Pak.J.Bot., 28(2): 195-202, 1996.

FOLIAR ABSORPTION OF $^{15}NH_3$ BY WHEAT AND RICE VARIETIES

MUHAMMAD ASHRAF, SYYED RAZI ABBAS SHAMSI* AND FAROOQ-E-AZAM

Soil Biology Division,
Nuclear Institute for Agriculture and Biology,
Faisalabad, Pakistan.

Abstract

Plant tops are considered as both sink and source of various N gases and other air pollutants which therefore, may influence the gaseous N content of the environment. Five varieties each of wheat (*Triticum aestivum* L. and *T. durum* L.) viz., Pak-81, LU-26, Sarsabz, Durum and M-143 and rice (*Oryza sativa* L.) viz., Bas-370, Bas-385, DM-25, Kashmir Basmati and IR-6, were compared for NH_3 absorption and its correlation with leaf area and N content of the plants. Eight week old plants grown in plastic pots were exposed to $^{15}NH_3$ for 24 h in an air-tight canopy able to provide a limited supply of CO_2. Labelled NH_3 was generated by reacting ^{15}N-labelled ammonium sulphate with NaOH in the canopy. Total N and ^{15}N content of the dried and powdered plant material was then determined. Upto 44% in rice and 50% in wheat of the $^{15}NH_3$ generated in the canopy was assimilated by the plants. Varietal differences in foliar absorption of NH_3 were observed. No correlation was obtained between leaf area and the amount of $^{15}NH_3$ absorbed by different varieties indicating that NH_3 absorption was not solely dependent on leaf area and/or photosynthetic activity of the plants.

Introduction

The estimates of NH_3 transfer from and into the atmosphere suggest that NH_3 is evolved in very large quantities into the atmosphere from anthropogenic, natural and agricultural sources. Wollenweber & Reven (1993) has quoted that during the past century, NH_3 concentration has increased more than 220 $\mu g\ m^{-3}$ over average ambient concentration of 1-10 $\mu g\ m^{-3}$. This high concentration of NH_3, on one side causes the perturbation of the atmosphere (Fangmeier *et al.*, 1994) and on the other hand it is also a source of N transfer between and within the ecosystems which may contribute to N nutrition of plants. A significant proportion of NH_3-N in the atmosphere is reported to be absorbed and assimilated in the foliar parts of the plants (Artyomov *et al.*,) 1994; Grundman *et al.*, 1993; Porter *et al.*, 1972. Hutchinson *et al.*, 1972; Lockyer & Whitehead, 1987; Rogers & Aneja, 1980). This will be particularly important in modern agriculture, where significant amounts of NH_3 are generated and lost to the atmosphere from nitrogenous fertilizers through volatilization and ammonification (Boumeester *et al.*, 1985, Lockyer & Whitehead, 1990; Torello *et al.*, 1983).

The absorbed NH_3 is assimilated into amino acids and various other macromolecules. The assimilation and synthesis of these molecules from ammonium is considered to be dependent on energy supplied from the photosynthetic activity of the plants (Evans, 1983; Pace *et al.*, 1990). This photosynthetic activity is in turn limited by the availability of the atmospheric CO_2. Therefore, the CO_2 is considered to affect the synthesis of amino acids and macromolecule from ammonium. However, the literature also shows a significant increase in N content of ensiling plant materials exposed to NH_3 which indicates that the assimilation of NH_3 is not dependent on photosynthetic activity of the plants. From the view point of nitrogen conservation and environmental cleanliness, it may be important to find out crop varieties efficient to absorb gaseous N from the atmosphere. According to the limiting factor theory when one or more factors are interrelated the decrease or increase of one factor will govern the behaviour of the other. Therefore, for studying the effect of one factor, the other factor/factors need to be kept constant. In case of CO_2 fixation, due to differential metabolic activity of the crops and/or varieties, it is very difficult to maintain the ambient concentration of CO_2 (15 mmole m^{-3}) in a close atmosphere. However, under limited CO_2, the plants have to grow at CO_2 compensation point (1.5-1.9 mmole m^{-3}; Bauer & Martha, 1981). It can be assumed that the potential of ammonia absorption of the plants is better examined under limited CO_2 supply. Moreover, the relationship of a crop also needs to be explored. The objectives of the present study were to assess the extent of NH_3 absorption by different varieties of wheat and rice under limited supply of CO_2 and to find out any relationship between the NH_3 absorption and leaf area of the plants.

