26.
EVALUATING THE ABILITY OF RICE TO EFFECTIVELY SUPPRESS THE ACTIVITY OF SOIL NITRIFIERS

W. S. Dandeniya

Department of Soil Science, Faculty of Agriculture, University of Peradeniya.
warshisd@gmail.com

INTRODUCTION

More than 20% of the N fertilizer produced worldwide is used in rice fields in Asia. The cost of N fertilizers usually represents 10-20 % of the total production cost of rice; however, the agronomic nitrogen use efficiency in rice farming is around 30% resulting yield reductions and waste of foreign exchange (Sirisena *et al.*, 2000). Nitrification is a microbiologically mediated transformation governing the pool sizes of plant available nitrogen forms (NH_4^+ and NO_3^-) in soil. The rate of nitrification reactions is affected by environmental factors such as substrate (NH_4^+ and NO_2^-) concentration, oxygen concentration, presence of toxic or inhibiting substances, pH, and temperature. Allelochemicals, a group of plant-derived secondary metabolites, interfere with growth of surrounding plants and microbial community dynamics in soil including nitrifiers (Subbarao *et al.*, 2007). The suppression of nitrification by biologically active plant derived compounds is referred to as biological nitrification inhibition (BNI) (Subbarao *et al.*, 2007). Nitrification in the rhizosphere can be suppressed by plants and other microbes by secreting inhibitory compounds, competing for NH_4^+ and/or by encouraging the growth of fast-growing heterotrophs in the rhizosphere that out-compete slow-growing nitrifiers. Considering the immense diversity of rice gene pool in Sri Lanka, there is a great opportunity of finding rice varieties with BNI potential. A study was conducted with ten rice varieties to determine the ability of rice to suppress nitrification.

METHODOLOGY

A soil sample was collected to isolate nitrifiers from the 0 -10 cm depth from a paddy field at the experimental farm of University of Peradeniya in Maha Illuppallama. Ammonia oxidizing bacteria, a group of microorganisms involved in the first step of nitrification, was isolated using a culture based approach with P buffer medium (pH 7.8) as described previously (Weaver *et al.*, 2007). Fourteen days old seedlings of ten rice varieties (BG 300, BG 352, BG 358, BG 94/1, AT 362, Suduru Samba, Sudu Heenati, Dahanala, Kalu Heenati and Suwandel) were used in the study. Roots of rice seedlings were immersed in distilled water (4 mg fresh roots/ ml), and shaken for 30 min to collect root wash (RW) and then the roots were pat dried, ground and dissolved in sterilized distilled water to obtain a root extract (RE) solution with a concentration of 10 mg fresh roots ml^{-1}. A bioassay was conducted with RW to test its effect on potential ammonia oxidation (PAO) activity of isolated ammonia oxidizing bacteria. A soil incubation study was conducted to test the effect of RW and RE on nitrifiers in the presence of other microorganisms. The potential nitrification rate (PNR) of soil nitrifiers after incubating with RW or RE (at a rate of 400 µg of fresh roots per milliliter of soil slurry) for 2h was measured using shaken slurry method as described previously by Hart *et al.* (1994). A control with 2 ml of sterilized distilled water to replace root derived compounds was performed in triplicates. Suppression of nitrification was calculated by dividing PNR with RW/RE from the PNR of control.

Data were statistically analyzed. Analysis of variance was performed for data generated from BNI experiment using MINITAB® Release 14.1 statistical software (Minitab Inc.). Means were compared by LSD mean separation ($p < 0.05$).

RESULTS AND DISCUSSION

Tested rice varieties differed in their ability to affect the activity of nitrifiers (Table 1). Root wash (RW), which mainly contain root exudates, enhanced the activity of ammonia oxidizers isolated from soil to varying degree depending on the variety. The observations made on the effects of RW on nitrifiers in the isolated culture did not agree always with the effect on nitrifiers in soil. Root extracts of all varieties obtained by grinding fresh roots and dissolving in distilled water suppressed the potential nitrification at the rate of RE applied in this study, which is nearly compounds from 400 µg fresh roots per milliliter of soil slurry (400 ppm). Interestingly, RW and RE application to soil affected nitrifiers differently for some rice varieties although the application rates of two different type of root derived compounds were similar (Table 1). For example, RW from BG352 stimulated the nitrifier activity while the RE from the same variety suppressed soil nitrifiers.

Table 1. The effect of root derived compounds on soil nitrifiers' activity (Values are given as Mean ± Standard Error mean). Values are calculated as [activity when applied RW or RE] / [activity of control]. Therefore, values <1 indicate that potential activity was suppressed by added compounds.

Variety	Effect of RW on potential activity of ammonia oxidizers *	Effect of RW on potential activity of nitrifiers in soil **	Effect of RE on potential activity of nitrifiers in soil **
BG300	1.72 ± 0.15 **b**	1.44 ± 1.18 **ab**	0.58 ± 0.10 **a**
BG352	25.50 ± 16.1 **b**	3.11 ± 0.40 **a**	0.69 ± 0.06 **a**
BG358	1.03 ± 0.02 **b**	0.52 ± 0.06 **b**	0.55 ± 0.05 **a**
BG94/1	1.04 ± 0.01 **b**	1.51 ± 0.17 **ab**	0.55 ± 0.07 **a**
AT362	1.16 ± 0.19 **b**	0.65 ± 0.24 **b**	0.62 ± 0.14 **a**
Suduru Samba	453.47 ± 6.51 **a**	0.59 ± 0.29 **b**	0.85 ± 0.16 **a**
Sudu Heenati	410.95 ± 2.83 **a**	1.36 ± 0.87 **ab**	0.67 ± 0.04 **a**
Dahanala	1.03 ± 0.01 **b**	0.22 ± 0.04 **b**	0.76 ± 0.07 **a**
Kalu Heenati	1.02 ± 0.04 **b**	0.98 ± 0.06 **b**	0.58 ± 0.06 **a**
Suwandel	435.56 ± 8.37 **a**	1.38 ± 0.67 **ab**	0.74 ± 0.25 **a**

* The effects on ammonia oxidizers were assessed on using a culture isolated from soil

** Suppression of nitrification was measured directly using soil.

Means in a given column followed by the same letter are not significantly different at $p < 0.05$.

