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Improvement of early seedling growth of dry direct-seeded rice by urease inhibitors application

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Abstract

Poor crop establishment associated with urea-induced ammonia volatilization inhibits the optimum plant growth of dry direct-seeded rice. The objective of this study was to examine the reduction of soil ammonia volatilization by urease inhibitors when urea is applied at seeding and to identify the sensitivity of shoot and root growth of dry direct-seeded rice to ammonia volatilization. Two Petri-dish incubation experiments were conducted using three different soils with three urease inhibitors. N as urea was applied to the Petri-dish at the rate of 1mg g^{-1} of soil. To simulate soil water content suitable for direct-seeded rice, soil inside the Petri-dishes was kept under aerobic conditions. Ammonia volatilization due to application of urea at seeding reduced early seedling growth of dry direct-seeded rice. Urease inhibitors significantly reduced ammonia volatilization. Among the three urease inhibitors, N-(N-butyl) thiophosphoric triamide (NBPT) was the most effective one in reducing ammonia volatilization, followed by phenyl phosphorodiamidate (PPD) and hydroquinone (HQ), respectively. The findings imply that application of suitable urease inhibitors can significantly improve seedling growth of dry direct-seeded rice by reducing ammonia volatilization from added urea. This study also suggests that root growth was more sensitive to ammonia volatilization than shoots when urea was applied at seeding.

Keywords: Ammonia volatilization; Dry direct-seeded rice; Early growth; Urea.
Abbreviations: NBPT: N-(N-butyl) thiophosphoric triamide; PPD: phenyl phosphorodiamidate and HQ: hydroquinone.

Introduction

Rice (*Oryza sativa* L.) is an important food crop for over half of the world's population (Li et al., 2011). To assure food security and preserve water resources, developing water saving technologies for reducing water usage and increasing water-use efficiency in rice production system is urgent. A technology, "dry direct-seeded rice" has been developed to reduce water input, save labor demand, and increase water productivity. For dry direct-seeded rice the dry seed is broadcasted onto soil surface and then incorporated either by ploughing or by harrowing while the soil is still dry (Pandey and Velasco, 2002). Direct seeded rice avoids the puddling and maintains continuous moist soil conditions and thus reduces the overall water demand for rice culture. Nearly 15% of all lowland rice is direct-seeded (Pandey and Velasco, 2002). Grain yield of 11.2 t ha^{-1} has been reported in dry direct-seeded rice system (Dong et al., 2005). Dry direct-seeded rice had higher grain yields and lower water use than conventionally transplanted rice (Zhao et al., 2007; Zhu, 2008; Sharma et al., 2002; Singh et al., 2002). However, poor early seedling growth is the limiting factor to the development of dry direct-seeded rice systems. The possible causes include unsuitable soil water content, improper seeding depth and ammonia toxicity due to urea application. In wide range of crops, ammonia toxicity induced by urea application has been reported, such as, maize, wheat, barley, oats, sorghum and rye (Stephan and Waid, 1963; Lee, 1979; Bremner and Krogmeir, 1988, 1989). In dry direct-seeded rice, the toxicity of ammonia volatilization is one of the main causes responsible for poor early seedling growth, when urea fertilizer is applied at seeding (Bremner, 1995; Fan and MacKenzie, 1995; Haden et al., 2011). Urea is the most widely used N fertilizer in the world because of its more solubility, high nutritive value and ease of handling, and accounts for over 50% of all N applied (Gilbert et al., 2006; Younis et al., 2008). Upon application to soil, urea is rapidly hydrolyzed by urease enzymes to form ammonia, the accumulation of excess ammonia can result in toxicity to plants (Clay et al., 1990; Court et al., 1962). The application of urease inhibitors has been considered as a promising method to reduce ammonia volatilization following urea application (Wang et al., 1991; Zhu, 2000). It has been reported that PPD is a very effective urease inhibitor which retards urea hydrolysis (Beyrouty et al., 1988; Buresh et al., 1988; Byrnes et al., 1983; Rao and Ghai, 1985; Simpson et al., 1985). While Bremner and Chai (1986, 1989) documented that NBPT is superior to PPD in retarding urea hydrolysis and reducing ammonia volatilization. HQ is

Table 1. Chemical and physical properties of the soils used in the two experiments.