Materials and Methods

Four varieties of bread wheat (*Triticum aestivum* L.) viz., Pak-81, LU-26. Sarsabz and Punjab-85, one variety of durum wheat (*Triticum durum* L.), and one month old nursery of the rice (*Oryza sativa* L.) varieities IR-6, Bas-370, Bas-385, DM-25 and Kashmir basmati (Kash-Bas) were grown in plastic pots containing 1.5 kg of a clay loam soil (C, 0.44%; N, 0.05%; pH (1:1) 7.8; E.C., 0.8 dS m^{-1}). The soil was obtained from an experimental field at the Nuclear Institute for Agriculture and Biology (NIAB). Faisalabad, Pakistan. The plants were fertilized with ammonium sulphate $(NH_4)_2SO_4$ and potassium dihydrogen phosphate (KH_2PO_4) at a rate sufficient to provide N, P and K concentrations of 30, 15, and 20μg^{-1} soil, respectively. The nutrients were applied in two equal doses, one at sowing and or/transplantation and the other at 45 days after sowing or transplantation.

Standardization of NH_3 generation: $^{15}NH_3$ used in the absorption studies on plants, the procedure for the production of NH_3 was standardized by using unlabelled $(NH_4)_2SO_4$ taken in 100-ml beakers, a 10 ml of 1N NaOH solution was added and the beakers were kept on a laboratory bench under normal conditions of temperature (23.4°C) and humidity (R.H. 79%). The reaction resulted in the loss of NH_3. The residual NH_4+-N in the beakers was determined by micro-Kjeldahl (Bremner & Mulvaney, 1982) method at 0.3 and 6 h intervals. The time and quentity of reagents required for the generation of appropriate amounts of NH_3 were determined.

Measurement of leaf area: The leaf area of all the wheat and rice varieties was measured just before exposing them to NH_3-enriched atmosphere. The method described by Yoshida *et al.*, (1972), was followed for leaf area measurements.

Exposure of plants to $^{15}NH_3$: Triplicate pots bearing 2-month old plants of wheat or rice

Table 1. Generation of NH_3 following reaction of ammonium solution with sodium hydroxide (10ml, 1M) for different periods.

Reaction Time (hrs.)	N added (mg)	NH_3-released (mg)
0	14.0	1.4 (10)
	28.0	1.4 (5)
3	14.0	13.5 (96)
	28.0	26.0 (93)
6	14.0	14.0 (100)
	28.0	27.7 (99)

Figures in the parentheses represent % of the NH_4+-N released.

were placed under an air-tight polyethylene chamber (0.76 x 0.76 x 0.61 meters, WxLxH) fabricated for ammonia exposure. This chamber was provided with an air circulating fan for uniform mixing of the enclosed air, a thermometer and a hygrometer for monitoring the temperature and relative humidity of the chamber. The temperature of the canopy during NH_3 exposure of wheat ranged from 15-28.8°C and for rice from 29.4-35.6°C and the corresponding values for R.H., were 62.5-75.5% for wheat and 42-96% for rice. Light intensity of the chamber was determined with the help of a lux meter (TES 1380, Taiwan) and was found to be more than 20000 lux. This chamber was enough for providing 5 mmoles m^{-3} of CO_2, therefore, the plants grew most of the time under CO_2 compensation point.

In each of the four corners of the chamber. 100 ml capacity beakers containing 10 ml of 1N NaOH were placed. Just before midday, 5 ml of a solution of $(^{15}NH_4)_2SO_4$ containing 25 mg N and having 21.4 atom % ^{15}N was injected into each beaker using a sterilized hypodermic syringe. The point of injection on the polyethylene sheet was immediately sealed with the help of scotch tape and the plants were allowed to grow for 24 h under these conditions before being harvested.

The plants were harvested after 24 h of exposure and partitioned into root and shoot portions. The plant material was dried to a constant weight at 70°C and finely powdered. Total N content in the plant components was determined by Kjeldahl method (Bremner & Mulvaney, 1982) and the distillates were subjected to isotope-ratio analysis using a double inlet system Mass Spectrometer (Varian MAT GD 150).

Table 2. Dry matter yield (DMY) and N content of different plant components in wheat (*Triticum aestivum* L.) varieties.

