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[Short Report]

Ammonia Emission from Leaves of Different Rice (*Oryza sativa* L.) Cultivars

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Abstract: NH₃ emission from leaves of three rice (*Oryza sativa* L.) cultivars, Akenohoshi, Shirobeniya and Kasalath, was examined using a simple open chamber system. In the three cultivars, NH₃ emission rate (AER) and NH₄⁺ content of leaves decreased with decreasing NH₄⁺ concentration in the root medium, but these values differed significantly with the cultivar. In the daytime, AER, NH₄⁺ content and glutamine synthetase (GS) activity in leaves changed similarly with maximum values around midday. Akenohoshi showed significantly lower AER and NH₄⁺ content but higher GS activity than Kasalath. The difference in AER among the rice cultivars may be related to the activity of GS involved in photorespiratory NH₃ recycling. Akenohoshi can be a breeding material useful for improving N recycling.

Key words: Ammonia assimilation, Ammonia emission, Glutamine synthetase, Nitrogen, Photorespiration, Rice (*Oryza sativa* L.) cultivars.

The concentration of NH₃ in atmospheric air normally ranges from below 1 to 20 nmol mol⁻¹ air. Despite this very low concentration, NH₃ gas has serious negative environmental impacts associated with acidification and eutrophication. Livestock and commercial fertilizers are major sources of NH₃, whereas little is known about the role of vegetation in atmospheric NH₃ budgets (Schjoerring et al., 2000).

The direction of the net NH₃ flux in plants depends on the difference between external (atmospheric) and internal (plant tissue) NH₃ concentrations: NH₃ is emitted when the molar fraction of NH₃ in the atmosphere is smaller than that of NH₃ in the substomatal cavities of leaves, while NH₃ absorption occurs in the opposite case (Farquhar et al., 1980). Plant communities on arable cropland represent a net source of NH₃ to the atmosphere. Net emission of NH₃ ranges from below 100 up to 700 kg NH₃-nitrogen (N) km⁻² season⁻¹ (Schjoerring et al., 2000). Thereby, NH₃ emission from crops may contribute to atmospheric NH₃ pollution. Furthermore, since N is essential for the plant growth, this phenomenon may depress the productivity of crops.

Although there have been many studies on NH₃ emission from upland field crops such as barley and oilseed rape (Schjoerring et al., 2000), those from rice are limited. Kamiji and Horie (1989) observed that NH₃ was emitted from flag leaves of rice during the ripening stage. Hayashi et al. (2008) reported that urea broadcast at a high rate (30 kg ha⁻¹) on a paddy field at the panicle initiation resulted in a considerable amount of NH₃ emission; however, the emission from the surface of paddy water accounted for only 30% of the total emission from paddy field. Rice plant probably emits NH₃ when excessive N is applied. Further agronomical and physiological evaluation of NH₃ emission from rice leaves is needed.

We investigated the diurnal change in NH₃ emission rate (AER) and the effects of NH₄⁺ concentration in the root medium on AER in rice plants by using a simple open chamber system. *Oryza sativa* L. is divided into some types or subspecies. The *japonica* and *indica* types differ in some agronomical characteristics, such as plant height, leaf area, tiller number, dry matter production and N accumulation (Takahashi, 1984). Thus, we selected three cultivars, Akenohoshi (a *japonica-indica* cross), Shirobeniya (a

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Abbreviations: AER, ammonia emission rate; AT, air temperature; GO, glycolate oxidase; GS, glutamine synthetase; LA, leaf area; PPF, photosynthetic photon flux density; RH, relative humidity; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase.

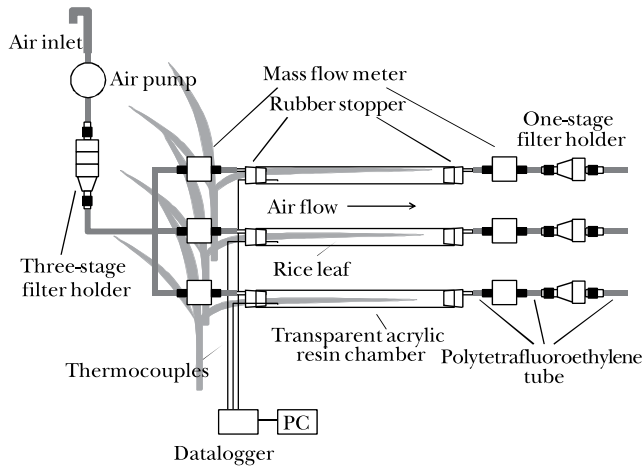


Fig. 1. A schematic diagram of the open chamber system used to measure NH_3 emission rate (AER) from leaves of rice plant.

traditional *japonica*) and Kasalath (a traditional *indica*) as materials for this study. Our data revealed that Akenohoshi shows lower AER than the other cultivars, and suggest that the cultivar difference in AER may be related to that in glutamine synthetase (GS) activity in leaves.

