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Differential responses of *Arabidopsis thaliana* accessions to atmospheric nitrogen dioxide at ambient concentrations

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To better understand the response of plants to atmospheric nitrogen dioxide (NO_2), we investigated biomass accumulation in 3 accessions of *Arabidopsis thaliana*: C24, Columbia (Col-0), and Landsberg erecta (Ler). Plants were grown in NO_2-free air for 1 week after sowing, followed by 3 (Col-0 and Ler) to 4 (C24) weeks in air with or without NO_2 (10 or 50 ppb). NO_2 treatment increased the biomass of all 3 accessions to varying extents. Treatment with 10 ppb NO_2 increased shoot biomass in C24, Col-0, and Ler by 3.2-, 1.4-, and 2.3-fold, respectively, compared with control. Treatment with 50 ppb gave similar increases, except in C24 (2.7-fold). The physiological, evolutionary, and genetic significance of these results are discussed below.

We reported previously that the exogenous application of nitrogen dioxide (NO_2) influences diverse physiological processes including nutrient uptake, photosynthesis, nitrogen metabolism, cell proliferation, cell enlargement, biomass accumulation, and the acceleration of flowering.[1,2] Therefore, we hypothesized that NO_2 positively regulates plant growth.[3] This is based on previous studies using concentrations of 50-200 ppb NO_2, which correspond to those in polluted urban air (OECD 2002).[4] It is important to understand whether plants respond to ground levels of NO_2 to increase our understanding of the biological and evolutionary relevance of this phenomenon. We reported previously that the *Arabidopsis thaliana* (Arabidopsis) C24 accession responded positively to treatment with 10 ppb NO_2,[1] which corresponds to the concentrations in rural air. In this study, we confirmed our previous observations using 2 additional Arabidopsis accessions: Columbia (Col-0) and Landsberg erecta (Ler). Importantly, these 3 accessions all possess distinct genetic backgrounds.[5]

Plants were grown and treated with NO_2, and shoot biomass was determined as reported previously.[1] Briefly, *Arabidopsis thaliana* (L.) Heynh. C24, Col-0, and Ler seeds were surface-sterilized with 1.0% sodium hypochlorite, rinsed in pure water (18.0 MΩ), and sown in a rectangular plastic tray (22 × 5 × 20 cm width, height, and depth, respectively) containing vermiculite and perlite (1:1, v/v), and held in a glass-walled NO_2-exposure chamber (1.3 × 1.0 × 0.65 min width, height, and depth, respectively; NOX-1000-SCII, Nippon Medical and Chemical Instruments Co) in a growth room. The temperature, CO_2 concentration, and relative humidity in the chamber were set at 22 ± 0.1°C, 360 ± 30 ppm, and 70 ± 1.5%, respectively. The air entering the chamber (at 1 L min^{-1}) from outside was stripped of NO, NO_2, and O_3 (to 0 ppb) using activated charcoal and $NaMnO_4$ (PureliteE30; Nippon Puretec Co). The tray was placed under fluorescent light (40 µmol photons m^2 s^{-1}) with a 16:8 h, light:dark cycle, and seeds were allowed to germinate and grow for 1 wk in air lacking NO_2. NO_2 was then added to the air entering the exposure chamber at concentrations of 0, 10 ± 0.2, or 50 ± 0.3 ppb. Seedlings were irrigated twice weekly with half-strength inorganic salts in Murashige and Skoog medium,[6] and grown for an additional 3–4 wk in the chamber. Plants were then harvested and shoot biomass was calculated. The results are summarized in **Table 1**.