Parameter	Acidic soil	Neutral soil	Alkaline soil
pH	4.8	7.3	7.9
Organic C (g/kg)	10.5	10.2	7.0
Total N (g/kg)	1.35	1.24	1.02
Olsen P (mg/kg)	8.4	24.0	9.7
Available K (mg/kg)	89	150	108
CEC ($cmol_c$/kg)	8.5	11.0	9.3
Clay (%)	23	30	17
Silt (%)	74	46	70
Sand (%)	3	24	13

normally used as a urease inhibitor because of its lower price (Yeomans and Bremner, 1986). The study will help to further elaborate the effectiveness of different urease inhibitors in reduction of ammonia and their impact on early seedling growth of dry direct-seeded rice. The objectives of this study were (1) to examine the effects of different urease inhibitors on ammonia volatilization and subsequent early seedling growth of dry direct-seeded rice, when N fertilizer is applied as urea at seeding, and (2) to identify the sensitivity of shoot and root growth of dry direct-seeded rice to ammonia volatilization at early seedling stage.

Results

Effects of urease inhibitors on ammonia volatilization

In experiment 1, three urease inhibitors (NBPT, PPD and HQ) reduced ammonia volatilization from soils with urea application except of HQ in neutral soil (Fig. 1). In acidic soil, both NBPT and PPD significantly reduced the ammonia volatilization starting from the rate of 0.50% on a urea basis and which was further decreased as the rates of NBPT or PPD increased, while the significant decrease in ammonia volatilization by application of HQ was only observed at the rate of 2.00% on a urea basis (Fig. 1a). When NBPT was applied at 0.50% on a urea basis, ammonia volatilization was 5466 μg per Petri-dish, while ammonia volatilization was 1111 μg per Petri-dish when PPD was applied at the same rate. These results suggest that PPD was more effective in reducing ammonia volatilization than NBPT in acidic soil.

In neutral soil, PPD or HQ did not significantly decrease ammonia volatilization; however, NBPT markedly reduced ammonia volatilization at the rate of 0.25% on a urea basis (Fig. 1b). Increasing the rate of NBPT from 0.25% to 2.00% on a urea basis did not further decrease ammonia volatilization. In alkaline soil, NBPT obviously reduced ammonia volatilization from the rate of 0.25% on a urea basis, while the magnitude of reduction in ammonia volatilization by PPD was smaller than NBPT, HQ significantly decreased ammonia volatilization at the rate of 2.00% on a urea basis. Results showed that in soils II and III, NBPT was most effective on causing a decrease in ammonia volatilization, followed by PPD and HQ. .

Effects of urease inhibitors on early seedling growth of dry direct-seeded rice

In experiment 2, urease inhibitors significantly improved root growth and development of rice seedlings, but did not significantly affect shoot growth, which was even inhibited at higher rates of urease inhibitors (Figs. 2-4). In acidic soil, the maximum shoot fresh weight under the application of NBPT, PPD and HQ was observed at 0.25%, 0.25% and 0.50% on a urea basis, respectively, the shoot fresh weight decreased as the rate of urease inhibitors increased (Fig. 2a). All three urease inhibitors significantly increased the root tip number (Fig. 3a) and root fresh weight (Fig. 4a). Significant increase in root tip number under the application of NBPT, PPD and HQ started from the rates of 0.50%, 0.25% and 1.00% on a urea basis, and the highest root tip number was observed at 1.00%, 1.00% and 2.00% on a urea basis, respectively. From 1.00% to 2.00% on a urea basis, the root tip number did not increase under the application of NBPT, but decreased under PPD. The response of root fresh weight to application of urease inhibitors was similar to that of root tip number (Fig. 4a). The root fresh weight was increased as the rates of urease inhibitors increased except that the application of PPD decreased root fresh weight from 1.00% to 2.00% on a urea basis. In neutral soil, NBPT and PPD did not improve the growth of shoot from 0 to 0.50% on a urea basis and even significantly inhibited the growth from 0.50% to 2.00% on a urea basis. While HQ had no effect on the shoot fresh weight (Fig. 2b). Although, both NBPT and PPD significantly increased root tip number at the rates of 0.25% to 2.00% on a urea basis, NBPT was much more effective on increase in root tip number than PPD, while root tip number did not respond to the application of HQ (Fig. 3b). Similarly, NBPT and PPD significantly increased root fresh weight, but HQ increased root fresh weight slightly (Fig. 4b). In alkaline soil, NBPT, PPD or HQ did not significantly improve shoot growth and HQ even decreased the growth at the rates of 1.00% and 2.00% on a urea basis (Fig. 2c). All three urease inhibitors significantly increased the root tip number and root fresh weight compared with zero urease inhibitor (control). NBPT showed the most pronounced effect on the improvement of root tip number and root fresh weight, followed by HQ and PPD (Figs. 3c and 4c). The results of this study indicate that roots of dry direct-seeded rice are more sensitive to ammonia volatilized from soil when urea is applied at seeding, compared with the shoots. Application of urease inhibitors significantly increased the growth and development of roots, but not the shoots.

