko YA, Yemets AI, Blume YB (2010) Functional role of nitric oxide in plants. Russian J Plant Physiol 57:451–461

Krönke K-D, Kolb-Bachofen V (1996) Detection of nitric oxide interaction with zinc finger proteins. Meth Enzymol 269: 279–284

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

Moncada S, Higgs EA (2006) The discovery of nitric oxide and its role in vascular biology. Br J Pharmacol 147:S193–S201

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

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

Tanaka O, Cleland CF, Ben-Tal Y (1983) Effect of ferricyanide, ferrocyanide and KCN on growth and flowering in the short-day plant *Lemna paucicostata* 6746. Plant Cell Physiol 24:705–711

Thomas B (2006) Light signals and flowering. J Exp Bot 57: 3387–3393

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

Wada KC, Takeno K (2010) Stress-induced flowering. Plant Signal Behav 5:944–947

Wink DA, Cook JA, Pacelli R, DeGraff W, Gamson J, Liebmann J, Krishna MC, Mitchell JB (1996) The effect of various nitric oxide-donor agents on hydrogen peroxide-mediated toxicity, a direct correlation between nitric oxide formation and protection. Arch Biochem Biophys 331:241–248