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Evaluation of Nitrogen Loss from Leaves of Different Varieties of Rice Using ^{15}N-Labelling Technique

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Article history: Received in revised form 10 October 2016 Accepted 30 October 2016 Available online Dec. 14, 2016

Cite this article as: CHEN N, Widjajanto DW, ZHENG Y, INAGA S and TASAKI A. 2016. **Evaluation of Nitrogen Loss from Leaves of Different Varieties of Rice Using ^{15}N-Labelling Technique**. J Applied Chem. Sci. 3: 300-305, doi: *http://dx.doi.org/10.22341/jacs.on.00301p*300

ABSTRACT

Losses of nitrogen from agricultural system into atmosphere may cause global warming through the contribution of N_2O, NO, and CH_4. Therefore, it is important to identify and quantify the source and amount of nitrogen loss in the soil-plant systems. Release of nitrogen from rice leaves throughout the growth period was studied by using ^{15}N-labelling technique. Indica and Japonica rice crops were evaluated throughout the experiment. Indica plants produced more biomass both shoot and root compared to those of Japonica. However, Indica produced lower weight of panicle compared to that of Japonica. The effect of rice varieties and nitrogen form became more evident with the growth stage. When labelled-N was applied at tiller stage, 16.2-38.7% of absorbed-N can be lost from rice leaves. When labelled-N was applied at panicle stage, the loss rate of absorbed-N ranged from 18.9% (Japonica, $^{15}NH_4^+$-N) to 62.1% (Indica, $^{15}NO_3^-$-N), and when it was applied at heading stage, the loss percentage of absorbed-N was 14.1% (Japonica, $^{15}NH_4^+$-N), 19.0% (Indica, $^{15}NH_4^+$-N), 59.1% (Japanica, $^{15}NO_3^-$-N), 60.8% (Indica, $^{15}NO_3^-$-N), respectively, at harvest, indicating that nitrogen loss was higher when rice was applied with $^{15}NO_3^-$-N than $^{15}NH_4^+$-N either in Indica or Japonica, and for variety, Indica had much more nitrogen loss from leaves than that of Japonica. Accordinglly, it may be concluded that ^{15}N-labelled technique showed high amount of nitrogen loss from leaves of rice.

Keywords: nitrogen loss, rice variety, growth period of rice, nitrogen form
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1. Introduction

Nitrogen is one of the most important elements in supporting crop growth and production. It often becomes the limiting factor of crops in achieving a maximum yield. Therefore, the application of nitrogen in both dry-land systems and cultivated-land has been practiced continuously, especially under intensive agricultural management. However, it was found that the application of chemical-N fertilizer has caused environmental problems such as excess residual N remains in agricultural farmland (Mashima *et al.*, 1996), increased greenhouse gases status such as N_2O, NO, and CH_4 (Goshima *et al.*, 1999; Rudaz *et al.*, 1999; Liu *et al.*, 2000; Hou *et al.*, 2000; Hao *et al.*, 2001).

Intensive agricultural practice is predicted to be one of pathways in generating greenhouse gases emission. In the cultivated-land system the process of denitrification may occur as the anaerobic condition may influence the reduction of nitrate and converted into N_2O. Leaching of nitrate into wider environment may pollute underground water and may cause eutrophication. To minimize the environmental problems due to application of high doses of chemical-N fertilizer, hence, nitrogen use efficiency (NUE) should be taken into consideration. The NUE is one of key roles in achieving maximum crop yield and maintaining the environment from being polluted. Evaluation of nitrogen loss from cultivated-land, fertilized grassland and forest has been studied intensively (Mashima *et al*., 1996; Rudaz *et al*., 1999; Liu *et al*., 2000; Hao *et al*., 2001). Using micrometeorological methods, the effect of inorganic N fertilizers urea, slow-release urea, and ammonium nitrate on fluxes of nitrogen loss from turf grass field plots was evaluated (Maggiotto *et al*., 2000). It was resulted that urea-based fertilizers seemed to minimize N_2O emissions, although long-term effects of slow-release urea still need to be studied. In grassland, it was estimated that for individual N applications, loss of nitrogen in the form of N_2O relative to the amount of applied-N ranged from 0.05% to 5.2% (Rudaz *et al*., 1999). However, nitrogen loss from plant leaves has been studied rarely.

