Iranian Journal of Weed Science 5 (2009) 13-25

مجله دانش علف‌های‌هرز

# Nitrification Inhibition Properties in Root Exudates of *Brachiaria humidicola* Plants

M. K. Souri[1*], G. Neumann [2]

[1*] Department of Horticultural Sciences, Tarbiat Modares University, Tehran- Iran

[2] Institute of Plant Nutrition, University of Hohenheim, Stuttgart-Germany

(Received 10 May 2009; returned 20 February 2010; accepted 6 December 2010)

## ABSTRACT

Nitrification inhibition (NI) by climax ecosystems has been suggested for decades, and this inhibitory effect seems to be a feature of wild genotypes rather than commercial cultivars. Many plants particularly grasses were suggested to have NI activity, and recently *Brachiaria humidicola* (BH) was shown to have promising control on nitrification rates through root exudates. In this study effects of different treatments such as N form ($NH_4^+$ vs $NO_3^-$) and N concentrations (1, 2 and 4 mM N), plant age, light intensity and different collecting mediums for root exudates on the NI activity of root washings were investigated. This was done using a series of nutrient solution experiments. The results showed that BH root exudates collected in distilled water, independent of light intensity, plant age, N-forms, N-concentrations and root exudates collection periods, had no significant inhibition on nitrification. However, root exudates collected in a 1 mM $NH_4Cl$ medium had significant inhibition on nitrification process in a soil bioassay. This inhibition was more highlighted when plants were grown in presence of ammonium rather than nitrate. In comparison to drying with rotary evaporator, freezed dried root exudates indicated significant NI in root exudates of plants which were grown in $NH_4^+$ under low light, while this effect was not seen under higher light intensity or nitrate nutrition. Measuring electric conductivity of solutions from root washing also showed higher conductivity when ammonium presented in root medium, particularly in root exudates collecting medium over extended time (24 instead 6 hours).

**Keywords:** root exudates, ammonium, nitrate, electric conductivity

[*] Corresponding author: E-mail: mk.souri@modares.ac.ir

## INTRODUCTION

Nitrogen management requires sufficient knowledge regarding spatial distribution of mineral nitrogen. Nitrification is the main cause of nitrogen losses in soil profile. This process can be retarded by different synthetic inhibitors. However, if plants could manage nitrification, it would offer important economic and environmental implications. Identification of such plants and related physiological and molecular traits can help to introduce such highly valuable properties from wild plants to crops. This consequently could lead to less application of fertilizers and higher N recovery rates (Subarrao *et al*., 2006). Plants are immobile in nature, but they have the ability to apply different strategies to coupe with unfavourable conditions surrounding them, and to optimize unfriendly conditions for their growth and development. Despite physiological and morphological changes, exudation of primary and secondary metabolites by roots as well as emission of chemicals through leaves is a well known phenomena in this case (Liljeroth & Baath, 1988; Jones *et al*., 2004).

*Brachiaria humidicola* Napper plants are $C_4$ species and among the most important pastures widely adapted to grow in tropical and subtropical parts of South America, Africa & Asia (CIAT, 1983). Different *Brachiaria* species consist 85% of the total planted pasture area in South America (Nakamura *et al*., 2005), and commercially an important economic player in the tropics, particularly in Brazil. The genus *Brachiaria* contains a wide range of species, which are adapted to poor acid soils and tolerant to drought and harsh environments. Vast diversity in *Brachiaria* plants could be the main reason of their adaptation capacity to different edaphic and climatic conditions. In comparison to other plants, in such conditions, they have relatively higher biomass production due to the ability to uptake and use nutrients more efficiently in poor soils (Nakamura *et al*., 2005; Cazetta & Villela, 2004). There is no report of growing such plants in Iran, however they can be introduced to subtropical parts in Southern provinces.

Observations during field surveys have shown that soils of BH generally have low levels of N-$NO_3^-$ (CIAT, 1985; Sylvester-Bradley *et al*., 1988). Recently experiments show that root exudates of *Brachiaria* plants specially BH (accession 26159) can efficiently suppress nitrification process in laboratory, soil and field conditions (Ishikawa *et al*., 2003; Wang *et al*., 2005; Subbarao *et al*., 2005 & 2008). They indicated that only BH particularly accession 26159 suppresses nitrification in soil, and this inhibition occurs under $NH_4^+$ rather than $NO_3^-$ nutrition. Nevertheless, it has been suggested that nitrification inhibition abilities are a plant's specific reaction to stress conditions, especially under low N level in the soil (Ishikawa *et al*., 2003; Subbarao *et al*., 2006).

(Subbarao *et al*., 2005, 2006 & 2007 a,b,c) mentioned strong inhibition of collected root exudates of BH on nitrification, which this inhibitory effect was lasting up to 70 days, which is more efficient than synthetic NIs such as N-Serve or DMPP (3,4-

dimethylpyrazol phosphate). Despite recent works, there is still not enough knowledge on nitrification inhibitory effect of plants root exudates. Therefore, the biological inhibition of nitrification by crop plants or pasture species particularly by BH plants is not well known, and still many questions are to be answered. In order to investigate whether the inhibitory effect of root exudates would change under different growth and physiological conditions (N-form, N-concentrations, different light intensities, plant age, differing collecting medium and collecting periods of root exudates), this study was conducted using BH plants under controlled condition in growth chamber.

