Nocturnal Uptake and Assimilation of Nitrogen Dioxide by C3 and CAM Plants

Misa Takahashi[a,b,*], Daisuke Konaka[a], Atsushi Sakamoto[a,b], and Hiromichi Morikawa[a,b]

[a] Department of Mathematical and Life Science, Graduate School of Science, Hiroshima University, Higashi-Hiroshima 739-8526, Japan. Fax: +81-82-424-0749.
E-mail: mtakahas@sci.hiroshima-u.ac.jp

[b] Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan

* Author for correspondence and reprint requests

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In order to investigate nocturnal uptake and assimilation of NO_2 by C3 and crassulacean acid metabolism (CAM) plants, they were fumigated with 4 μl l^{-1} ^{15}N-labeled nitrogen dioxide (NO_2) for 8 h. The amount of NO_2 and assimilation of NO_2 by plants were determined by mass spectrometry and Kjeldahl-nitrogen based mass spectrometry, respectively. C3 plants such as kenaf (*Hibiscus cannabinus*), tobacco (*Nicotiana tabacum*) and ground cherry (*Physalis alkekengi*) showed a high uptake and assimilation during daytime as high as 1100 to 2700 ng N mg^{-1} dry weight. While tobacco and ground cherry strongly reduced uptake and assimilation of NO_2 during nighttime, kenaf kept high nocturnal uptake and assimilation of NO_2 as high as about 1500 ng N mg^{-1} dry weight. Stomatal conductance measurements indicated that there were no significant differences to account for the differences in the uptake of NO_2 by tobacco and kenaf during nighttime. CAM plants such as *Sedum* sp., *Kalanchoe blossfeldiana* (kalanchoe) and *Aloe arborescens* exhibited nocturnal uptake and assimilation of NO_2. However, the values of uptake and assimilation of NO_2 both during daytime and nighttime was very low (at most about 500 ng N mg^{-1} dry weight) as compared with those of above mentioned C3 plants. The present findings indicate that kenaf is an efficient phytoremediator of NO_2 both during daytime and nighttime.

Key words: Assimilation of NO_2, CAM Plant, Kenaf, Stomatal Conductance

Introduction

Plants take up nitrogen dioxide (NO_2), a major urban air pollutant, and assimilate its nitrogen to organic compounds. Accordingly, several investigations proposed that plants should be utilized to reduce the pollutant concentrations in the atmosphere (Hill, 1971; Yoneyama *et al.*, 1979, 2002; Okano *et al.*, 1986; Weber *et al.*, 1995; Morikawa *et al.*, 1998, 2002, 2003a,b; Takahashi *et al.*, 2001; Morikawa and Erkin, 2003). NO_2 has been thought to be taken up through stomata (Saxe, 1986; Thoene *et al.*, 1991; Neubert *et al.*, 1993). The abilities of NO_2 uptake by plants varied by environmental conditions such as air temperature, relative humidity, illumination intensity, wind, CO_2 concentration and NO_2 concentration (Srivastava *et al.*, 1974; Geßler *et al.*, 2000). Since the opening of stomata is regulated by light, the amount of NO_2 taken up by plants should be reduced during nighttime, and the amount of NO_2 taken in the dark condition is reportedly 10 to 36% of its light-condition value in various plants species (Srivastava *et al.*, 1974; Saxe, 1986; Thoene *et al.*, 1991; Neubert *et al.*, 1993; Segschneider *et al.*, 1995) except for spinach (Kaji *et al.*, 1980). Spinach is reported to take up NO_2 at the same level during daytime and nighttime (Kaji *et al.*, 1980). In this study we investigated nocturnal uptake and assimilation of NO_2 by C3 and crassulacean acid metabolism (CAM) plants. Since CAM plants are known to open stomata and take up carbon dioxide nocturnally, we expected that these plants could exhibit an active nocturnal uptake and assimilation of NO_2.

Material and Methods

Plant materials

Seeds of tobacco (*Nicotiana tabacum*) and kenaf (*Hibiscus cannabinus*) were sown in pots containing vermiculite and perlite (1:1, v/v) and grown at 22 ± 0.3 °C and 70 ± 4% relative humidity for 6 to 9 weeks in a growth chamber under the light of 100 μmol photons m^{-2} s^{-1} (16 h light/8 h dark). Ground cherry (*Physalis alkekengi* var. *franchetii*),

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Sedum sp., kalanchoe (*Kalanchoe blossfeldiana*) and *Aloe arborescens* were purchased from local shops and were maintained in a greenhouse for 1–2 weeks before use.

