Australian Journal of Crop Science

AJCS ISSN:1835-2707

# Effect of deep placement of nitrogen fertilizer on growth, yield, and nitrogen uptake of aerobic rice

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#### Abstract

Recent studies have reported that poor growth of aerobic rice associated with urea induced ammonia toxicity when applied at early growth stage. The objective of this study was to examine the effect of different methods of nitrogen application on plant growth of aerobic rice grown in continuous aerobic rice system. Micro-plot experiment was conducted in 2008 dry season in a field where aerobic rice has been grown for fourteen seasons in International Rice Research Institute (IRRI) farm. Two Pot and pot-diffusion incubations experiments were done with the soil from same field where micro-plot experiment was conducted and aerobic rice has been grown for 14 seasons in greenhouse and growth chamber. Apo, an upland rice variety, was grown under aerobic conditions with different application method of nitrogen in field and pot experiments. The field micro-plot experiment showed that urea and urea super granules (USG) deep placement increased grain yield of aerobic rice by 1.66 t ha<sup>-1</sup> in continuous aerobic rice cultivation. Pot experiments studying the effects of different application methods of nitrogen indicated that N incorporation into soil and placement at a depth of 5-10cm in the soil increased the vegetative growth parameters, and plant growth parameters of aerobic rice under ammonium sulfate were significantly higher than urea at all applied treatments. In pot-diffusion incubations experiment, N placement at a depth of 5.0 cm in the soil significantly reduced nitrogen loss by ammonia volatilization. Our results suggested that there is a possibility of improving aerobic rice yield in the continuous aerobic rice system by using right N source or changing conventional method of nitrogen application to deep placement.

**Keywords:** aerobic rice, ammonium sulfate, ammonia, deep placement, urea. **Abbreviations:** USG: urea super granules, nitrogen: N.

## Introduction

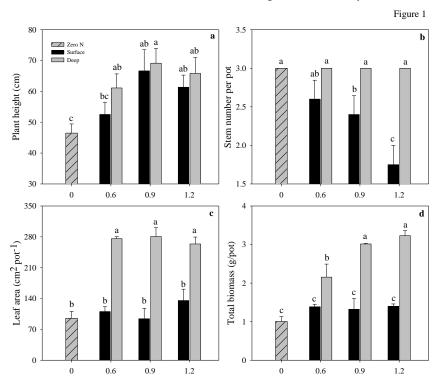
To avert the future water scarcity and safeguard food security, water-saving alternatives to conventional flooded rice are needed. One adaptation strategy is the cultivation of "aerobic rice," which is grown on non-puddled and non-flooded soil (Bouman and Tuong, 2001). In aerobic rice production system, total water usage can be reduced by 27-51% and water productivity can be increased by 32-88% (Bouman et al., 2005). High yield potential (5-7 t ha<sup>-1</sup>) can be achieved in the aerobic rice system under the use of modern input-responsive cultivars (Lafitte et al., 2002). However, yield decline under continuous cropping of aerobic rice was observed (Nishizawa et al., 1971; Guimaraes and Stone., 2000; George et al., 2002; Peng et al., 2006; Nie et al., 2012). The long term experiment (LTE) of aerobic rice from 2001 to 2007 was conducted at IRRI, a 0.6 t ha<sup>-1</sup> y<sup>-1</sup> decline was observed in annual grain yield over the 7 years period (Peng et al., 2006). The exact causes of yield decline in the continuous aerobic rice system are still unknown. The growth of aerobic rice was possible limited by poor N uptake. Yield decline under continuous aerobic rice cropping can be reversed by nitrogen application (Nie et al., 2008).