Discussion

Dry direct-seeded rice suffers from ammonia toxicity when urea is applied at seeding. Among the three soils used in this study, more ammonia was volatilized from the acidic and neutral soils than the alkaline one (Fig. 1). The pH values of soils were 4.8, 7.3 and 7.9 for acidic, neutral and alkaline soil, respectively (Table 1). Ammonia volatilization increases with soil pH and ammonia toxicity is most often observed on alkaline soils when urea fertilizer is banded or broadcasted in close proximity to recently sown seeds (Bremner, 1995; Fan and MacKenzie, 1995). However, soil properties affecting ammonia volatilization include ammonium concentration, pH, texture, organic matter, cation exchange capacity and buffering capacity. While examining

Figure 1

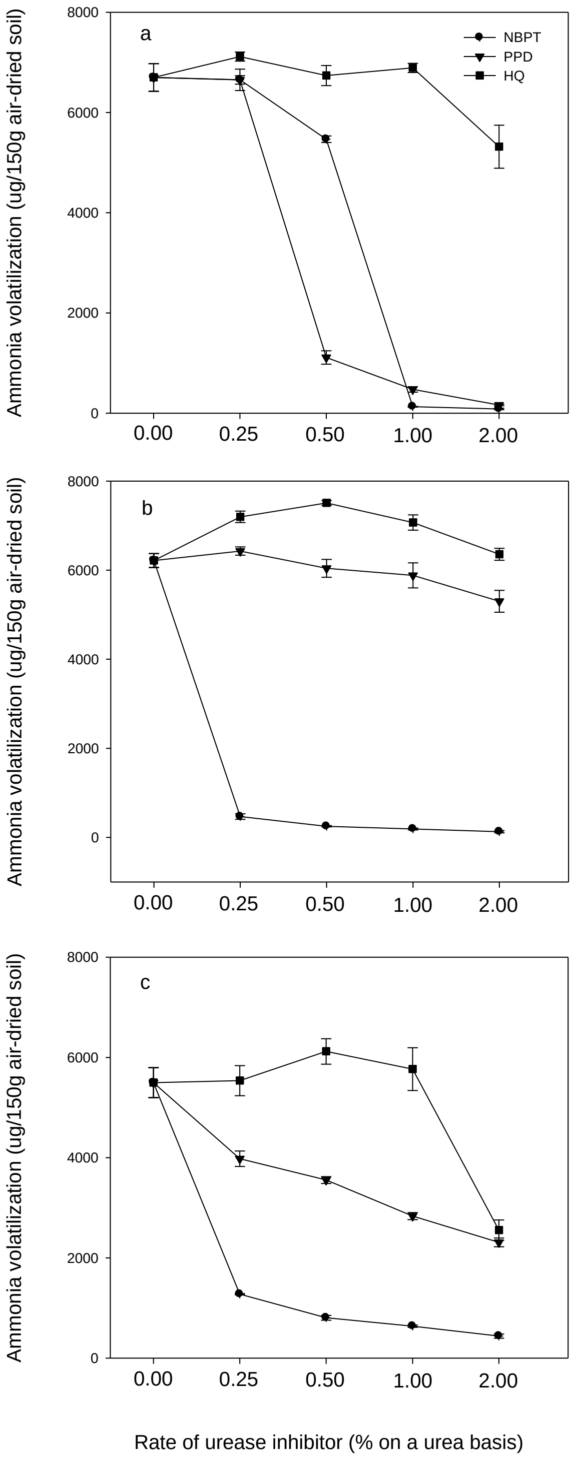

Figure 2