Wheat	DMY (g pot-1)			N content (mg pot-1)		
variety	Shoot	Root	Total	Shoot	Root	Total
Pak-81	10.9e	4.1b	15.0e	268.4e	29.9b	298.3d
	(72.7)	(27.3)		(90.0)	(10.0)	
Lu-26	14.0a	4.2b	18.2a	275.2d	22.6d	297.8d
	(76.9)	(23.1)		(92.4)	(7.6)	
Durum	11.2d	4.7a	15.9d	301.7a	35.1a	336.8a
	(70.4)	(29.6)		(89.6)	(10.4)	
M-143	12.3c	4.1b	16.4c	284.2c	25.5c	309.7c
	(75.0)	(25.0)		(91.8)	(8.2)	
Sarsabz	13.4b	4.1b	17.5b	293.1b	24.2c	317.2b
	(76.6)	(23.4)		(92.4)	(7.6)	

In a column, the values followed by the same letters are not significantly different from each other at 5% level of significance according to DMR test. Figures in parentheses are percent of the total.

Results and Discussion

Reaction of $(NH_4)_2SO_4$ and NaOH was found to be quick under laboratory conditions and without agitation a complete loss of NH_4+ was observed after 6 h of reaction between 5 ml of $(NH_4)_2SO_4$ solution (14 mg N) and 10 ml of 1N NaOH (Table 1). This combination of solutions was therefore, used for the actual studies.

$$(NH_4)_2SO_4 + 2NaOH \text{ -----> } 2NH_3 + 2H_2O + Na_2SO_4$$

Significant differences was observed in the dry matter yield of shoot and total dry matter (shoot + root) in different varieties of wheat (Table 2), with maximum yield found in LU-26. However, the dry matter yield of roots was statistically similar in all varieties except Durum. In rice, maximum dry matter yield was obtained in IR-6 with minimum in DM-25 (Table 3). Although, the distribution of dry matter into shoot and root varied among the varieties of wheat and rice, the major part was always located in the shoot portion.

Nitrogen yield in wheat cvs, Pak-81 and LU-26 (Table 2), and rice cvs. Bas-370 and Bas-385 (Table 3) was statistically similar with significant differences in other varieties. However, N contained in shoot portion was significantly different in 5 varieties of wheat with maximum N yield observed in Durum. In rice cvs., in contrast to dry matter yield, total N yield was maximum in Kash-Bas (Table 3). In the varieties of wheat >85% and in rice varieties >80% of the total N yield was attributable to shoot portion.

Maximum leaf area among wheat varieties was found in Sarsabz which was significantly different from other varieties (Table 4), which among themselves showed

Table 3. Dry matter yield (DMY) and N content of different plant components in rice (*Oryza sativa* L.) varieties.

Wheat	DMY (g pot-1)			N content (mg pot-1)		
variety	Shoot	Root	Total	Shoot	Root	Total
IR-6	29.6a (71.7)	11.7a (28.3)	41.3a	214.4b (81.0)	50.2a (19.0)	264.6b
Bas-385	19.6b (69.5)	8.7b (30.5)	28.2b	166.3d (83.1)	33.9c (16.9)	200.2d
Bas-385	19.0c (75.7)	6.1c (24.3)	25.1cd	173.2c (85.4)	29.6d (14.6)	202.8d
DM-25	17.7d (71.7)	7.0d (28.3)	24.7d	170.6d (82.0)	37.4b (18.0)	208.0c
Kash-Bas	18.6c (74.1)	6.5c (25.9)	25.1c	239.6a (88.8)	30.2d (11.2)	269.8a

In a vertical column. the values followed by the same letters are not significantly different from each other at 5% level of significance according to DMR test. Figures in parentheses are percent of the total.

non-significant differences. Maximum leaf area in rice varieties was attained by IR-6 with minimum in Bas-370. Maximum uptake of $^{15}NH_3$ was observed in Durum, although Sarsabz had the maximum leaf area. Maximum absorptin of $^{15}NH_3$ among the rice varieties was observed in Kash-Bas. Per cent contribution of $^{15}NH_3$ among the rice varieties was observed in Kash-Bas. Percent contribution of $^{15}NH_3$ to the total N

Table 4. Leaf (LA), mg and/per cent N derived from atmosphere (mgNdfa, %Ndfa) by the wheat and rice varieties.