However, ammonium sulfate was more effective in improving the plant growth of aerobic rice than urea at the high N rate (Nie et al., 2009). Urea as high nitrogen concentration (46%) fertilizer is used widely for agricultural production. However, more than 40% of N lost through ammonia gas when urea was applied on soil surface (Catchpoole et al., 1983; Nommik, 1973). Urea was much quickly hydrolysis by urease to ammonia and carbon dioxide (NH<sub>2</sub>CONH+H<sub>2</sub>0 $\rightarrow$ 2NH<sub>3</sub>+CO<sub>2</sub>) in the soil solution, ammonium ions in the soil solution exist in equilibrium with ammonia ( $NH_4^+$  +  $OH^- \rightarrow NH_3 + H_2O$ ). Many factors influence the nitrogen lost through ammonia such as soil pH, texture, organic matter, cation exchange capacity, and buffering capacity. Soil pH is one of important factors affecting amount of ammonia volatilized (Hargrove, 1988), the activity of both  $NH_4^+$  and  $OH^-$  ions increase with the soil pH, thus driving the equilibrium to the right and much more ammonia lost (Wahhab et al, 1957), the ammonia volatilization increased markedly with soil pH increasing (Ernst et al., 1960). We observed that the negative yield trend in IRRI's LTE was a prominent increase in soil pH, which rose from 6.4 at the outset

Parameter	Mean
pH	7.03
Organic C (%)	1.5
Total N (%)	0.18
Available P-Olsen (mg kg <sup>-1</sup> )	31.0
Available K (meq $100 \text{ g}^{-1}$ )	1.09
Active Fe (%)	2.03
Active Mn (%)	0.14
Available Zn (mg kg <sup>-1</sup> )	2.0
Exch. K (meq $100 \text{ g}^{-1}$ )	1.26
Exch. Na (meq $100 \text{ g}^{-1}$ )	1.27
Exch. Ca (meq $100 \text{ g}^{-1}$ )	27.3
Exch. Mg (meq $100 \text{ g}^{-1}$ )	15.3
Exch. Al (meq 100 $g^{-1}$ )	Nil
$CEC \ (meq \ 100 \ g^{-1})$	41.5
Clay (%)	58.3
Silt (%)	33.0
Sand (%)	8.7

Table 1. Soil chemical and physical properties of soil used for the field-microplot and pot experiments.

The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons.





**Fig 1.** Plant height, stem number per pot, leaf area, and total biomass of Apo grown aerobically in soil under urea at 0, 0.6, 0.9, and  $1.2 \text{ g N pot}^{-1}$ , at two methods of N application (surface application: S, deep placement of urea: UD) and in a no nitrogen control (Zero N) and in pot experiment 1. The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons. Error bars represent the standard error. The different letters indicate significant difference among the treatments at the 5% level.

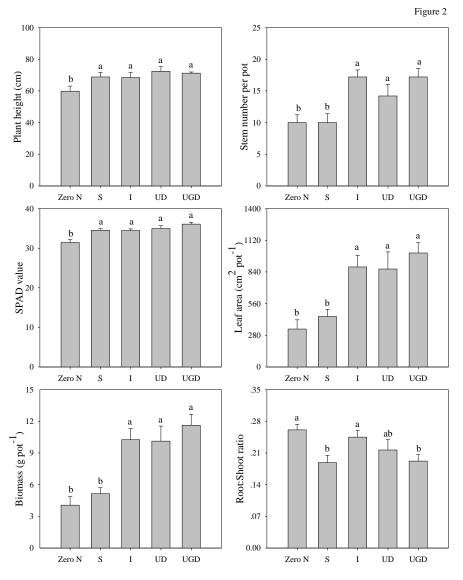
of the experiment to nearly 7.1 after 13 seasons of aerobic cultivation. Yield failure of aerobic rice also been reported on a site where the soil pH increased from 6.5 to 7.4 in 2006 (Kreye et al., 2009). Further research reported that soil acidification can improve the growth of aerobic rice and nitrogen uptake (Xiang et al., 2009). Recent studies have reported that poor growth of aerobic rice associated with urea application induced ammonia toxicity when applied at seeding (Haden et al, 2011; Qi et al., 2012a). The loss of ammonia after urea application may lead to poor growth in monocropped aerobic rice. In high soil pH condition, it is much important that improving nitrogen use efficiency and reducing nitrogen loss by ammonia volatilization. Ammonia loss from ammonium sulfate occurred