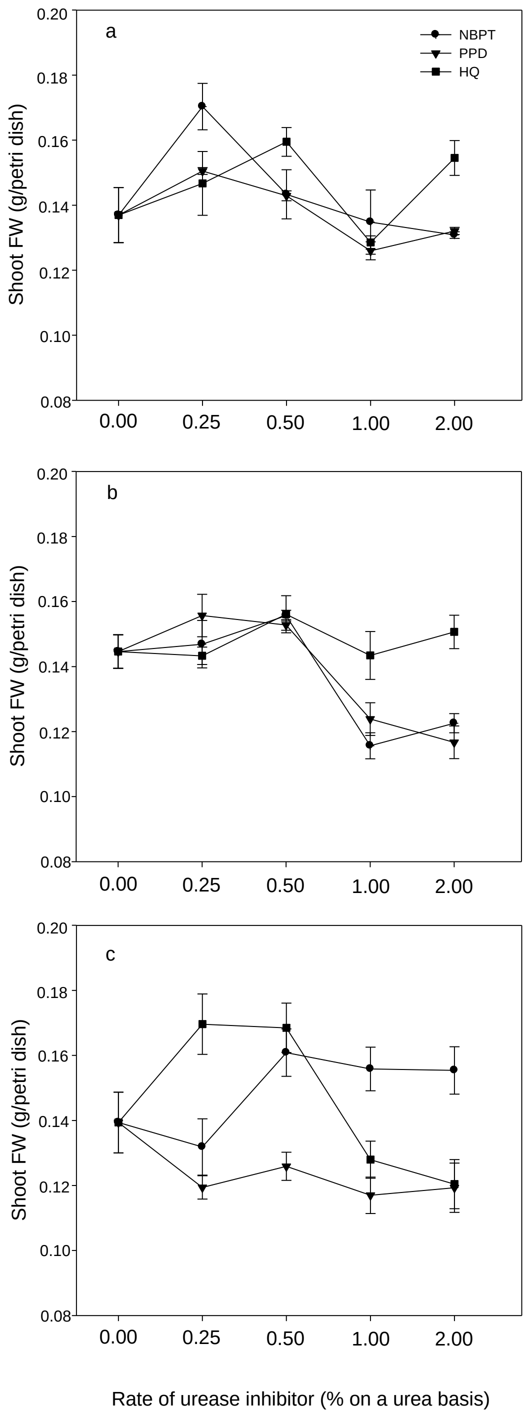

Fig 1. Ammonia volatilization from three different soils (a, b, c = acidic, neutral soil and alkaline soil, respectively) with urea under five rates of NBPT, PPD and HQ each (0-2.00% on a urea basis) in experiment 1. The rate of urea is 1mg of N per gram of soil. Ammonia volatilization was determined after four days after incubation. Error bars represent the standard error.

Fig 2. Shoot fresh weight of rice variety Apo grown in three different soils (a, b, c = acidic, neutral soil and alkaline soil, respectively) with urea under five rates of NBPT, PPD and HQ each (0-2.00% on a urea basis) in experiment 2. The rate of urea is 1mg of N per gram of soil. The presoaked seeds were not in contact with soil but exposed to ammonia gas volatilized from soils. Error bars represent the standard error.