Materials and Methods

Imbibed seeds of the three rice cultivars were sown in nursery boxes in a greenhouse in the summer of 2008. After three wk, seedlings were transplanted into water culture baths of a 400 L capacity according to the method described by Kumagai et al. (2009). The baths contained the hydroponic solution recommended by Yoshida et al. (1972) with a slight modification. The NH_4^+ concentration of the solution was set at two levels: standard- NH_4^+ (SN) and low- NH_4^+ (LN), which was 2.86 and 1.43 mM, respectively. The concentrations of nutrients other than NH_4^+ were adjusted to the standard level. De-ionized water was used as a medium. The pH of the solution was adjusted every day to 5.0–5.5 using HCl and NaOH. Each solution in the water culture baths was renewed at a two-wk interval.

The following experiments were performed for plants at the vegetative stage using uppermost fully expanded leaves: i) the diurnal change of AER from leaves in Akenohoshi as related to those in environmental variables, ii) the effect of NH_4^+ concentration in the root medium on AER and NH_4^+ content in leaves of the three cultivars, and iii) the diurnal changes of AER, NH_4^+ content, and activities of glycolate oxidase (GO) and GS in leaves of Akenohoshi and Kasalath.

AER from leaves was measured using an open chamber system (Fig. 1). This system was constructed referring to the dynamic chamber method with a dry NH_3 collector used for measurement of NH_3 volatilization from paddy field (Hayashi et al., 2006). The system consisted of an inlet to take ambient air, an air pump, filter holders (NLI,

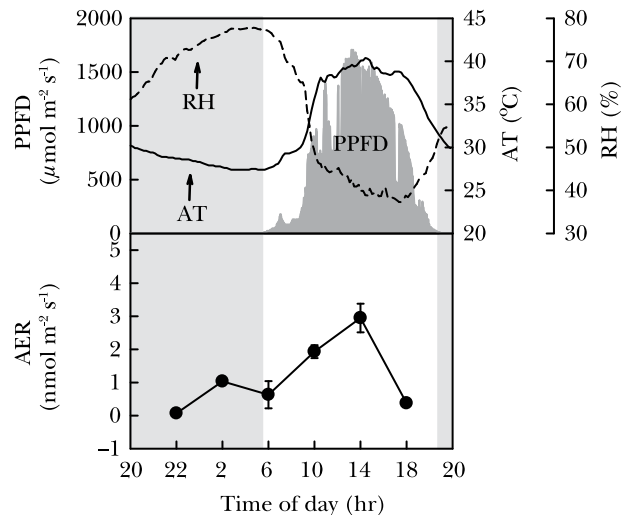


Fig. 2. Diurnal changes in photosynthetic photon flux density (PPFD), air temperature (AT), relative humidity (RH) and NH_3 emission rate (AER) from leaves of Akenohoshi grown under the standard-N condition on a clear day. The filters that absorbed NH_3 were collected six times per day at intervals of 4 hr, and mean AERs were calculated for each period. Values of AER are given as the mean \pm SE of three replicates.