to a lesser constant rate due to a lack of alkalinity and the ammonia loss was less compare with urea (Vlek and Craswell, 1979). Deep placement of urea and slow release nitrogen can reduce ammonia loss (Mikkelsen et al, 1978). In this study, urea, urea super granules (USG) and ammonium sulfate was as nitrogen source, and three methods of nitrogen application were used. The objectives of this study were 1) to determine the effects of different nitrogen source and the methods of nitrogen application on growth and yield of aerobic rice grown in continuously cropped aerobic soil, and 2) to examine the effects of different methods of urea application and USG on ammonia volatilization.

**Table 2** Grain yield and yield components of Apo grown aerobically in soil with application of urea and USG under two placements in micro-plot experiment in the dry season of 2008.

Parameters	Urea	Urea	USG	LSD 5%
	(surface)	(deep placement)	(deep placement)	
Grain yield (t ha <sup>-1</sup> )	3.68b	5.20a	5.48a	1.07
Aboveground total biomass (g m <sup>-2</sup> )	928.3b	1189.4a	1247.1a	191.40
Harvest index (%)	37.6b	41.7a	41.9a	3.13
Panicles m <sup>-2</sup>	204b	240a	241a	21.30
Spikelets panicle <sup>-1</sup>	121.4a	125.1a	129.9a	6.13
Spikelets $m^{-2}$ (x 10 <sup>3</sup> )	24.8b	30.1a	31.2a	3.71
Filled grain (%)	61.6b	71.3a	72.2a	9.06
1000-grain weight (g)	20.8a	21.1a	21.2a	0.39

Micro-plots were arranged in a field where aerobic rice has been grown continuously for fourteen seasons. Within a row, means followed by different letters are significantly different at 0.05 probability level according to least significant difference (LSD) test.



Application methods of urea and USG

**Fig 2.** Plant height, stem number per pot, SPAD value, leaf area, total biomass, and root:shoot ratio of Apo grown aerobically in soil under urea and urea supergranule at 1 g N pot<sup>-1</sup>, at different method of N application (surface application: S, incorporation with soil: I, deep placement of urea: UD, and deep placement of urea supergranule: UGD, respectively) and in a no nitrogen control (Zero N) and in pot experiment 2. The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons. Error bars represent the standard error. The different letters indicate significant difference among the treatments at the 5% level.

# Results

## Field micro-plot experiment

In micro-plots experiment, the surface placement of urea treatment produced grain yield of 3.68 t ha<sup>-1</sup> in the 14th season continuous aerobic rice field (Table 2). Deep placement of urea significantly increased grain yield, aboveground total biomass, harvest index, panicles m<sup>-1</sup> and spikelet m<sup>-1</sup> and filled grain percentage compared with the surface placement of urea. USG deep placement increased grain yield by 48.9% and urea deep placement increased grain yield by 41.3% compared with the surface placement of urea, respectively. There was no significant difference in spikelet panicle<sup>-1</sup>, and 1000-grain weight among the three treatments. Deep placement of USG increased all parameters compared with deep placement of urea but the difference was statistically insignificant. These results suggested that it had greater possibility to improve plant growth by changing nitrogen application method in the continuous aerobic rice system.