application of urea to various soils, some scientists found that soil initial pH was not a good predictor of ammonia volatilization (Buresh, 1987; Whitehead and Raistrick, 1993). The present study does not concentrate on the relationships between ammonia volatilization and soil properties. Application of urease inhibitors significantly alleviated ammonia volatilization following urea application in dry direct-seeded rice system. Application of NBPT at 2.00% on a urea basis decreased ammonia volatilization by 11.5 to 80.0 times compared with without inhibitor i.e. control, while application of PPD and HQ at 2.00% on a urea basis decreased ammonia volatilization by 1.4 to 40.4 and 0 to 1.2 times, respectively. These results suggest that among the three urease inhibitors, NBPT was the most effective one in decreasing ammonia volatilization, followed by PPD and HQ (Fig. 1). These findings are consistent with those of Wang et al. (1991), who reported that NBPT generally exceeded PPD and HQ in the ability to reduce or delay urea hydrolysis and ammonium accumulation under aerobic conditions and HQ was less effective than the other two urease inhibitors. Application of urease inhibitors significantly improved the root growth of rice seedlings in dry direct-seeding system. Application of NBPT increased root fresh weight by 1.1 to 20.7 times compared with the treatments with urea application (Fig. 4). However, urease inhibitors slightly increased shoot fresh weight at lower rates. At higher rates, shoot fresh weight did not respond to urease inhibitors or was reduced. The symptoms of ammonia toxicity following urea application include root damage and poor seedling growth (Bremner, 1995; Bremner and Krogmeir, 1988, 1989), which are confirmed in this study. However, two pieces of evidence from this study suggest that roots of dry direct-seeded rice are more sensitive to ammonia toxicity than the shoots. First, root growth of dry direct-seeded rice is more seriously inhibited by ammonia volatilization than shoot growth (Figs. 3-4). Second, application of urease inhibitors significantly improved the root growth parameters, but not those of shoot (Figs. 2-4). Since ammonia volatilization inhibits the establishment of dry direct-seeded rice due to urea application at seeding, appropriate N management strategies to minimize the adverse effects should be considered. In addition to application of urease inhibitors, some other practical options, such as delaying the first urea application until two weeks after emergence, optimizing the timing and placement of urea, and using N fertilizers acidifying the soil are encouraged. Sun et al. (2004) reported that slow-released N fertilizer (e.g. coated urea or big granule urea) reduced the ammonia volatilization by 30-60% compared with conventional urea. The principal cause was that slow-released N fertilizer lowered the activity of soil urease enzymes. Improvement of fertilizer practices, such as, splitting the application of urea into three or more doses, banding urea so that the granules are not placed too close to seed rows and deep placement of urea should be adopted. The activity of soil urease enzymes is higher within the 0-20 cm of plough layer and decreases as the soil depth increases, so urea should be placed in the plough pan with lower activity of soil urease enzymes (Fenn et al., 1992; Gillman et al., 1995). The N rate in these two Petri-dish incubation experiments is meant to examine the soil ammonia volatilization and early seedling growth of dry direct-seeded rice if seeds are sown close to concentrated fertilizer bands and does not reflect typical field application rate when calculated at the aggregate scale. The results from the Petri-dish incubation experiments need to be confirmed under real field conditions. The mechanisms of ammonia