Wheat	LA(cm^2)	MgNdfa	%Ndfa	Rice	LA(cm^2)	mgNdfa	%Ndfa
Pak-81	27.1c	2.26c (0.08)	0.84	IR-8	23.8a (0.08)	1.9bc	2.6
Lu-26	27.9b	2.17d (0.08)	0.78	Bas-370	22.2d (0.09)	2.1b	3.0
Durum	27.0c	3.44a (0.13)	1.14	Bas-385	19.6cd (0.09)	1.8c	2.7
M-143	27.7b	2.13d (0.08)	0.75	DM-25	20.7c (0.11)	2.2b	3.0
Sarsabz	33.5a	2.64b (0.08)	0.90	Kash-Bas	17.4b (0.17)	3.0a	3.0

In a column, the values followed by the same letters are not significantly different from each other at 5% level of significance according to DMR test. Figures in parenthesis are mgNdfa per unit leaf area.

content of wheat plants ranged between 0.75 and 1.14 and maximum value being recorded for Durum and minimum for M-143, while for rice plants, the values ranged from 2.6 to 3.0 with minimum in IR-6. Percent contribution of $^{15}NH_3$ was greater in rice varieties as compared to wheat while the reverse was true for leaf area. Although, per unit leaf area absorption of ^{15}N-labelled ammonia varied among wheat and rice varieties, but it was always greater in rice. Per cent contribution of atmospheric NH_3 would seem to be low in both the crop varieties, but in this study the plants were exposed to $^{15}NH_3$ only for 24 h. It would suggest that during the entire growth period, atmospheric NH_3 could make significant contribution to the total plant N. Plants of all the varieties of wheat taken together showed an uptake of 50.5% and of rice varieties 44% of the total $^{15}Nh_3$-N introduced into the system. Substantial absorption of NH_3 from the atmosphere that ranges from 30 to 77% has also been reported (Janzen & Bruinsma, 1989; Porter *et al.*, 1972; Whitehead & Lockyer, 1987; Wollenweber & Raven, 1993).

It is generally believed that the absorptin of atmospheric NH_3 is dependent on leaf area of the plants, but in our study, the leaf area did not show a good correlation (Table 5) with the amount of NH_3 absorbed in both wheat and rice. Moreover, the assimilation of this absorbed ammonia is thought to be dependent on the supply of carbon compounds from the photosynthetic activity of the plants. However, a linear absorption by intact plants (Whitehead & Lockyer, 1987) and its assimilation by ensiling plant materials (Ashbell & Winberg, 1993) suggests that this might not be true. Therefore, this type response and the results observed in our study suggest that NH_3 absorption might not be dependent on photosynthetic activity of the plants. But still for assimilation

Table 5. Correlation (r) of different plant parameters with absorption of ammonia.

	mgNdfa	%Ndfa
Wheat		
Leaf area	0.017	0.047
Shoot N yield	0.832	0.749
Total N yield	0.931*	0.878*
Rice		
Leaf area	0.706	0.462
Shoot N yield	0.671	0.108
Total N yield	0.532	0.232
Wheat + Rice		
Leaf area	0.148	0.872**
Shoot N yield	0.568	0.882**
Total N yield	0.582	0.847**

*, ** significant at P = 0.05 and P = 0.01, respectively.

of ammonia metabolically, active sites of the plant are required. A greater, per unit leaf area absorption in rice than wheat suggested that rice plants for assimilation of NH_3 are physiologically more active, therefore, it provides greater sink for atmospheric ammonia. However, the source of the carbon compounds for incorporation of ammonia is questionable, whether these are of respiratory origin or produced due to the transaminating activity of the ammonium assimilatory enzymes. The results of the present study are contrary to other reports which show a significantly positive relatinship between leaf area and absorption of NH_3 (Hutchinson *et al.*, 1972; Cowling & Lockyer, 1981).

Significant corelations (r = 0.931) were found between N derived from NH_3 and total N uptake in wheat varieties, while in rice the correlation was not so significant. However, the leaf area, shoot N content and total N content of the crops taken together showed a highly significant correlation with percent N content of the plants derived from atmospheric ammonia. This type of behaviour could be explained on different response of the crop towards ammonia absorption.

The results of our experiments and other studies (Artyomov *et al.*, 1994; Wollenweber & Raven, 1993), show that plants do actively act as sink of atmospheric NH_3. Moreover, it is also known that significant amounts of fertilizer N are lost to the atmosphere in the form of NH_3 and this N will be available to plants depending upon their ability to absorb and assimilate NH_3. It may be reasonable to assume, therefore, that ability to assimilate atmospheric NH_3 may be considered as a selection criteria for crop varieties more efficient in making use of fertilizer N and it will be more meaningful where an increasing concentration of atmospheric pollutants like ammonia are going to put the atmosphere in danger. There is need to study the relationship of NH_3 uptake with plant phenology and physiology, as well as the availability of N in soil in both quantitative and qualitative terms.