## Pots experiment

In pot experiment 1, the application of urea improved plant height and total biomass of Apo compared with the untreated control in all N rate treatments (Fig.1). The leaf area and total biomass were significantly higher on plants fertilized by deep placement of urea than control (Zero urea) plants and plants fertilized by surface application of urea. There was no difference in the stem number of the control and plants fertilized by deep placement of urea in all N rate treatment. When the urea was applied on soils surface, the stem number decreased from 0.6 to 1.2 g N pot<sup>-1</sup>. In pot experiment 2, deep placement of urea and USG improved plant growth compared with the control (Zero N) (Fig. 2). Application of nitrogen improved all variables of plant growth except stem number under surface application of urea compared with control. Urea incorporation with soil, deep placement of urea and USG significantly increased stem number, leaf area, and total biomass compared with surface application of urea, but there was no significantly difference in plant height and SPAD value. There was no significantly difference in all measures among the urea incorporation with soil, deep placement of urea and USG. Significantly decreases in root/shoot ratio when urea applied on soil surface and deep placement of USG compared with control, but there was no difference among control, urea incorporation with soil, and deep placement of urea. All ammonium sulfate treatments significantly improved plant height, stem number, SPAD value, leaf area, and total biomass compared with control (Fig. 3). There was no significant difference in plant height, root/shoot and SPAD value among the ammonium sulfate surface placement, incorporated placement and deep placement. Aboveground N uptake was higher with ammonium sulfate than with urea and USG regardless nitrogen placement (Fig. 4). Incorporation of urea into soil, deep placement of urea and USG significantly increased aboveground nitrogen uptake compared with control (Fig. 4a). But there was no significant difference between urea surface and control. Three placements of ammonium sulfate significantly improved aboveground N uptake (Fig. 4b), there was no difference among three placements.

#### Pot-diffusion incubation experiment

In pot-diffusion incubation experiment, deep placement of USG significantly reduced nitrogen loss by ammonia volatilization (Table 3). At 3 days and 9 days after incubation, the amount of

ammonia volatilization under urea application significant higher compared with control and USG, but there was no difference among three treatments at 6 days after incubation.

# Discussion

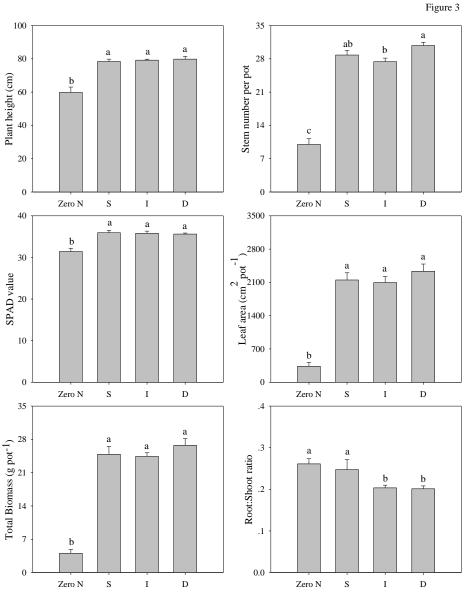
The field micro-plot experiment showed that deep placement of urea and USG increased grain yield of aerobic rice by 1.66 t ha<sup>-1</sup> (Table 2). The increase in grain yield was due to the improvement in aboveground biomass, panicle m<sup>-2</sup>, grain filling and spikelet m<sup>-2</sup>. The grain yield of 15th-season aerobic rice in main plot was 3.96 t ha<sup>-1</sup> (data not shown), which was much higher than control in micro-plot experiment. These results suggest that deep placement of urea and USG can reduce the yield decline in continuous aerobic rice system. It was observed that stem number decreased with nitrogen rate increased from 0.6 to 1.2 g N per pot when urea was applied on soil surface, but the stem number was no different among four N rates when urea was placed in deep of soil (Fig.1b). This observation suggests that urea-induced toxicities may be the main cause of poor growth when urea was applied on soil surface during the early growth stage. Deep placement of urea reduced the adverse effects of urea, this is because deep placement of urea can reduce nitrogen loss by ammonia volatilization and ammonia is known the main cause of urea-induced toxicities (Haden et al., 2011). The plant growth of Apo fertilized with USG was larger than plants fertilized with urea and the control (Fig. 2). Almost growth parameters were best with USG application among all treatments. USG as a slow-release nitrogen reduces total N concentration in surface soil and is likely to minimize loss through volatilization and surface runoff (De Datta and Craswell, 1982). There was a low root/shoot ratio under urea surface application compare with other treatments, because root growth was poor (data not shown) under urea surface, recent research also reported that root growth is more sensitive to ammonia volatilization than shoots when urea is applied (Qi et al., 2012b). When ammonium sulfate was as nitrogen source, plant growth was no significantly different in all application methods (Fig. 3). However, the plant growth of Apo fertilization with ammonium sulfate was much greater than with urea in all placement. It is known that ammonium sulfate as acidic nitrogen contribute to much less ammonia compared with urea and could be associated with its greater effect on plant growth of aerobic rice (Haden et al., 2011; Nie et al., 2009). Soil acidification and urea-induced ammonia toxicity might explain the big difference between ammonium sulfate and urea applied on surface soil. Urea and ammonium sulfate improved aboveground N uptake of aerobic rice regardless N placement (Fig. 4). When ammonium sulfate was as nitrogen source, aboveground N