NILU products, Kjeller, Norway), cylindrical transparent acrylic chambers (inner diameter, 35 mm; length, 400 mm), and mass flow meters (SEF21A, Horiba Stec Co. Ltd., Kyoto, Japan). Three chambers were set in parallel, and leaves of plants growing in the water culture baths were inserted into the chambers through rubber stoppers. The rubber stoppers were sealed with bond. A single leaf of a plant was set in each chamber. A filter holder in the upstream, consisting of three stages of a cellulose filter (51A, ADVENTEC, Tokyo, Japan) impregnated with phosphoric acid (H_3PO_4) to trap NH_3 , was used to remove NH_3 in entering air. A one-stage filter in the downstream was used to collect NH_3 emitted from the leaf. The filter was impregnated with a mixed solution of 5% (v/v) H_3PO_4 and 2% (v/v) glycerol and then dried in NH_3 -free air. The airflow rate in each sampling line was approximately 2 L min^{-1} . Air temperature (AT) in each chamber was measured using a thermocouple and recorded using a datalogger. Relative humidity (RH) and photosynthetic photon flux density (PPFD) in the greenhouse were also recorded. NH_3 trapped onto H_3PO_4 -impregnated filter was extracted in 10 mL of de-ionized water. The concentration of extracted NH_3 was determined according to the indophenols-blue method (Scheiner, 1976). AER was expressed as NH_3 (nmol) emitted from unit leaf area (LA, m^2) per s. The NH_3 collection efficiency of this system was examined with measurement of known amounts of NH_3 gas (1 to 200 μgN) generated in the chamber by addition of 1 M NaOH to NH_4Cl solution. The average of NH_3 collection efficiency was $98.0 \pm 0.2\%$ (mean \pm SE, $n=9$),

indicating a high reliability of this method.

For measurements of NH_4^+ content and activities of GO and GS, leaves were sampled and immediately frozen in liquid N_2 . NH_4^+ content was assayed according to the method described by Manderscheid et al. (2005). GO activity was measured as described by Ueno et al. (2005). GS activity was measured according to Nguyen et al. (2005) with a slight modification.

All statistical tests were performed using the Sigmastat software (Sigmastat 3.1, Systat Software, Inc., Richmond, USA). The statistical methods used are shown in the figure legends.

Results and Discussion

Fig. 2 shows the diurnal changes in PPF, AT, RH and AER from leaves of Akenohoshi grown under the SN condition on a clear day. PPF and AT increased from 0600 in the morning and then reached to the maximum around midday; thereafter, these gradually decreased. RH changed in a reflecting image to AT ranging from 39.5 to 78.5%. AER increased from a low value in the morning and then reached a maximum value during midday; thereafter, it rapidly decreased. The range of AER (0.06 to $3.59 \text{ nmol m}^{-2} \text{ s}^{-1}$) and its daily pattern were similar to those in rice reported by Kamiji and Horie (1989).

In barley and oilseed rape plants, NH_3 emission increased with an increase in the NH_4^+ concentration in the root medium (Mattsson et al., 1998). In the three rice cultivars also, plants grown under the SN condition showed higher AERs than those grown under the LN condition (Fig. 3A). Moreover, NH_4^+ contents in leaves of the former plants were higher than those of the latter plants (Fig. 3B). These results indicate that AER in the rice cultivars largely depends on the NH_4^+ content in the leaf tissue. Since the excessive accumulation of NH_4^+ has a toxic effect on plants (Britto and Knorzucker, 2002), NH_3 emission from rice leaves may be significant in preventing from excessive accumulation of NH_4^+ in the tissues. It is important to note that there were significant cultivar differences in AER and NH_4^+ content in leaves (Fig. 3). In addition, these parameters correspondingly changed among the cultivars; these were highest in Kasalath and lowest in Akenohoshi under both SN and LN conditions. Thus, the data suggest that these cultivars may have somewhat different traits of NH_3 assimilation.

Fig. 4 shows the diurnal changes in AER, NH_4^+ content and activities of GO and GS in leaves of Akenohoshi and Kasalath grown under the SN condition. We confirmed again that AER in both cultivars increased with time in the morning and then reached a maximum around midday; thereafter, they gradually decreased (Fig. 4A). The diurnal patterns in NH_4^+ content were similar to those in AER (Fig. 4B). These results indicate that high NH_3 emission from leaves in the daytime is correlated with high