Once added to a medium the availability of an organic compound is determined by the complexity of the medium. Soil is a highly complex medium due to soil matrix itself and the presence of soil microorganisms that use plant metabolites as C and energy sources. Physicochemical reactions like chelation, adsorption to clay and organic matter, dilution by soil moisture, and root exudation as affected by plant stress and plant age can change the active plant-derived secondary metabolite concentration in soil (Gopalakrishnan *et al.*, 2009). Therefore, the results from laboratory bioassays conducted using artificial growth media may not agree with studies conducted using soil as a medium (Tanaka *et al.*, 2010). Results from laboratory bioassays on allelopathy are relative and provide information about potential BNI only (Tanaka *et al.*, 2010; Subbarao *et al.*, 2007). It was clearly indicated in the present study that although some varieties like Suduru Samba, Sudu Heenati and Suwandel stimulate the activity of ammonia oxidizer isolated from soil, similar results were not obtained when applied the root exudates to soil. Root exudates in Suduru Samba suppressed the

nitrifiers activity in soil by nearly 50 % compared to control even though the same extract stimulated the ammonia oxidizers activity nearly 450 times in laboratory bioassay.
Dominant nitrifying bacteria in soil are chemolithoautotrophs that use CO_2 as the cellular C source but some members have the ability to grow as a chemolitho organotroph using fructose, pyruvate and amino acids like organic compounds (Clark and Schmidt, 1967). The composition of root wash of rice varieties was not analyzed in the present study. However, a previous research conducted with traditional and improved rice varieties in Sri Lanka confirmed that rice varieties have varying root exudation profiles and some varieties exude more organic compounds like amino acids than others (Dandeniya, 2007). The rate of application of root derived compounds in the present study was 0.4 mg ml^{-1} (final concentration in soil slurry) and suppression of nitrifier activity was observed even at this level. Root derived organic compounds might stimulate the growth of other fast growing bacteria in the rhizosphere exerting a pressure to slow growing nitrifiers. This may contribute to the observed low nitrification rates in soil media compared to ammonia oxidizer growth media used in the laboratory bioassay.

Results indicate that the diversity of rice gene pool in Sri Lanka present great potential to search for biological nitrification inhibition. Information on ability of rice to suppress nitrification will be useful in developing rice varieties with better N use efficiency to use in environments with excessive nitrification such as rain-fed and upland rice cultivation systems.

CONCLUSION

Root derived secondary metabolites in tested rice varieties possess the ability to suppress nitrification to different extent. The suppression of nitrifiers' activity may have achieved via manipulation of other soil microorganisms that can exert a competition for slow growing nitrifiers and this should be further investigated.

ACKNOWLEDGEMENT

Data published here is part of an ongoing study funded by University Research Grants-2011 of University of Peradeniya.

REFERENCES

Clark C. and Schmidt E.L. 1967. Growth response of *Nitrosomonas europaea* to amino acids. J. Bacteriol. 93:1302-1308.

Dandeniya, W.S. 2007. Diversity of Microbial Communities Associated with Rhizosphere of Selective Improved and Traditional Rice Varieties Grown in Sri Lanka. M.Phil. Thesis. Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka.

Gopalakrishnan S., Watanabe T., Pearse S.J., ITO O., Hossain Z.A.K.M. and Subbarao G.V. 2009. Biological nitrification inhibition by *Brachiaria humidicola* roots varies with soil type and inhibits nitrifying bacteria, but not other major soil microorganisms. Soil Sci. Plant Nutr. 55:725-733.

Hart, S.C., Stark, J.M., Davidson, E.A., Firestone, M.K. (1994). Nitrogen mineralization, immobilization and nitrification. pp. 985-1018.In: Weaver, R.W., Angle, J.S., and Bottomley, P.S. (Eds.) Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Soil Science Society of America. Madison, WI, USA

Tanaka J.P., Nardi P. and Wissuwa M. 2010. Nitrification inhibition activity, a novel trait in root exudates of rice. AoB Plants.

Sirisena D.N., Wickramasinghe W.M.A.D.B., Weerakoon W.M.W., Kumaragamage D. and Bandara S.T. 2000. Evaluation of the leaf-N based nitrogen fertilizer management in irrigated transplanted rice. Available Online < http://agrilearning. goviya.lk/Paddy/Paddy_Research/Paddy_pdf/A15.pdf> [Accessed 04-11-2012].

Subbarao G.V., Wang H.Y., Ito O., Nakahara K. and Berry W.L. 2007. NH_4 triggers the synthesis and release of biological nitrification inhibition compounds in *Brachiaria humidicola* roots. Plant Soil 290:245-257.

Weaver, R.W., Angle, J.S., and Bottomley, P.S. (Eds.). 2007. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties. Soil Science Society of America. Madison, WI, USA.