Fumigation with NO_2

Plants were fumigated with 4 μl l^{-1} ^{15}N-labeled NO_2 at 22.0 ± 0.3 °C and a relative humidity of 70 ± 4% for 8 h in a fumigation chamber as described previously (Morikawa *et al.*, 1998). Fumigation in the light (70 μmol photons m^{-2} s^{-1}) was performed during daytime (9:00 am–5:00 pm), and that in the dark was performed during nighttime (9:00 pm–5:00 am). Prior to NO_2 fumigation, plants were transferred to the fumigation chamber and adapted in the dark or light for 12 h for daytime or nighttime fumigation, respectively.

Analysis of total, Kjeldahl and inorganic nitrogen derived from NO_2

After fumigation with NO_2, the leaves were harvested and lyophilized. The total nitrogen (reflecting NO_2 uptake) was measured by an EA-MS analyzer consisting of an elemental analyzer (EA1108 CHNS/O; Fisons Instruments, Milan, Italy) directly connected to a mass spectrometer (Delta S; Thermo-Finnigan, Bremen, Germany) as reported previously (Morikawa *et al.*, 2004). The Kjeldahl-N based mass spectrometry by the EA-MS analyzer was performed to determine the reduced nitrogen (reflecting NO_2 assimilation) in leaves as reported elsewhere (Takahashi *et al.*, 2003; Morikawa *et al.*, 2004).

Nitrate-nitrogen and nitrite-nitrogen derived from NO_2 in fumigated leaves were determined as reported previously (Kawamura *et al.*, 1996; Takahashi *et al.*, 2003; Morikawa *et al.*, 2004). Briefly, the amount of nitrate and nitrite content in fumigated leaf samples was first determined by capillary electrophoresis (Kawamura *et al.*, 1996). The atomic percentage of ^{15}N in the nitrate and nitrite was then determined as follows; nitrate and nitrite were collected using ion exchange chromatography and then reduced to ammonia by Devarda's alloy. The ammonia was then concentrated by the Conway diffusion method (Conway and Byrne, 1933) and the concentrated ammonia was analyzed using the EA-MS analyzer.

Measurement of stomatal conductance

Stomatal conductance to water vapour was measured with fully-expanded leaves of kenaf and tobacco using an LI-6400 (LI-COR, Lincoln, NE) under the same conditions for fumigation describe above in the presence or absence of NO_2.

Results

Table I summarizes the uptake and assimilation of NO_2 upon fumigation with 4 μl l^{-1} NO_2 for 8 h in the light during daytime and in the dark during nighttime. Three C3 plants such as kenaf, tobacco and ground cherry showed a similar daytime up-

Table I. Uptake and assimilation of nitrogen dioxide by various plants during daytime and nighttime.

Species	Uptake [ng N mg^{-1} dry weight]		Assimilation [ng N mg^{-1} dry weight]	
	Daytime	Nighttime	Daytime	Nighttime
Hibiscus cannabinus (kenaf)	2705 ± 214	1546 ± 618	2755 ± 215	1780 ± 117
Nicotiana tabacum (tobacco)	1752 ± 941	221 ± 113	1140 ± 574	244 ± 182
Physalis alkekengi (ground cherry)	2479 ± 415	155 ± 40	1703 ± 181	137 ± 29
Sedum sp.	452 ± 238	358 ± 31	298 ± 194	277 ± 67
Kalanchoe blossfeldiana (kalanchoe)	77 ± 17	36 ± 11	64 ± 16	29 ± 11
Aloe arborescens	43 ± 10	23 ± 4	29 ± 7	13 ± 2

Data represent mean ± SD. The number of replicates is 4 to 17.

take of NO_2 as high as 1800 to 2700 ng N mg^{-1} dry weight. Nighttime uptake of NO_2 by tobacco and ground cherry was greatly decreased (less than 13% of the respective daytime value). In a sharp contrast, kenaf exhibited a noticeable nocturnal uptake of NO_2 more than 50% of its daytime value.