uptake was much higher than urea and USG as nitrogen source, it was also reported by Nie et al. (2009). The relative increase in N uptake was greater when urea was the N source than with ammonium sulfate. This was because plant growth was much poorer with urea application on surface soil than with ammonium sulfate. USG deep placement significantly reduced N loss by the ammonia volatilization. 3 days after N application (Table 3), the ammonia volatilization was the most high among sampling. Then, the amount of ammonia volatilization reduced compared with the first sampling. The loss was maximum during the first 3 days after N application, many researchers have reported similar results (Mikkelsen et al., 1978 and Reddy et al., 1986). The cause was possible that the high amount of ammonia was hold in soil in first 3 days, the equilibrium with ammonia ( $NH_4^++OH^- \rightarrow NH_3+H_2O$ ) was drive to left. Fertilizer efficiency was greatest when USG were placed at deep of soil (Table 2 and Fig. 4a). These results indicate that the urea

**Table 3.** Volatilized ammonia measured during a 3, 6, 9 days after aerobic incubation as influenced by N source (Urea or USG) in pot-diffusion incubation experiment.

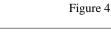
xperiment.				
NH3 volatilization	3	6	9	Total
mg N kg⁻¹				
Zero N	0.91b	0.59a	0.25b	1.74b
USG	1.05b	0.38a	0.30b	1.73b
Urea	7.29a	0.34a	2.48a	10.11a
LSD 5%	3.59	0.26	1.16	4.65
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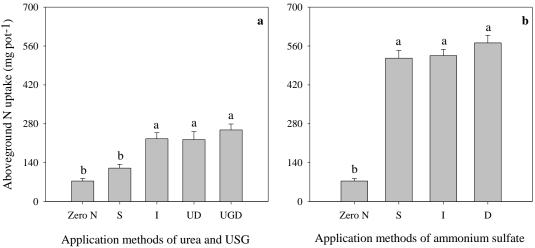
The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons. Within a column, means followed by different letters are significantly different at 0.05 probability level according to least significant difference (LSD) test.



Methods of ammonium sulfate application

**Fig 3.** Plant height, stem number per pot, SPAD value, leaf area, total biomass, and root:shoot ratio of Apo grown aerobically in soil under ammonium sulfate at 1 g N pot<sup>-1</sup>, at different method of N application (ammonium sulfate applied on soil surface, incorporated with soil: I, deep placement: D, respectively) and in a no nitrogen control (Zero N) and in pot experiment 2. The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons. Error bars represent the standard error. The different letters indicate significant difference among the treatments at the 5% level.