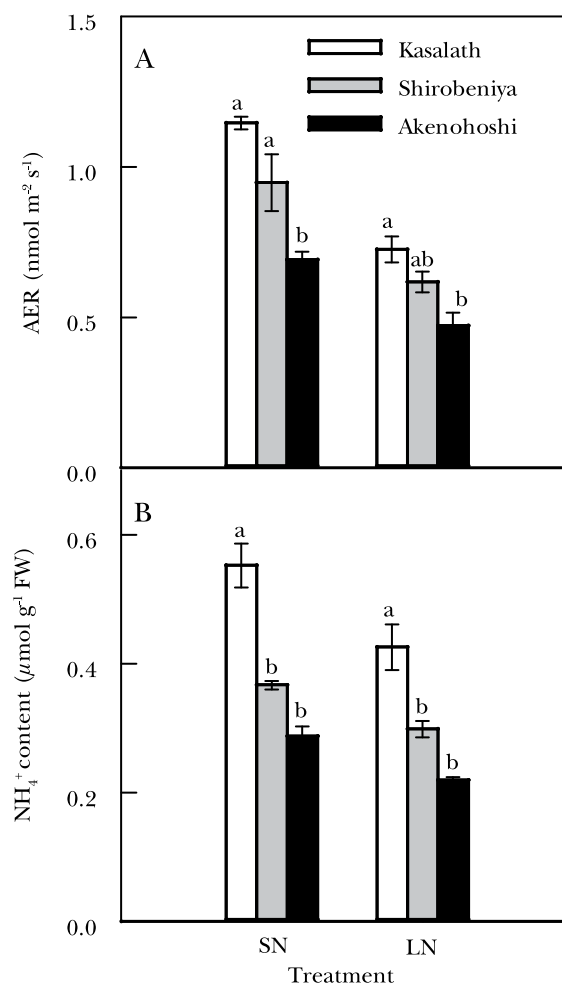


Fig. 3. NH_3 emission rate (AER) (A) and NH_4^+ content (B) in leaves of three rice cultivars grown under the standard-N (SN) and low-N (LN) conditions. The NH_3 emission per day (from 2000 to 2000) was measured, and mean AER was calculated. At midday, leaves other than those used for the AER measurement were sampled from the same plants for assays of NH_4^+ content. The integral amount of solar radiation and daily mean temperature were 121 MJ m^{-2} and 31°C , respectively. Values are given as the mean \pm SE of three replicates. Bars followed by the same letters in each treatment had no significant difference as determined by Tukey's test at $P < 0.05$.

accumulation of NH_4^+ within leaf tissues. In addition, AER and NH_4^+ content were significantly lower in Akenohoshi than in Kasalath from 1100 to 1500 (Fig. 4A, B).

$\text{NH}_3/\text{NH}_4^+$ is constantly generated in large amounts from various physiological processes in leaf tissues, such as photorespiration, nitrate reduction, protein turnover and lignin biosynthesis (Lea et al., 1992; Leegood et al., 1995). In the photorespiratory cycle NH_4^+ is released during glycine decarboxylation in mitochondria (Keys et al., 1978). Some generated NH_3 penetrates membranes and may be lost to the atmosphere via the apoplasmic solution of mesophyll cells (Husted et al., 2002). Therefore, activity of GO, a key enzyme of the photorespiratory cycle