All of the three C3 plants showed a daytime assimilation of NO_2 more than 65% of the daytime uptake. This result keeps line with our previous report that more than 50% of NO_2 taken up into plant leaves is assimilated to organic nitrogen (Takahashi *et al.*, 2003). Nighttime assimilation of NO_2 by tobacco and ground cherry was 8–21% of respective daytime value. To our surprise, kenaf exhibited a nocturnal assimilation of NO_2 as high as 1780 ng N mg^{-1} dry weight on average, which corresponded to well more than 50% of the daytime value of this plant.

On the other hand, obligate CAM plants studied here, kalanchoe and *A. arborescens*, and a C3/CAM intermediate plant, *Sedum* sp., appeared to have a low NO_2 uptake both during daytime and nighttime; their uptake level (at most 500 ng N mg^{-1} dry weight) was more or less one or two orders of magnitude less than that of the C3 plants investigated here (see Table I). Their nocturnal assimilation of NO_2 was close to 50% or even greater than respective daytime value. This is an indication that although the absolute values of NO_2 uptake by these CAM plants are low, their daytime and nighttime NO_2 assimilation activity does not largely differ.

Table II summarizes the amount of nitrate-nitrogen and nitrite-nitrogen derived from NO_2 in the leaves of kenaf and tobacco after daytime and nighttime fumigation with NO_2 for 8 h. Since the nitrogen derived from NO_2 taken up into plant body is rapidly converted into organic nitrogen (Morikawa *et al.*, 2004), the nitrate-nitrogen and nitrite-nitrogen detected in kenaf and tobacco leaves shown in this table can be considered to reflect "extra" NO_2 that remained unassimilated.

The level of nitrate-nitrogen in kenaf after nighttime fumigation was approximately four times greater than that after daytime fumigation, suggesting that the level of "unassimilated NO_2-nitrogen" was four times greater after nighttime fumigation than after daytime fumigation. It is likely that kenaf retains a high capability of NO_2 uptake even in the dark during nighttime. In tobacco leaves, the level of nitrate-nitrogen after nighttime fumigation was approximately four times less than that after daytime fumigation. This suggests that tobacco has a limited NO_2 uptake capability during nighttime.

The level of nitrate-nitrogen after daytime fumigation was almost the same between kenaf and tobacco, whereas it was more than ten times greater in kenaf than in tobacco after nighttime fumigation. This agrees with the abovementioned suggestion that kenaf leaves retain a high uptake capability of NO_2 in the dark during nighttime.

On the other hand, no or very low level of nitrite-nitrogen was detected in both, plant leaves after daytime- and nighttime-fumigation. Since some part of NO_2 taken up into plant body is thought to be converted to nitrite (Wellburn, 1990), the present finding of no or very low nitrite implies that nitrite formed from NO_2 was rapidly detoxified (assimilated) in the plant leaves both during daytime and nighttime.

Fig. 1 shows the changes in stomatal conductance observed in the leaves of kenaf and tobacco before and after fumigation with NO_2. Stomatal conductance of kenaf in the light during daytime was 0.3 ± 0.09 mol H_2O m^{-2} s^{-1} before NO_2 fumigation. Upon the onset of NO_2 fumigation the

Table II. Nitrate-nitrogen and nitrite-nitrogen derived from NO_2 detected in the leaves of kenaf and tobacco after fumigation with ^{15}N-labeled NO_2 for 8 h in the light during daytime and in the dark during nighttime.

	Nitrate-N [ng N mg^{-1} dry weight]		Nitrite-N [ng N mg^{-1} dry weight]	
	Light during the day	Dark during the night	Light during the day	Dark during the night
Kenaf	63.8 ± 14.8[a]	246 ± 46	n.d.[b]	n.d.
Tobacco	87.4 ± 11.9	22.2 ± 33.3	1.33 ± 1.54	n.d.

[a] Data represent means of three replicates ± SD.
[b] n.d., not determined.