All accessions responded positively to treatment with 10 ppb NO_2 with a characteristic response. Shoot biomass in the C24, Col-0, and Ler accessions was increased by 3.2-, 1.4-, and 2.3-fold, respectively, compared with control. This increase was similar to, or even higher than, the increase observed after treatment with 50 ppb: treatment increased shoot biomass by 2.7-, 1.7-, and 2.5-fold in C24, Col-0, and Ler, respectively (**Table 1**). The increases in shoot biomass, except that in Col, between 0 and 10 ppb were statistically significant (**Table 1**). Therefore, plants are highly sensitive to NO_2, whereby a concentration as low as 10 ppb

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Table 1. Effects of nitrogen dioxide (NO_2) concentration on shoot biomass yield in *Arabidopsis thaliana* accessions C24, Columbia (Col-0), and Landsberg erecta (Ler)

NO2 concentration (ppb)	Average dry wt. of shoots (mg per plant) ± SD (n)		
	C24	Col-0	Ler
0	25.2 ± 6.8 (4)	14.3 ± 2.5 (5)	11.3 ± 2.6 (5)
10 ± 0.2	80.8 ± 17 (10) ***	20.2 ± 5.5 (15)	26.2 ± 4.0 (15) ***
50 ± 0.3	68.5 ± 9.3 (10) ***	24.2 ± 5.5 (5) *	28.2 ± 2.3 (5) ***

C24, Col-0, and Ler plants were harvested at 5, 4, and 4 wk after sowing, respectively. Statistical significance assessed using the Dunnett *t* test: *, $P < 0.05$; ***, $P < 0.001$. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA USA).

significantly stimulated the accumulation of shoot biomass in all 3 Arabidopsis accessions.

The weighted mean NO_2 concentration in cities from over 141 countries is reported to be 50.6 $\mu g/m^3$ (~27 ppb).[7] Approximately half of atmospheric NO_2 is derived from human activities such as the combustion of fossil fuels, and the remaining half is derived from non-anthropogenic sources such as soil microbial emissions.[8] Therefore, it is conceivable that 10 ppb is a reasonable estimate of ground levels of NO_2 derived from natural sources, which is consistent with reported NO_2 values (5-10 ppb) in rural air.[9,10] Our present finding that Arabidopsis plants responded to NO_2 at ambient concentrations suggests that atmospheric NO_2 at the ground level exerts physiological effects on plants by acting as a positive growth signal.

Unlike the current Earth atmosphere, that of primitive Earth is unlikely to have contained nitrogen oxides (nitric oxide [NO] and NO_2), because it contained nitrogen but not free oxygen.[11,12] Geochemical studies indicate that appreciable increases in atmospheric oxygen occurred in the Archean eon ~2.5-3 billion years ago,[11,12,13] and in the Proterozoic eon ~0.75 billion years ago.[13] The former increase is known as the great oxidation event (GOE), which was triggered by oxygen production by oxygenic photosynthetic cyanobacteria. Furthermore, the ancestors of modern denitrifiers are likely to have arisen before,[14] or at the same time as,[15] the GOE and released NO into the atmosphere. In atmospheric chemistry, the rapid interconversion of NO and NO_2 (the Leighton relationship) involves the oxidation of NO by ozone and the photolysis of NO_2 by sunlight.[16] The ancestors of land plants appeared ~0.4-0.5 billion years ago[17]; therefore, atmospheric NO_2 is likely to have influenced the evolution of plants, given that air is important for the coevolution of life and Earth.[18] However, when and how plants evolved traits to use atmospheric NO_2 as a growth signal, and whether the ancestors of terrestrial plants such as green algae[19] possess this trait remains unclear.

