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# Progress and Problems in Producing Nitrogen Dioxide-Philic Plants

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*Dear Editor,*

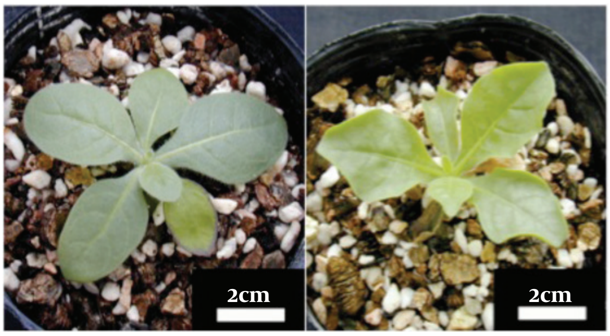
The recently published articles in the Iranian Journal of Biotechnology inspired us to write this short communication on nitrogen dioxide $NO_2$-philic plants (1).

To cope with environmental pollution, we thought (still think) that development of methods that mitigate pollution is an urgent issue, and that $NO_2$-philic plants that can grow with $NO_2$ as the sole nitrogen source are instrumental for the implementation of mitigation of air pollution.

The main point of this idea is that mitigation, rather than monitoring or observation, of environmental pollution is of primary importance. In other words, our point is that "plants that rely on pollution", rather than "plants that are resistant to pollution", are more meaningful to implement air remediation by plants. In the very beginning of this study, we attempted screening of such plants among naturally occurring plants (2). Screening study revealed that several plant species have a high ability to uptake and assimilate $NO_2$ (2). But it was not high enough for these species to grow with $NO_2$ as the sole nitrogen source (3). We also performed genetic engineering of nitrite reductase (NiR), which is a second enzyme of nitrate reduction pathway and a key enzyme of assimilation of $NO_2$. We have produced genetically modified plants of *Arabidopsis thaliana* (a model plant species) (4) and *Rhaphiolepis umbellata* (a roadside tree species) (5) that significantly bear higher NiR activities compared with non-transformed control plants. However, their ability to uptake $NO_2$ was less than two times higher than that of non-tranformed plants, and again this was not enough for them to be $NO_2$-philic (3).

We also investigated the growth of plants in the presence of $NO_2$ as the sole nitrogen source, using *Nicotiana plumbaginifolia* as a test species. The Plants were grown in the complete absence of root nitrogen under exposure to 0, 0.15, 1.0 and 4.0 ppm $NO_2$ and natural light at 22°C for 9 – 13 weeks. Control plants were grown with 10 mM $KNO_3$ in ambient air containing < 0.05 ppm $NO_2$. Plants grown at 0 ppm $NO_2$ died after 6 weeks. The relative growth rate in biomass (RGR) of plants grown at 1.0 Pm $NO_2$ and that of those grown at 4.0 ppm $NO_2$ were very similar to the RGR of control plants (about 0.1.day $^{-1}$). However, plants grown at 1.0 and 4.0 ppm $NO_2$ exhibited some morphological abnormalities in leaves (Figure 1, Nakagawa, Takahashi and Morikawa, unpublished results), that indicates the toxic effects of $NO_2$. Interestingly, plants grow n at 0.15 ppm $NO_2$ did not show such damages and grew normally up to 13 weeks. Furthermore, we also noticed that $NO_2$ at 0.15 ppm is somewhat stimulatory for the growth of plants.

**Figure 1.** An Attempt to Produce a $NO_2$-Philic Plant




Five-week-old *Nicotiana plumbaginifolia* plants that were grown either with 1 ppm $NO_2$ as the sole nitrogen source (right), or with 10 mM $KNO_3$ in ambient air (<0.05 ppm $NO_2$) (left).

Clearly, the effect of $NO_2$ on plant growth changes as a function of its concentrations; it was inhibitory at high concentrations but stimulatory at low concentrations. Thus, clarification of these toxic and stimulatory effects of

$NO_2$ may provide an important key to produce $NO_2$-philic plants. The investigation on the stimulatory effect of $NO_2$ led to the finding of a plant-hormone like effect of $NO_2$ on the growth and development of plants (6). Ambient concentrations of $NO_2$ stimulated growth and biomass production of plants that were well fed root nitrogen. Uptake per plant of nutrients was also stimulated. Carbon and nitrogen metabolisms also were stimulated by the presence of $NO_2$. This effect was confirmed in several plant species including Arabidopsis and horticultural species (3).

The investigation on the toxic effect of $NO_2$ led us to the investigation on metabolic fate of $NO_2$ in plants, and to the finding of novel oxidized nitrogen compounds including nitro- and nitroso-organic compounds formed from $NO_2$ (7, 8). We also found that fumigation of Arabidodpsis plants with a high concentration (4 - 40 ppm) of $NO_2$ induces nitration of specific proteins involved in photosystem II in chloroplasts (Takahashi and Morikawa, submitted). Protein tyrosine nitration is an important posttranslational protein modification, and thus protein nitration induced by $NO_2$ may be of important biological relevance. Currently, which one(s) of such nitro- and nitroso-organic compounds including nitrated proteins is responsible to the toxic effect of $NO_2$ has yet to be identified. Nonetheless, the abovementioned results on these two effects of $NO_2$ implicate that exogenous $NO_2$ plays a distinct role in cellular signaling. Clarification of the mechanisms and identification of genes involved in both toxic and stimulatory effects of $NO_2$ will pave the way for production of $NO_2$-philic plants for air remediation.

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## Authors' Contribution

Misa Takahashi developed the original idea and the protocol, abstracted and analyzed data, wrote the manuscript, and are guarantor. Hiromichi Morikawa developed the idea, abstracted, and wrote the manuscript together with M.T. Makiko Nakagawa mainly contributed to the analysis of data.

## Financial Disclosure

No relevant financial interests or financial conflicts within the past 5 years and for the foreseeable future.

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