

Nitrogen dioxide is a positive regulator of plant growth

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Atmospheric nitric oxide (NO) and nitrogen dioxide (NO₂) have long been recognized as either detrimental or beneficial for plant development. Recent research has established that NO is a phytohormone. Our present knowledge of the physiological role of NO₂ is incomplete. We do know, however, that exogenous NO₂ positively regulates the vegetative and reproductive growth of plants. We may therefore postulate that NO₂ is a positive growth regulator for plants. We are now in a position to coherently summarize what is known of NO₂ physiology; collated information on the topic is presented here.

The air or atmosphere of the Earth is a layer of gases surrounding the planet that is retained in place by the force of gravity. In addition to major gases, there are trace amounts of others, such as the nitrogen oxides. Nitrogen oxides comprise nitric oxide (NO) and nitrogen dioxide (NO₂), which occur at concentrations in the ppb to tens of ppb range (or even higher). Atmospheric NO and NO₂ have long been recognized as either detrimental or beneficial for plant development.^{1,2} However, the molecular mechanisms of action underlying the effects have remained elusive. The 2 nitrogen oxide species readily interconvert *in vivo*,³ and *in vitro*,⁴ which suggests that there are similarities in their effects on plants.

Recent research has established that NO is a phytohormone that influences diverse physiological processes in plants. This finding has provided solutions to some long-unanswered questions on

NO activities (reviewed by Santner and Estelle⁵). However, these advances pose a new question: does NO₂ play a hormonal role similar to or different from that of NO?

Our present knowledge of the physiological role of NO₂ is incomplete. We do know, however, that exogenous NO₂ positively regulates the vegetative and reproductive growth of plants.² We may therefore postulate that NO₂ is a positive growth regulator for plants. We are now in a position to coherently summarize what is known of NO₂ physiology; collated information on the topic is presented in the following discussion.

Nitrogen dioxide triggers plant growth and development

Exogenous NO₂ influences diverse physiological and developmental processes in a range of plants, including *Arabidopsis thaliana* (Table 1, see also below). Exposing plants that are well supplied with soil nitrogen to gaseous NO₂ increases the uptake of nutrients, photosynthesis, and nutrient metabolism so that shoot biomass, total leaf area, and the contents per shoot of C, N, P, K, Ca, Mg, and S (or Fe), free amino acids and crude proteins approximately double over those of control plants, with some exceptions (Table 1). Fruit yield is also increased 1.4-fold compared with control plants (Table 1). An increase in photosynthetic rate under the influence of NO₂ has also been reported by Xu et al.⁶ There are differences in NO₂ effect sizes on plant biomass among *Arabidopsis* accessions; effects were greater in accession C24 (≤ 2.8-fold) than in accession Columbia (Col-0) (≤ 1.7-fold) (Table 1).

Keywords: nitrogen dioxide, nitric oxide signaling, hormone signaling, organ growth, cell proliferation, cell enlargement, organ size control

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Table 1. Plant responses to NO₂ exposure measured as: changes in shoot biomass, total leaf area, fruit yield, and shoot contents of C, N, P, K, Ca, Mg, S (or Fe), free amino acids, crude proteins, and NO₂-derived nitrogen (NO₂-N). Shoot content responses other than NO₂-N are expressed as fold changes. NO₂-N is expressed as the proportion of N in the whole plant