**Fig 4.** Aboveground N uptake of Apo grown aerobically in soil under urea, urea supergranule (a) and ammonium sulfate (b) at 1 g N pot<sup>-1</sup>, at different method of N application (surface application: S, incorporation with soil: I, deep placement: D of urea and ammonium sulfate, and deep placement of urea supergranule: UGD, respectively) and in a no nitrogen control (Zero N) and in pot experiment 2. The soil was from an aerobic field where aerobic rice has been grown continuously for 14 seasons. Error bars represent the standard error. The different letters indicate significant difference among the treatments at the 5% level.

incorporated with soil and deep placement increases the urea use efficiency at the vegetative stage of aerobic rice. The importance of deep placement of ammonium-nitrogen has been well documented (Reddy et al., 1976; Watanabe et al., 1979). It is also evident that deep placement is one kind of method for improving the vegetative growth and yield of aerobic rice when urea is as nitrogen source.

# Materials and methods

#### Field micro-plot experiment

Micro-plots experiment was conducted in the field where 14th season aerobic rice was grown since 2001 at International Rice Research Institute (IRRI) farm at Los Baños, Laguna, Philippines (14° 11'N, 121° 15 'E, 21 m above sea level) in the dry season of 2008. Yield decline has been observed under mono-cropped aerobic rice in this field (Peng et al., 2006). The property and chemicals of aerobic soil was shown in table 1.

Forty twenty-day-old seedlings taken from wet bed were transplanted in every micro-plot at three seedlings per hill and keep a spacing of 25cm x 10cm. The upland rice variety Apo (formerly IR55423-0) was chosen because it does well under aerobic conditions. Phosphorus as solophos 60 kg P ha<sup>-1</sup>, potassium as potassium chloride 40 kg K ha<sup>-1</sup>, and zinc as zinc sulfate 5 kg ha<sup>-1</sup> were broadcasted in all plots one day before transplanting. The plots had been keep aerobic conditions and were irrigated when the soil water pressure at 15cm depth reached -30KPa. Weeds were removed by hands if necessary.

In field micro-plots experiment, three nitrogen treatments were arranged in a completely randomized design with four replicates. Each replication included three micro-plots, and micro-plots were encircled by  $1m \times 1m \times 0.3m$  metal plates inserted 15cm deep in the soil. Urea and urea super-granules (USG, 0.5-0.7cm diameter) as nitrogen fertilizer were applied in three equal splits (50kg N ha<sup>-1</sup> as basal (1 day before transplanting), at 25, and 45 days after transplanting (DAT)). Urea broadcast, urea deep placement, and USG deep placement was as three nitrogen treatments. For deep placement, urea and

USG was put 10cm depth in the soil between two rows. At maturity, all 40 hills in each micro-plot were harvested to determine grain yield. Plants were separated into panicles and straw. Plant height, stem number, and panicle number were determined. Straw was over-dry in oven at 70°C for dry weight. Panicles were hand-thresh and spikelets were separated to filled, half filled, and empty by submerging them in tap water. Three subsamples each of 30-g filled spikelets and 2-g unfilled spikelets were taken to count the number of spikelets. Dry weights of rachis and filled and unfilled spikelets were determined after oven-drying at 70°C to constant weight. Aboveground total biomass was the total dry matter of straw, rachis, and filled and unfilled spikelets. Spikelets per panicle, filled grain percentage (100  $\times$  filled spikelet number/total spikelet number), 1000 grain-weight, and harvest index (100  $\times$ filled spikelet weight/aboveground total biomass) were calculated.

#### Pot experiment

#### Soils description

Two pot experiments and one pot-diffusion incubations experiment were conducted in greenhouse and growth chamber. Soil was collected from field where aerobic rice has been grown for 14 seasons at IRRI farm. The soil was air-dried for 2 weeks, crushed into small pieces ( $<1 \text{ cm}^3$ ), and mixed thoroughly to homogenize the large composite sample. This air-dried aerobic rice soil was placed in large plastic sacks and stored in the dark in a dry location prior to its use in the following pot experiments. In all experiments, 4-L porcelain pots were filled with 3.0 kg of air-dried aerobic soil.