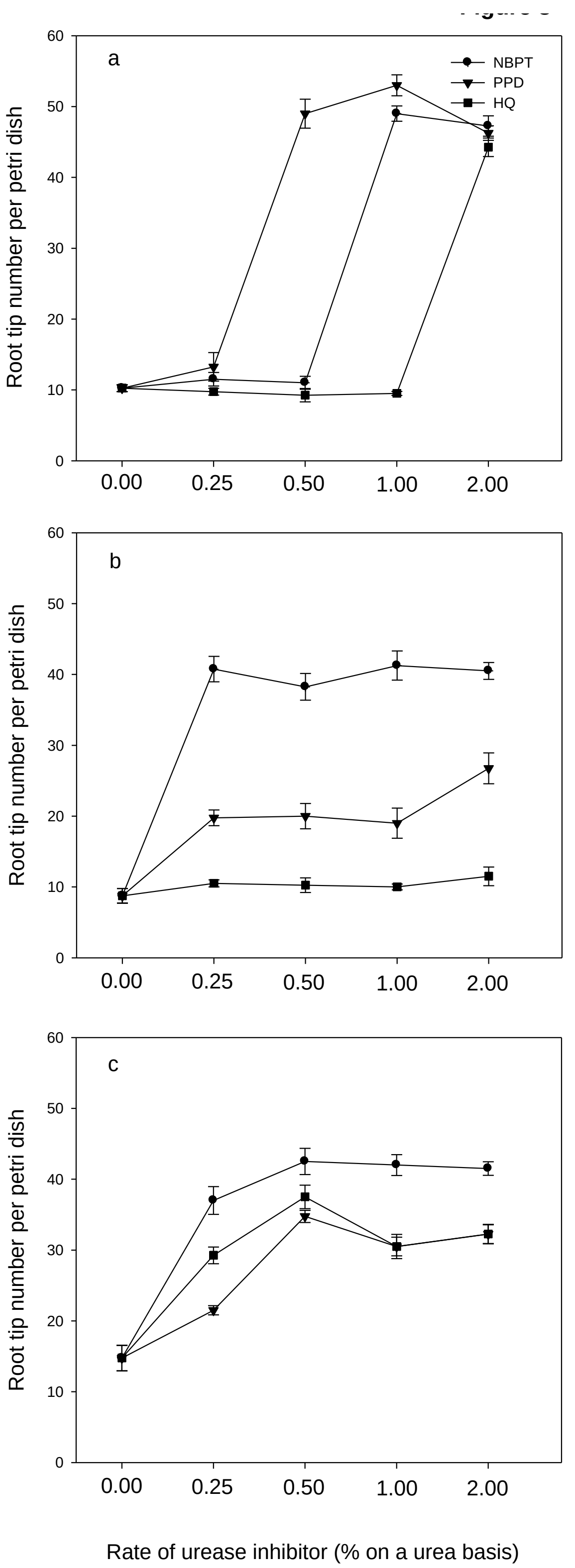

Fig 3. Root tip number of rice variety Apo grown in three different soils (a, b, c = acidic, neutral soil and alkaline soil, respectively) with urea under five rates of NBPT, PPD and HQ each (0-2.00% on a urea basis) in experiment 2. The rate of urea is 1mg of N per gram of soil. The presoaked seeds were not in contact with soil but exposed to ammonia gas volatilized from soils. Error bars represent the standard error.

toxicity have been reported in several researches. Gaseous and/or dissolved ammonia diffuses directly into plant cells wherein it disrupts cellular metabolism by interfering with intracellular pH regulation (e.g. between the cytosol and vacuole) (Kosegarten et al., 1997; Wilson et al., 1998). Vine and Wedding (1960) documented that the toxicity of ammonia is partly due to its ability to specifically inhibit the oxidation of diphosphopyridine nucleotide (DPNH) and then block the transport of electrons from oxidized substrates to oxygen. However, the exact mechanisms about ammonia toxicity to dry direct-seeded rice are still unclear. The physiological mechanisms responsible for the toxicity of ammonia to seed germination and root growth of dry direct-seeded rice will be addressed in the future.

Materials and methods

To clearly examine soil ammonia volatilization from urea application and its damage to early seedling growth of dry direct-seeded rice when urea is applied at seeding, two Petri-dish incubation experiments were conducted.

Soils description

The soils for the two Petri-dish incubation experiments were collected from the top 25 cm of three fields in Hubei province, where tea tree, wheat and vegetables were grown, respectively. Chemical and physical properties of the three soils are listed in Table 1. Before filling the Petri-dish, the soil was air-dried, pulverized, crushed to pass through a 2 mm sieve and well mixed. Two sizes of plastic Petri-dishes with diameters of 5.5 and 14.5 cm were used. A 150 g of air-dried soil from each soil was placed in a larger plastic Petri-dish.

Application of Urease inhibitors

In both experiments, 1mg N as urea was applied per gram of soil. Three urease inhibitors, N-(N-butyl) thiophosphoric triamide (NBPT), phenyl phosphorodiamidate (PPD) and hydroquinone (HQ) were applied at rates of 0, 0.25, 0.50, 1.00 and 2.00% on a urea basis. Urea and different urease inhibitors at various rates were applied to the soil in each Petri-dish in solution. In order to keep the soil in Petri-dishes under aerobic conditions, the total volume of solution was kept as 50 ml.

Experiment 1

In experiment 1, ammonia volatilization was measured using the method of Conway microdiffusion incubation adapted for soil by Bremner and Krogmeier, (1989). A smaller uncovered Petri-dish with a diameter of 5.5 cm containing 10 ml of 2% boric acid and 1-2 drops of mixture indicators (bromcresol green and methyl red) was placed in the center of the larger Petri-dish to serve as a trap for ammonia gas. Then the lid of the larger Petri-dish was covered and sealed so that the ammonia gas could be trapped by the boric acid without gas leakage from this system. Petri-dishes were placed inside a darkened growth chamber for 4 days at 28°C and 70% humidity using a completely randomized design. Each treatment was replicated four times with four Petri-dishes. This experiment was started on 14 July 2009 and incubated for 4 days. The amount of ammonia volatilized from soil was determined by titrating the boric acid using 0.01N HCl (Bremner and Mulvaney, 1982).