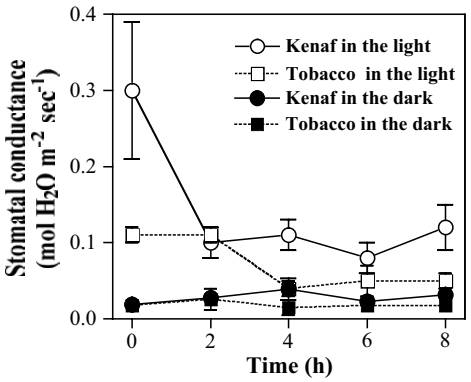

Fig. 1 Stomatal conductance of kenaf and tobacco leaves in the light and dark before and after NO_2 fumigation. Data represent mean of the values of three leaves, each of which was taken from different individual. The bars show represent SD when their value exceeds the size of the circle or square symbol.

stomatal conductance immediately decreased to about 30% of the value before the fumigation, and thereafter it did not change appreciably throughout the experiment. In the dark, kenaf showed stomatal conductance as low as 0.02 to 0.04 mol H_2O m^{-2} s^{-1} both in the presence and absence of atmospheric NO_2.

In the light tobacco showed a stomatal conductance of 0.11 ± 0.01 mol H_2O m^{-2} s^{-1} before NO_2 fumigation and the first 2 h of NO_2 fumigation, and thereafter it decreased to 0.04 mol H_2O m^{-2} s^{-1} or less, which is very close to the level of kenaf in the dark. In the dark, the level of stomatal conductance of tobacco was similar to or slightly smaller than that of kenaf in the dark (see Fig. 1).

Discussion

Our present finding that tobacco and ground cherry showed a very limited nighttime uptake of NO_2 keeps line with the results reported by the previous authors with various plant species (Srivastava *et al.*, 1974; Saxe, 1986; Thoene *et al.*, 1991; Neubert *et al.*, 1993; Segschneider *et al.*, 1995; Morikawa *et al.*, 1998).

Furthermore, our finding that kenaf exhibited a noticeable nocturnal uptake and assimilation of NO_2 more than 50% of its respective daytime value is in line with the result of spinach reported by previous authors (Kaji *et al.*, 1980).

Since CAM plants are known to open stomata and take up carbon dioxide nocturnally, we expected that these plants could exhibit an active nocturnal uptake and assimilation of NO_2. Those CAM plants such as *Sedum* sp., kalanchoe and *A. arborescens* studied here did show nocturnal uptake and assimilation of NO_2 that was close to 50% or even higher than respective daytime value, confirming that they possess a similar activity during day- and nighttime as expected. However, uptake and assimilation of NO_2 by the CAM plants appeared to be one or two orders of magnitude less than that of the C3 plants investigated here (see Table I).

The NO_2-nitrogen taken up into the plant body is rapidly converted into organic nitrogen (Morikawa *et al.*, 2004). The present analysis of inorganic nitrogen derived from NO_2 (Table II) showed that upon nighttime fumigation kenaf leaves accumulate greater level of "unassimilated NO_2-nitrogen" than tobacco leaves. This finding is in accordance with the above mentioned conclusion that the capability of kenaf to take up NO_2 nocturnally is much greater than that of tobacco.

NO_2 has been thought to be taken up through stomata (Saxe, 1986; Thoene *et al.*, 1991; Neubert *et al.*, 1993), and therefore stomatal resistance to diffusion of NO_2 is thought to be the main determinating factor for the uptake of NO_2 by plants (Saxe, 1986; Neubert *et al.*, 1993). Our present study of stomatal conductance (Fig. 1) indicated that kenaf possesses a high level of stomatal conductance during daytime fumigation, but that its nighttime value was very similar to that of tobacco. It has been reported that NO_2 is taken up not only through stomata but also the outer leaf surface such as the cuticle layer (Kisser-Priesack *et al.*, 1987; Larcher, 1995; Geßler *et al.*, 2002). It is therefore likely that the cuticle layer of kenaf leaves is somehow more permeable to NO_2 than that of tobacco leaves.

Our present finding that kenaf keeps about 50% of its daytime level may in turn suggest that the present illumination condition (70 μmol photons m^{-2} s^{-1}) may not be strong enough for NO_2 assimilation. We therefore performed fumigation experiments under natural light using a similar fumigation chamber that was installed in a confined greenhouse. It was found that the daytime uptake of NO_2 by kenaf under the natural light was very similar to the values shown in Table I. This result implies that kenaf plants had enough reducing power for NO_2 assimilation supplied by the pre-

sent fluorescent lamp illumination. This behavior of kenaf plants deserves further investigation.

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