Species	NO ₂ (ppb)	Fold change ^{b)}													NO ₂ -N ^d (%)	References	
		Shoot biomass	Total leaf area	Fruit yield	Element contents per shoot									Free amino acids per shoot			Crude proteins per shoot
					C	N	P	K	Ca	Mg	S	Fe					
<i>Arabidopsis thaliana</i> C24	10	2.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
<i>Arabidopsis thaliana</i> C24	50	2.8	2.6	-	2.3	2.4	2.6	2.1	2.4	1.9	2.5	-	-	-	-	4.1	2
<i>Arabidopsis thaliana</i> Col-0	50	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
<i>Arabidopsis thaliana</i> Col-0	250	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
<i>Brassica campestris</i>	250	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
<i>Corchorusolitorius</i>	50	1.7	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	19
<i>Cucumis sativus</i>	100	1.7	3.5	-	1.7	1.8	1.1	1.6	1.3	1.7	-	2.2	-	-	-	14	18
<i>Cucurbitamoschata</i>	200	1.6	1.1	-	1.8	2.1	1.3	1.8	1.5	1.9	-	2.5	-	-	-	2.2	18
<i>Helianthus annuus</i>	200	2	2.3	-	2.1	2.3	2.1	2	1.4	1.5	-	1.3	-	-	-	12	18
<i>Hibiscus cannabinus</i>	100	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
<i>Lactuca sativa</i>	50	2.4	1.5	-	2.4	2.3	2.3	2.2	2.7	2.5	-	0.9	-	-	-	0.23	18
<i>Nicotianaplumbaginifolia</i>	150	1.7	1.9	-	1.7	1.5	1.6	2	1.6	1.7	1.5	-	1.6	1.6	<3	1	
<i>Nicotianatabacum</i>	40	1.0 ^{a)}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
<i>Solanumlycopersicum</i>	20	1.7 ^{a)}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
<i>Solanumlycopersicum</i>	50	-	-	1.4	-	-	-	-	-	-	-	-	-	-	-	9.5	11

^{a)}Total biomass rather than shoot biomass.¹⁶ ^{b)}Fold change was estimated by dividing the value for NO₂-treated plants by the corresponding value for control plants. ^{c)}Plants were fed ¹⁵N-labeled NO₂ and unlabeled nitrate; the content of nitrogen derived from NO₂ (NO₂-N) in aboveground parts of plants was determined by mass spectrometry.² NO₂-N is expressed here as a percentage of total N in the whole plant N.

The fact that NO₂-derived N (NO₂-N) comprises < 5% of total plant N in some, though not all, species (Table 1), suggests that NO₂ functions as a signal to stimulate the growth of plants rather than as a N source for metabolite production.

Nitrogen dioxide positively controls cell proliferation and enlargement

We recently demonstrated that NO₂ regulates organ growth in *Arabidopsis* by controlling cell proliferation and enlargement.² We harvested, fixed, and analyzed leaves from positions 1 (the oldest) through 25 (the youngest) taken from 5-wk-old *Arabidopsis* C24 plants that had been grown in the presence (50 ppb) or absence (0 ppb) of NO₂. Leaf areas were significantly larger (1.3–8.4-fold) on NO₂-treated plants than on control plants. The size of an organ, like a leaf, is determined by the number and size of its constituent cells. Therefore, we analyzed the numbers and sizes of palisade cells in the adaxial subepidermal layer, where cells are neatly aligned in the paradermal plane throughout leaf development. NO₂ treatment significantly increased cell size by 2.0–3.2- and 1.3–1.9-fold in younger and in older leaves, respectively. Cell numbers

in younger leaves increased by 1.2–3.1-fold under NO₂ treatment, but this was not the case for older leaves.² Pearson's correlation analyses demonstrated that NO₂-induced increases in leaf areas were largely attributable to cell proliferation in developing leaves; in maturing leaves, the effect was attributable to both cell proliferation and enlargement. These results were corroborated by kinematic analysis of leaf growth in NO₂-treated and untreated plants (M. Takahashi et al., unpublished results).

Genes involved in NO₂ control of organ size

Endoreduplication (the replication of chromosomes without subsequent cell division) allows plants to increase the sizes of cells and organs. However, analysis of ploidy levels in *Arabidopsis* by flow cytometry has demonstrated that NO₂-induced cell enlargement is not correlated with endoreduplication.²

We focused on 23 cell proliferation and/or enlargement genes that are reportedly involved in increases in organ size and biomass (reviewed by⁷); we analyzed (by quantitative real-time PCR) the average transcript expression levels of these

genes in young (leaf 21–25), mature (leaf 12–20), and old (leaf 1–11) leaves from 5-wk-old *Arabidopsis* C24 plants that had been raised with or without NO₂ treatment. No single gene was constantly significantly up- or downregulated. However, NO₂-induced expression of different sets of these genes depended on the leaf developmental stage.²

Xu et al.⁶ reported that a salicylic acid (SA)-altering *Arabidopsis* Col-0 mutant *sncl* with high SA levels failed to respond to NO₂ (at 250 ppb) due to its increased antioxidant capacity.