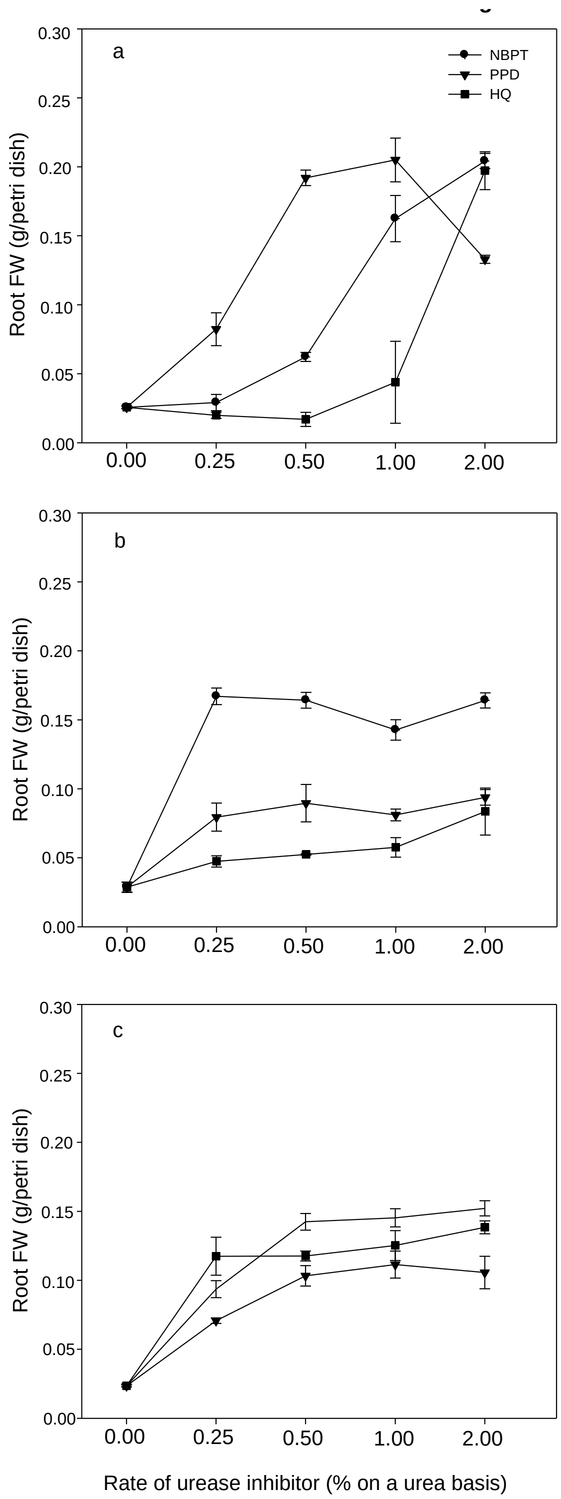

Fig 4. Root fresh weight of rice variety Apo grown in three different soils (a, b, c = acidic, neutral soil and alkaline soil, respectively) with urea under five rates of NBPT, PPD and HQ each (0-2.00% on a urea basis) in experiment 2. The rate of urea was 1mg of N per gram of soil. The presoaked seeds were not in contact with soil but exposed to ammonia gas volatilized from soils. Error bars represent the standard error.

Experiment 2

In experiment 2, the treatments and incubation methods were the same to those of Petri-dish incubation experiment 1. A smaller uncovered Petri-dish containing 10 pre-soaked seeds of Apo with moistened filter paper was placed in the center of a larger Petri-dish, instead of boric acid. Inside the sealed Petri-dish, the pre-soaked seeds were exposed to ammonia volatilized, but without any contact with the soil. The Petri-dish incubation was started on 14 July 2009 and lasted for 4 days under the same conditions as in experiment 1. The fresh shoot and root were weighed and root tip number was counted using a WinRHIZO scanner (Regeant Instrument, Quebec, Canada).

Statistical analysis

Data were analyzed according to completely randomized design following analysis of variance (SAS, 2003) and mean comparison between treatments was performed based on the Least Significant Difference (LSD) test at the 0.05 probability level.

Conclusion

Ammonia volatilization from urea at seeding reduced early seedling growth of dry direct-seeded rice. Application of urease inhibitors significantly alleviated ammonia volatilization following urea application. Among the three urease inhibitors, NBPT was the most effective one in decreasing ammonia volatilization, followed by PPD and HQ. The evidence from this study suggests that roots of dry direct-seeded rice are more sensitive to ammonia toxicity than the shoots when urea is applied at seeding. Future studies should focus on the physiological mechanisms responsible for the toxicity of ammonia to seed germination and root growth of dry direct-seeded rice.

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