# Plant material and experiment details

In pot experiment 1, urea was used, the treatments consisted of two methods of nitrogen application, top dressing and deep placement, at three N rates, 0.6, 0.9, and 1.2 g N pot<sup>-1</sup>. In pot

experiment 2, 1g N pot<sup>-1</sup> as urea, USG, and ammonium sulfate were applied one day before sowing. For urea and ammonium sulfate, three different placements included broadcast on soil surface, incorporated with soil, and placed 5cm depth in soil were used:, and USG was only placed 5cm depth in soil. In both pot experiments, no nitrogen input treatment was as control. One day before sowing, fertilizers were applied to the pots and mixed well with the soil and then the soil was soaked with tap water. In both pot experiments, an upland rice variety, Apo was used because of its good performance under aerobic conditions (George et al. 2002; Lafitte et al. 2002). Six pre-germinated seeds were sown in each pot, after one week, seedlings were thinned and keep three uniform seedlings per pot. Each treatment was replicated five times with five pots. Throughout the experiment, 100-150 mL of water was added to each pot twice a day for keeping aerobic conditions (between -0.30 and -0.40 bars). Weeds were removed by hand as needed. Insect pressure was low so no pesticides were required. The plants were sampled at 30 and 50 days after sowing for experiments 1 and 2, respectively. Before plant sampling, plant height, stem number per pot was measured. Three SPAD value were taken from each pot in pot experiment 2. Then, plants were cut, and separated into leaves, stems with sheath, and roots. Leaf area was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska, USA). Roots were washed carefully with tap water to remove soil. The dry weight of leaves, stems, and roots were determined after oven-drying to constant weight at 70°C, the sum of leaves, stems, and roots dry weights was as total biomass.

#### Pot-diffusion incubations experiment

In pot-diffusion incubations experiment, 1g N pot<sup>-1</sup> as urea, USG was used. Urea was applied on soil surface, and USG was placed like pot experiment 1. No nitrogen input treatment was as control. After nitrogen application, 1000 ml water was put in each pot for keeping soil moisture around field water capacity between -0.30 and -0.40 bars. 200ml of 4% boric acid was added in glass dish (8cm diameter) for trapping ammonia. The pots were sealed with tape and incubated in a darkened growth chamber at 30°C and 70 % humidity. Within the growth chamber a completely randomized design was used with 3 replicates for each treatment. Ten ml boric acid will be taken out at 3, 6 and 9 days after incubation, then change the new boric acid after every sampling. The amount of ammonia was determined direct measurement using an ammonium ion selective electrode (Beckman Coulter Inc., Ca, USA).

The tissue nitrogen concentration was determined by micro kjldahl digestion, distillation, and titration (Bremner and Mulvaney, 1982). The aboveground N uptake was calculated based on the tissue nitrogen concentration.

#### Statistical analysis

Data were analyzed according to completely randomized design following analysis of variance (Statistix 8.0) and mean comparison between treatments was performed based on the least significant difference (LSD) test at the 0.05 probability level.

# Conclusion

Poor growth of aerobic rice was observed when urea was applied on soil surface at early seedling growth stage. Field and pot experiments demonstrated that the plant growth of aerobic rice was better with the deep application than surface application of urea. Furthermore, ammonium sulfate was more effective in improving the vegetative plant growth, N uptake of aerobic rice than urea in the aerobic soil. These results suggest that there is a possibility of improving aerobic rice yield in the continuous aerobic rice system by using right N source or changing conventional method of nitrogen application to deep placement.

#### Acknowledgement

This work is part of the CGIAR Challenge Program on Water and Food through the project "Developing a System of Temperate and Tropical Aerobic Rice in Asia (STAR)." It was also supported by the Conservation Food and Health Foundation through the Cornell University project "More Rice with Less Water." Financial support was also provided by National Rice Production Foundation of China (CARS-01-10B) and Special Fund for Agro-scientific Research in the Public Interest (201203031).

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