It is one of pathways of nitrogenous compound release into the atmosphere (Goshima *et al*., 1999; Smart and Bloom 2001). Nitrogen has been released from leaves of crop supported from N_2O produced by plant NO_3-assimilation directly (Smart and Bloom 2001). About 87.3% and only 17.5% of N_2O was released from leaves of rice crop, at the flooded soil and un-flooded soil, respectively (Yan *et al*., 2000). Nitrogen loss in the form of N_2O was also found at the Genetic Modified-Tobacco (GMT) clone 271 but was undetected at wild-type tobacco (WTT) that cultured on medium containing NO_3^-. No nitrogen loss was found either from the GMT clone 271 or WTT grown on medium containing NH_4^+ (Goshima *et al*., 1999).

This experiment was conducted to evaluate nitrogen loss from rice crop leaves grown hydroponically by employing ^{15}N-labelling technique.

2. Materials and Methods

Experiment was carried out inside a green house of Plant Nutrition Laboratory, Kagoshima University, Kagoshima Japan in 2003. Two varieties of rice, Indica and Japonica were evaluated hydroponically under Kimura solution. Its elements concentration is shown at Table 1. The solution was renewed weekly and the pH of it was maintained at 5.5. Rice crops were allowed to grow throughout the growth period.

Table 1. Kimura solution used for of culture rice crop grown hydroponically

Elements		Concentration (mg/L)
N	:	20.0
P	:	2.2
K	:	8.3
Mg	:	7.1
Ca	:	6.0
Fe	:	1.0
Mn	:	0.3
B	:	0.1
Zn	:	0.1
Cu	:	0.1
Mo	:	0.01

Seeds of rice both Indica and Japonica were germinated on May 16th 2003, and one seedling was transplanted into pot on June 18th 2003. Different forms of nitrogen, $^{15}NH_4^+$-N and $^{15}NO_3^-$-N were added into the solution, respectively, at tillering stage (TI) on July 17th 2003, panicle initiation stage (PI) on July 31st 2003, heading stage (HD) on August 21st 2003, and maturing stage (MS) on October 2nd and 7th 2003 for Japonica and Indica. Crops were harvested at TI on July 24th 2003, PI on August 6th and on 8th 2003, HD on August 28th 2003, and MS on October 9th and 14th 2003 for Japonica and Indica, respectively.

Samples taken at TI, PI and HD stages were divided into shoot and root, while samples of MS were grouped into shoot, root and ears. Dry matter (DM) content, total N and ^{15}N contents then were determined. Sub samples then were oven at 70°C for about 48 hours to determine DM content. Kjedahl method was used to determine total N, while mass-spectrophotometry was employed to analysis ^{15}N. Nitrogen absorbed by rice crops was calculated by subtracting N left in the solution in the pots from applied-N. Nitrogen loss was measured as follows: (%)N loss = (N absorbed – N left in crops)/N absorbed x 100). A completely randomized design under factorial pattern 2 x 2 x 3 was used to arrange the experiment. The first factor was kind of rice crops, Indica and Japonica, meanwhile the second factor consisted of different form of chemical-N fertilizers, $^{15}NH_4^+$–N and $^{15}NO_3^-$–N. Each combination treatment then was replicated 3 times. Data was col lected throughout the growth period of rice. Collecteddata was analyzed using SPSS 10.0 package of Microsoft Office.

