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

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In order to investigate nocturnal uptake and assimilation of NO2 by C3 and crassulacean acid metabolism (CAM) plants, they were fumigated with 4 µlL-1 15N-labeled nitrogen dioxide (NO2) for 8 h. The amount of NO2 and assimilation of NO2 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 NO2 during nighttime, kenaf kept high nocturnal uptake and assimilation of NO2 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 NO2 by tobacco and kenaf during nighttime. CAM plants such as Sedum sp., Kalanchoe blossfeldiana (kalanchoe) and Aloe arborescens exhibited nocturnal uptake and assimilation of NO2. However, the values of uptake and assimilation of NO2 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 NO2 both during daytime and nighttime.

Key words: Assimilation of NO2, CAM Plant, Kenaf, Stomatal Conductance

Introduction

Plants take up nitrogen dioxide (NO2), 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). NO2 has been thought to be taken up through stomata (Saxe, 1986; Thoene et al., 1991; Neubert et al., 1993). The abilities of NO2 uptake by plants varied by environmental conditions such as air temperature, relative humidity, illumination intensity, wind, CO2 concentration and NO2 concentration (Srivastava et al., 1974; Geßler et al., 2000). Since the opening of stomata is regulated by light, the amount of NO2 taken up by plants should be reduced during nighttime, and the amount of NO2 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 NO2 at the same level during daytime and nighttime (Kaji et al., 1980). In this study we investigated nocturnal uptake and assimilation of NO2 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 NO2.

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 NO₂

Plants were fumigated with 4 µl l⁻¹ ¹⁵N-labeled NO₂ 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 NO₂ 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 NO₂

After fumigation with NO₂, the leaves were harvested and lyophilized. The total nitrogen (reflecting NO₂ 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 NO₂ assimilation) in leaves as reported elsewhere (Takahashi et al., 2003; Morikawa et al., 2004).

Nitrate-nitrogen and nitrite-nitrogen derived from NO₂ 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 ¹⁵N 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 described above in the presence or absence of NO₂.

Results

Table I summarizes the uptake and assimilation of NO₂ upon fumigation with 4 µl l⁻¹ NO₂ 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-
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 C$_3$ 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 C$_3$/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 C$_3$ 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$_2$O 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. |
|---------------------------------|----------------|----------------|
| [ng N mg$^{-1}$ dry weight]     | Light during the day | Dark during the night |
| Kenaf                          | 63.8 ± 14.8*     | 246 ± 46        |
| Tobacco                        | 87.4 ± 11.9      | 22.2 ± 33.3     |
| Nitrate-N                      |                   |                 |
| [ng N mg$^{-1}$ dry weight]    | Light during the day | Dark during the night |
| Kenaf                          | n.d. b           | n.d.            |
| Tobacco                        | 1.33 ± 1.54      | n.d.            |
| a Data represent means of three replicates ± SD. |
| b n.d., not determined. |
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$_2$O 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$_2$O 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$_2$O 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 determining 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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