We recently found that disruption of the *PLANT HORMONE LIKE EFFECT OF NITROGEN DIOXIDE (PHLENDI)* gene rendered *Arabidopsis* plants insensitive to NO₂; we are investigating the role of this *PHLENDI* gene in relation to the positive regulatory function of NO₂ in plants.

Similarities and differences between the effects of NO and NO₂

A shoot biomass increase similar to that induced by NO₂ was obtained in *Arabidopsis* plants exposed to NO (at 50 ppb concentration) gas.² This outcome is congruent with previous studies reporting

that treatment of *Arabidopsis* seedlings with the NO donor sodium nitroprusside enhances vegetative growth⁸ and that exposure to NO gas promotes expansion of pea leaf discs⁹ and vegetative growth of spinach.¹⁰ Thus, NO and NO₂ likely stimulate vegetative growth through similar mechanisms.

On the contrary, NO and NO₂ have the opposite effects on flowering time. Exogenous NO delays flowering of *Arabidopsis* Col-0,⁸ whereas exogenous NO₂ significantly accelerates the flowering time of *Arabidopsis* Col-0 and C24, which are early and late flowering accessions, by ~6 and 2 d, respectively.² There are also reports of similar accelerations in flowering time, and increases in flower number and fruit yield when tomatoes are treated with NO₂.¹¹

The opposite effects of NO and NO₂ on flowering time in *Arabidopsis* provide evidence that their interconversion inside and outside cells is limited. Positive effects of NO₂ on both vegetative growth and flowering mimic those of gibberellic acid (GA), which also stimulates vegetative and reproductive growth.¹²

Does NO₂ meet the criteria for classification as a phytohormone?

NO has been considered a phytohormone based on its qualitative dependence

on hormone dosage, site of action, kinetics of synthesis, metabolism and transport, and interactions with other regulators (reviewed by Shapiro¹³).

NO₂ meets some of these criteria. For example, in addition to the fact that NO₂ is either beneficial or detrimental to plants (Takahashi et al.² and references therein), our study has demonstrated: 1) that NO₂ induces increases in organ size and biomass at concentrations as low as 10 ppb (Table 1), and 2) that higher levels (≥ 200 ppb) of NO₂ significantly inhibit the growth of *Arabidopsis*.² Therefore, the effects are qualitatively dependent on NO₂ dosage.

NO reportedly fits the criteria required of a phytohormone: ease of transport (due to small molecular size) and rapid diffusion through biological membranes.¹³ NO₂ also meets these criteria. There are interactions between the effects of NO₂ and hormones such as SA⁶ and GA (see above). Besides the involvement of NO₂ in protein tyrosine nitration, an important posttranslational protein modification,¹³ the metabolism of NO₂ in plants has also been reported.¹

Although more than half a dozen NO production pathways or routes¹⁴ exist in plants, and many studies have reported on NO₂ emission by plants, in

vivo biosynthetic pathways for NO₂ are largely unexplored, other than reports on the enzymatic production of NO₂.¹⁵ However, in vitro production of NO₂ via single-electron oxidations of nitrite by heme proteins such as horseradish peroxidase and leukocyte peroxidases, *Arabidopsis* hemoglobins, and human hemo/myoglobins have been demonstrated (Shapiro 2005,¹³ and references therein). However, the extent to which these enzyme-mediated reactions contribute to the in vivo production of NO₂ remains unknown.

The action sites and receptors of NO₂ in plants have yet to be identified. Our demonstration of induced selective nitration of specific proteins following exposure of *Arabidopsis* plants to exogenous NO₂ (M Takahashi et al., unpublished results), and a report of NO₂ involvement in protein tyrosine nitration as a proximal intermediate in plants¹³ make it likely that nitrated proteins are a target of NO₂ action. In summary, whether NO₂ functions as a hormone has yet to be determined. Nevertheless, almost all current experimental data support such a role.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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