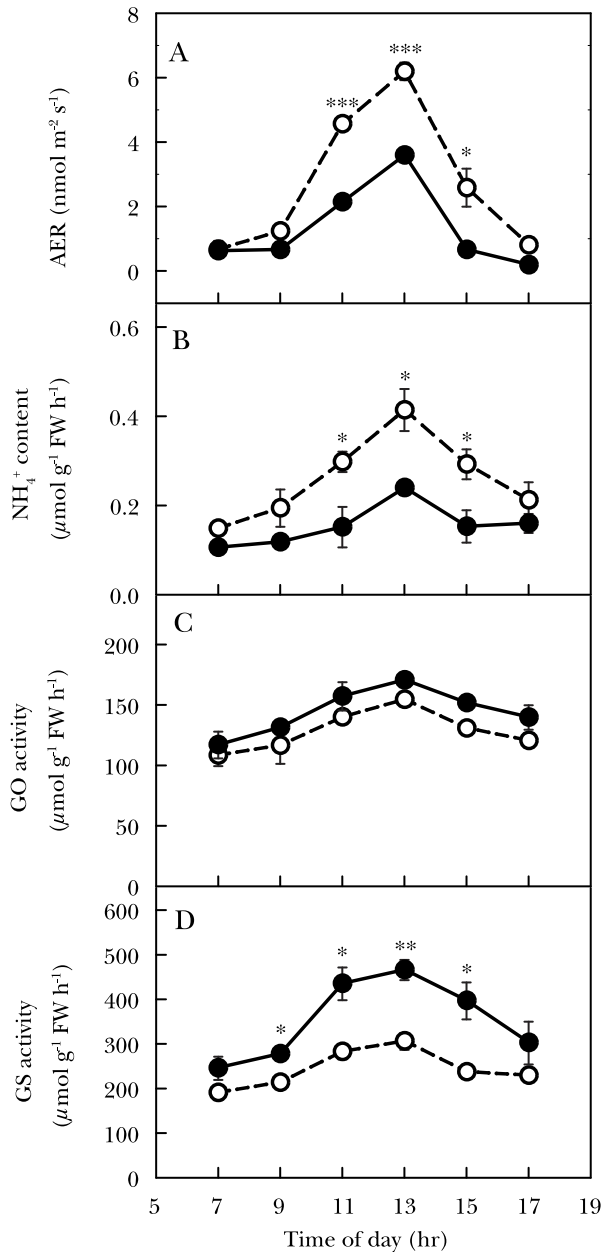


Fig. 4. Diurnal changes in NH_3 emission rate (AER) (A), NH_4^+ content (B) and activities of glycolate oxidase (GO; C) and glutamine synthetase (GS; D) in leaves of Akenohoshi (●) and Kasalath (○) grown under the standard-N condition on a clear day. The filters that absorbed NH_3 were collected six times at intervals of 2 hr in the daytime. Simultaneously, leaves were sampled from other plants in the water culture bath at intervals of 2 hr for measurements of NH_4^+ content and activities of GO and GS. The integral amount of solar radiation and daytime mean temperature were 119 MJ m^{-2} and 34°C , respectively. Values are given as the mean \pm SE of three replicates. *, ** and *** indicate significant differences at $P < 0.05$, 0.01 and 0.001 , respectively, between the two cultivars by Student's *t*-test.

(Leegood et al., 1995), was investigated as one of indicators of photorespiratory activity (Fig. 4C). GO activities of the two cultivars also reached the maximum levels at the midday, but the activity levels did not significantly differ

between the cultivars, indicative of no large difference in the photorespiratory activities. In fact, we have recently confirmed that there is almost no difference in photorespiration rate, which was estimated from the response of photosynthetic rate to changing O_2 concentration, between the two cultivars (Kumagai et al., 2010).

GS is a key enzyme in the GS/glutamate synthase cycle and involved in the synthesis of glutamate from glutamine and NH_3 (Leegood et al., 1995). Thereby GS plays a critical role in the recycling of NH_3 released from photorespiration (Schjoerring et al., 2000). Therefore, GS activity was compared between the two rice cultivars (Fig. 4D). GS activities also reached its maximum level at the midday, but the activity levels were higher in Akenohoshi than in Kasalath from 0900 to 1500. These data suggest that lower AER and NH_4^+ content found in leaves of Akenohoshi may be, at least in part, caused by higher GS activity. GS of higher plants consists of two isoforms, cytosolic GS (GS1) and chloroplastic GS (GS2), and the recycling of photorespiratory NH_3 depends on GS2 (Keys and Leegood, 2002). Obara et al. (2000) have reported that Kasalath had lower GS2 activity in leaves than *japonica* and *javanica* cultivars. This fact supports our suggestion. At present, there are conflicting reports on the involvement of GS2 in NH_3 emission. A study on barley mutants for GS2 showed that GS2 played an important role in controlling NH_3 emission and NH_4^+ content in leaves (Mattsson et al., 1998), whereas that on GS2 antisense oilseed rape suggested no involvement of GS2 in these traits (Husted et al., 2002). Our data on rice are consistent with the results of barely of the same grass family.

In previous studies Akenohoshi showed as higher N content per unit LA than other cultivars (Ookawa et al., 2003; Kumagai et al., 2007, 2010). Ookawa et al. (2004) also observed that contents of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and N in leaves of Akenohoshi are slowly reduced during leaf senescence. These traits of Akenohoshi may be related to some extent to the lower AER. Kamachi et al. (1991) showed that GS2 content declined in parallel with the loss of other chloroplastic enzymes such as Rubisco during leaf senescence in the rice cultivar Sasanishiki. At present, how GS2 content changes during leaf senescence in Akenohoshi remains unknown. A comparative study on several soybean cultivars with varying yield potential showed that low-yielding cultivars lost more N than high-yielding cultivars during the reproductive stage (Weiland and Stutte, 1985). Further studies will be needed to clarify the cultivar difference in NH_3 emission and its significance in crop production. This system for measuring AER is expected to be useful for elucidating the N assimilation in rice plants.

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