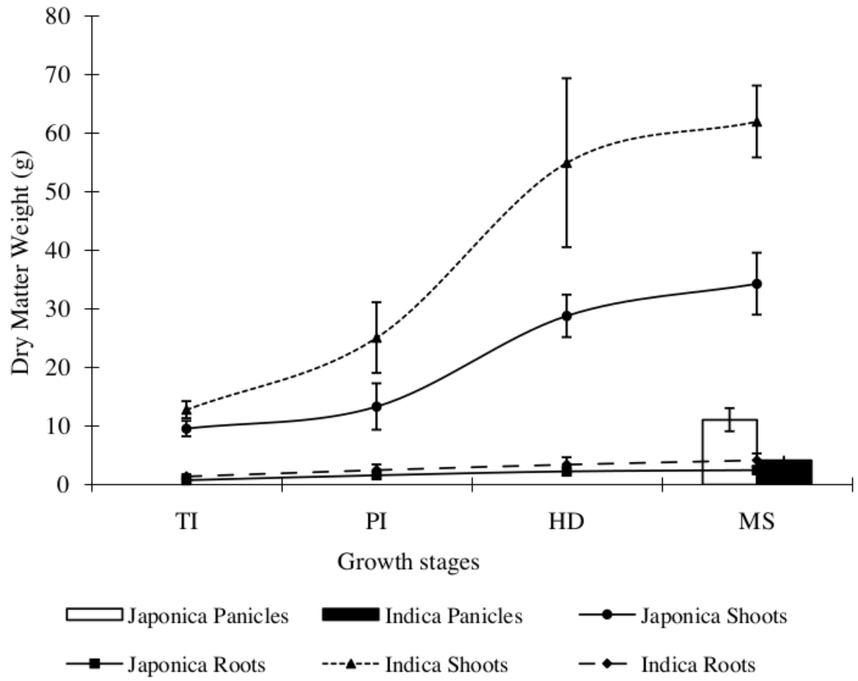

Fig. 1. Dry matter crop production of Indica and Japonica at different growth stages

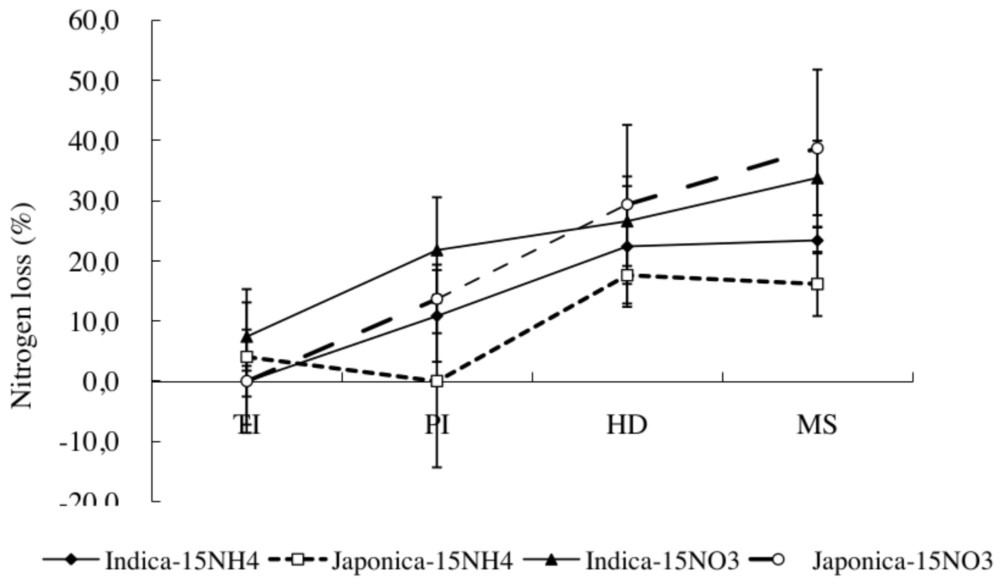

Fig. 2. Nitrogen loss from applied-N at TI stages

3. Results and Discussion

3.1. Dry matter crop production

On the basis of the collected-data it was found that Indica produced more biomass both shoot and root compared to those of Japonica. However, Indica produced lower weight of panicle compared to that of Japonica (Fig. 1). More biomass of shoots produced by Indica may be responsible for more nitrogen loss than that of Japonica.

Table 2. Significance coefficient of N loss in relation to varieties, N forms and stages due to applied-^{15}N at TI, PI and HD stages

Sources of Variance	applied-^{15}N at growth stages		
	TI	PI	HD
Variety	0.957	0.000	0.147
N Forms	0.056	0.000	0.000
Stages	0.000	0.000	0.693

3.2. Nitrogen Loss from Leaves at different growth stages

About 20 to 60% absorbed-N by rice crop was loss from leaves throughout the growth stages (from TI to HD stages) (Fig. 2; 3; 4). There was significantly differences of nitrogen loss due to application of different form of nitrogen at TI, PI, and HD ($p<0.001$) and growth stages ($p<0.01$) (Table 2).

Application of N at the TI stage (Fig. 2) showed that nitrogen loss was found unstable both of Indica and Japonica treated-$^{15}NH_4^+$, vice versa, for those two crops treated-$^{15}NO_3$. Nitrogen loss from leaves of Indica was found higher than that of Japonica. Application of N at the PI (Fig. 3) and HD stage (Fig. 4) showed that nitrogen loss from leaves of Indica and Japonica increased throughout the growth periods. On the basis of the findings data it may be concluded that nitrogen loss from leaves both of Indica and Japonica at each stage was differ. This is may be due to the fact that there were differences in physiological metabolisms of rice at each stage of rice growth periods (TI, PI, and HD). The amount of N loss found in this experiment is not in agreement with N loss found at tobacco crops as rice that has been used in this experiment may have different physiological metabolism compared to that of tobacco (Goshima *et al.,* 1999). However, these two-finding proved that there was N loss from leaves of crop into the atmosphere.