The NO_2-induced shoot biomass increase is probably a multigenic trait; therefore, quantitative trait locus (QTL) analysis followed by the identification of the specific genes that underlie QTL is important to understand the molecular mechanisms of this plant behavior. Our present finding of variation in this trait among Arabidopsis accessions might provide an important resource for QTL analysis using recombinant inbred lines[19] to clarify the molecular mechanisms and evolutionary origin of the genes involved in this NO_2-induced plant behavior.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Takahashi M, Furuhashi T, Ishikawa N, Horiguchi G, Sakamoto A, Tsukaya H, Morikawa H. Nitrogen dioxide regulates organ growth by controlling cell proliferation and enlargement in Arabidopsis. New Phytol 2014; 201:1304-15; PMID:24354517; http://dx.doi.org/10.1111/nph.12609
2. Takahashi M, Nakagawa M, Sakamoto A, Ohsumi C, Matsubara T, Morikawa H. Atmospheric nitrogen dioxide gas is a plant vitalization signal to increase plant size and the contents of cell constituents. New Phytol 2005; 168:149-54; PMID:16159329; http://dx.doi.org/10.1111/j.1469-8137.2005.01493.x
3. Takahashi M, Morikawa H. Nitrogen dioxide is a positive regulator of plant growth. Plant Signal Behav 2014; 9: http://dx.doi.org/10.4161/psb.28033; PMID:24525764
4. OECD environmental data compendium 2002. Paris: Organization for Economic Cooperation and Development, 52-5.
5. Sheldon CC, Burn JE, Perez PP, Metzger J, Edwards JA, Peacock WJ, Dennis ES. The FLF MADS box gene: a repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell 1999; 11:445-58; PMID:10072403
6. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 1962; 15:473-7; http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x
7. http://www.nationmaster.com/index.php.
8. Aneja VP, Roelle PA, Murray GC, Southerland J, Erisman JW, Fowler D, Asman WAH, Patni N. Atmospheric nitrogen compounds II: emissions, transport, transformation, deposition and assessment. Atmos Environ 2001; 35:1903-11; http://dx.doi.org/10.1016/S1352-2310(00)00543-4
9. Campbell GW. Measurements of nitrogen dioxide concentrations at rural sites in the United Kingdom using diffusion tubes. Environ Pollut 1988; 55:251-70; PMID:15092495; http://dx.doi.org/10.1016/0269-7491(88)90249-7
10. Shooter D, Brimblecombe P, Brasell MR. Ground level nitrogen dioxide concentrations in the rural Waikato Valley, New Zealand. Environ Monit Assess 1993; 25:159-68; PMID:24225665; http://dx.doi.org/10.1007/BF00549137
11. Kaufman AJ, Johnston DT, Farquhar J, Masterson AL, Lyons TW, Bates S, Anbar AD, Arnold GL, Garvin J, Buick R. Late Archean biospheric oxygenation and atmospheric evolution. Science 2007; 317:1900-3; PMID:17901329; http://dx.doi.org/10.1126/science.1138700
12. Crowe SA, Døssing LN, Beukes NJ, Bau M, Kruger SJ, Frei R, Canfield DE. Atmospheric oxygenation three billion years ago. Nature 2013; 501:535-8; PMID:24067713; http://dx.doi.org/10.1038/nature12426
13. Frei R, Gaucher C, Poulton SW, Canfield DE. Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. Nature 2009; 461:250-3; PMID:19741707; http://dx.doi.org/10.1038/nature08266
14. Garvin J, Buick R, Anbar AD, Arnold GL, Kaufman AJ. Isotopic evidence for an aerobic nitrogen cycle in the latest Archean. Science 2009; 323:1045-8; PMID:19229033; http://dx.doi.org/10.1126/science.1165675
15. Betlach MR. Evolution of bacterial denitrification and denitrifier diversity. Antonie Van Leeuwenhoek 1982; 48:585-607; PMID:6762849; http://dx.doi.org/10.1007/BF00399543
16. Wallington TJ, Nielsen OJ. Atmospheric degradation of anthropogenic molecules. In: Handbook of environmental chemistry. Vol. 2. Reactions and processes. Part 1. Hutzinger O, ed. Berlin, Germany: Springer-Verlag, 1998: 64-96.
17. Lewis LA, McCourt RM. Green algae and the origin of land plants. Am J Bot 2004; 91:1535-56; PMID:21652308; http://dx.doi.org/10.3732/ajb.91.10.1535
18. Nisbet EG, Sleep NH. The habitat and nature of early life. Nature 2001; 409:1083-91; PMID:11234022; http://dx.doi.org/10.1038/35059210
19. Koornneef M, Alonso-Blanco C, Vreugdenhil D. Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu Rev Plant Biol 2004; 55:141-72; PMID:15377217; http://dx.doi.org/10.1146/annurev.arplant.55.031903.141605