References

Artyomov, V.M., E.M. Artyomov and S.D. Fridman. 1994. Ammonia uptake by plants. *Environ. Monitoring and Assessment*, 29: 221-228.

Ashbell, G and Z.G. Winberg. 1993. The effect of applying ammonia to corn wheat and sorghum upon ensiling. *Can. Agric. Eng.*, 35: 113-117.

Bauer, A. and P. Martha. 1981. The CO_2 compensation point of C-3 plants---a re-examination. I. Interspecific variability. *Zeitschrift fur Pflanzen-physiologia*, 103: 445-450.

Boumeester, R.J.B., P.L.G. Vlek and J.M. Stumpe. 1985. Effect of environmental factors on ammonia volatilization from a urea-fertilized soil. *Soil Sci. Soc. Am. J.*, 49: 376-381.

Bremner, J.M. and C.S. Mulvaney. C.S. 1982. Total nitrogen. pp. 594-624. *In: Methods of soil analysis* (Eds.) Page *et al.*, 2: 594-624. Am. Soc. Agron., Madison, Wisconsin.

Cowling, D.W. and D.R. Lockyer. 1981. Increased growth of ryegrass exposed to ammonia. *Nature*,292: 337-338.

Evane, J.R. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol.*, 72: 297-302.

Fangmeier Andreas, Angelike Hadwiger-Fangmeier, Ludger Van Der Eerden and Hans-Juergen Jaegger. 1994. Effect of atmospheric ammonia on vegetation: A review. *Environ. Pollut.*, 86: 43-82.

Grundman, G.L., R. Lensi and A. Chalmet. 1993. Delayed ammonia and nitrous oxide uptake by maize leaves. *New Phytol.*, 124: 259-263.

Hauck, R.D., 1989. A human ecosphere perceptive of agricultural nitrogen cycling. pp. 3-19. *In: Advances in nitrogen cycling in agricultural ecosystems* (Ed.,) J.R. Wilson. UK.

Hutchinson, G.L., R.J. Millington and D.B. Peters, 1972. Atmospheric ammonia: Absorption by plant leaves. *Science*, 175: 771-772.

Jenzen, H.H. and Y. Bruinsma. 1989. Methodology for the quantification of root and rhizosphere nitrogen dynamics by exposure of shoot to ^{15}N-labelled ammonia. *Soil Biol. Biochem.*, 21: 189-196.

Lockyer, D.R. and D.C. Whitehead, 1987. Gaseous ammonia as a source of nitrogen for grass. *J. Sci. Food Agric.*, 38: 329-330.

Lockyer, D.R. and D.C. Whitehead. 1990. Volatilization of ammonia from cattle urine applied to grassland. *Soil Biol. Biochem.*, 22: 1137-1142.

Pace, G.M., R.J. Volk and W.A. Jackson. 1990. Nitrate reduction in response to CO_2-limited photosynthesis. Relationship to carbohydrate supply and nitrate reductase activity in maize seedlings. *Plant Physiol.*, 92: 286-292.

Porter, L.K., F.G. Viets Jr. and G.L. Hutchinson. 1972. Air containing nitrogen-15 ammonia: Foliar absorption by corn seedlings. *Science*, 175: 759-761.

Rogers, H.H. and V.P. Aneja. 1980. Uptake of atmospheric ammonia by selected plant species. *Environ. Exp. Bot.*, 20: 251-257.

Torello, W.A., J.D. Wehner and A.J. Turgeon. 1983. Ammonia volatilization from fertilized turfgrass stands. *Agron. J.*, 75: 454-456.

Whitehead, D.C. and D.R. Lockyer. 1987. The influence of the concentration of gaseous ammonia on its uptake by the leaves of Italian ryegrass, with and without an adequate supply of nitrogen to roots. *J. Exp. Bot.*, 38: 818-827.

Wollenweber, B. and J.A. Raven. 1993. Nitrogen acquisition from atmospheric NH_3 by *Lolium perenne*: Utilization of NH_3 and implications for acid base balance. *Bot. Acta.*, 106: 42-51.

Yoshida, S., D.A. Forno, J.H. Cock and K.A. Gomez. 1972. *Laboratory manual for physiological studies of rice*. IRRI, Philippines, pp. 59-61.

(Received for Publication 31 July 1995)