3.3. Nitrogen Loss from Leaves at Different form of N Application

Two different form of N were applied into the grown medium of rice crops, $^{15}NH_4^+$–N and $^{15}NO_3^-$–N. Both Indica and Japonica were grown at the added-$^{15}NH_4^+$–N medium showed lower in nitrogen loss than that of both Indica and Japonica were grown at the added-$^{15}NO_3^-$–N medium. These findings indicated that there were differences in physiological processes of NO_3^- and NH_4^+. These differences of N behaviours at each growth phase of rice crop may affect the nitrogen

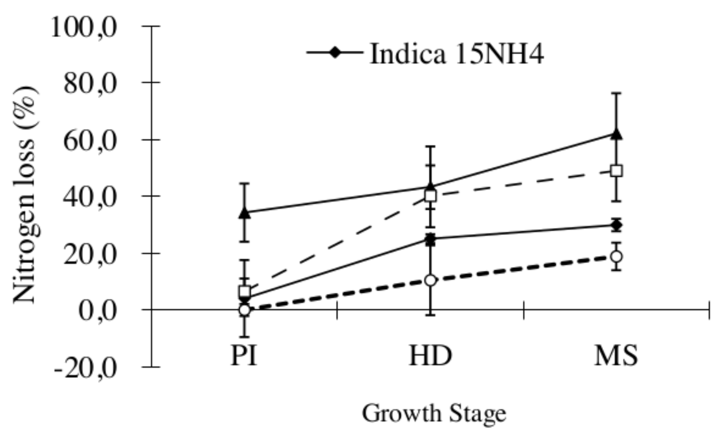

Fig. 3. Nitrogen loss from applied-N at PI stages

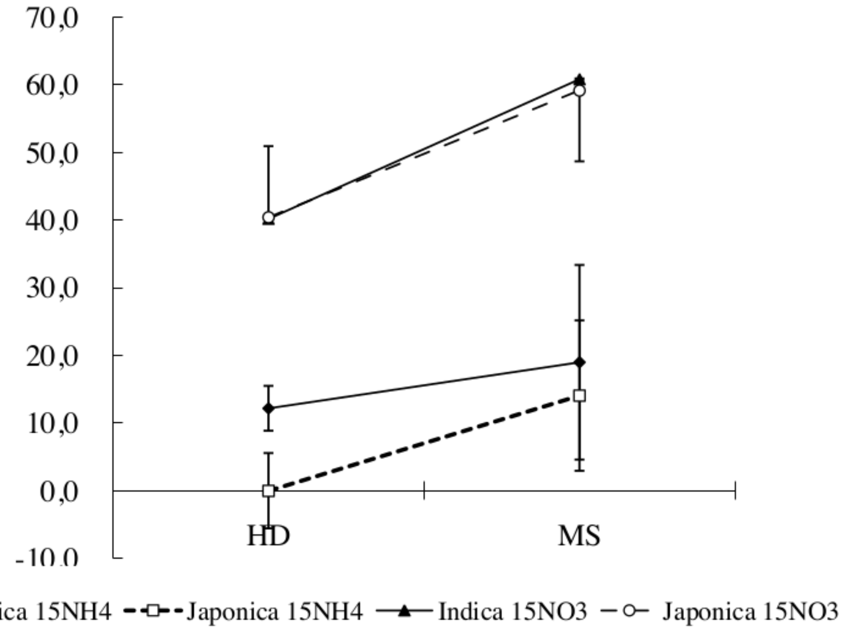

Fig. 4. Nitrogen loss from applied-N at HD stages

losses. These were in agreement with previous finding that N_2O has been emitted from leaves and it was indicated in correlated with leaf nitrate assimilation activity. Nitrogen loss in the form of N_2O from leaves was detected due to support of N_2O produced by plant NO_3-assimilation directly (Smart and Bloom 2001).

4. Conclusion

On the basis of analyzed-data it may be concluded that ^{15}N-labelled technique showed high amount of nitrogen loss (20-60%) from leaves of rice crops. It increased in correlation with rice varieties, growth stages, and N forms.

Acknowledgment

Authors would like thanks to the Japan Society for the Promotion of Science that funded this work through Project with ID No. P. 02214.

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Source of support: None Declared

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