## MATERIALS AND METHODS

### Plant Culture

This study was conducted during 2005-2007 in the Institute of Plant Nutrition, University of Hohenheim, Stuttgart-Germany. Seeds of *Brachiaria humidicola* (Rendle) accession 26159 germinated at 25 ºC in fine sand (0.02-0.5 mm) for the first experiment. For the next experiments, due to long germination period (4 weeks), heterogeneous germination, as well as low germination rate (15%), vegetative new tillers from older plants were used as new seedlings. Seedlings more than 10 cm (3-4 leaves) were cut and transferred to treatment condition in nutrient solution (2.5 litres in black plastic pots). The composition of nutrient solution was 10 µM $H_3BO_3$, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.1 µM $CuSO_4$, 0.01 µM $Mo_7O_{24}(NH_4)_6$, 83 µM Fe-EDTA, 0.7 mM $K_2SO_4$, 0.5 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 1.2mM KCl. For ammonium treatments 1 mM $CaCl_2$ was used (Souri, 2008). Treatments were N-form ($NH_4^+$ or $NO_3^-$) in form of $(NH_4)_2SO_4$ or $Ca(NO_3)_2$, with 1, 2, and 4 mM N, depending on experimental set up. However, normal N concentration was 2 mM N as ammonium sulphate or calcium nitrate. Each treatment consisted of four replicates. Ammonium treatment was applied either at the beginning of transferring the seedlings to nutrient solution or for short periods, after being pretreated in nitrate (2 mM N-$NO_3^-$ as pre-culture) for two weeks (Prof. Dr. Volker Römheld, personal communication).

In addition to N status, the effect of different variable factors such as light intensity and plant age (young three-weeks-old vs seven-weeks-old plants) on production and release of NIs were tested.

Different light intensity conditions were applied using the same growth chamber, in which for high light intensity (400 µmol/$m^2s^{-1}$), plants were placed at the centre of the table in the growth chamber with constant light intensity. For intermediate light intensity plants were placed at the corner of the growth chamber, where light intensity was 240 µmol/$m^2s^{-1}$. Low light intensity was achieved in the same growth chamber by locating plants inside a box and under different layers of plastic, where they received 180 µmol/$m^2s^{-1}$ light intensity. Plants were grown under a light/dark regime of 16/8, and a temperature of 28/25 ºC in nutrient solution culture. Nutrient solutions were renewed thoroughly every 3 days. In treatments with pH control, a solution of 3 mM

Morpholinoethanesulfonic acid (MES) and double daily pH checking and adjusting with KOH, $Ca(OH)_2$ and $H_2SO_4$ were performed.

**Collection of Root Exudates**

Root exudates were collected 2 hours after starting the light period, either for 6 hours from 10 am to 4 pm, or for 24 hours from 10 am to 10 am in the next day. Before collection, plant roots were washed for 1-2 minutes in distilled water. Collecting medium generally was 500 ml distilled water, however, a solution of 1mM $NH_4Cl$ was also used as the collection medium. After collection, root exudates were concentrated at 35 ºC using rotary evaporator to a volume of 15 ml, from which 2.5 ml was applied per replicate in soil incubation bioassay for rapid detection of potential nitrification (Kandeler, 1993). Four samples (replicates) for incubation as well as two samples which were kept under freezing conditions for original $NO_2^-$ concentrations were used to determine potential nitrification inhibitory of root exudates. In this study we used two controls in our bioassay; first water control in which distilled water was applied instead of root exudates in soil nitrification bioassay, and second 3,4 dimethylpyrazol phosphate (DMPP) control as a standard synthetic nitrification inhibitor which was used in 10 times of its normal concentration (1% of N-$NH_4$) in all incubations during this study. Plants were exposed to 6 or 24 hours root exudates collection in 1 mM $NH_4Cl$, and the electric conductivity (µS/m) of root washings after 3 hours collection was determined.

Excel and SPSS soft wares were used to draw and analysis the data. Comparison of means was performed at P= 0.05 using Duncan test. In figures data are presented as average of four replicates ± SD.

## RESULTS

### A) Collecting Root Exudates in Distilled Water

Root exudates of plants which were grown in nutrient solution under $NO_3^-$ and $NH_4^+$ or under $NH_4^+$ but with buffered pH 6 (Figure 1), and were collected after a 24 hours period in distilled water, showed no significant nitrification inhibition (NI) compared to control. Significant changes in the pH of nutrient solution were occurred for both ammonium and nitrate grown plants within only 24 hours where a pH of 2 has been also recorded (data not presented). Similarly, different effects of plant age (Figure 2); collecting periods (Figure 3); nitrogen concentrations (Figure 4); different light intensities (Figure 5), when collected in distilled water, showed no significant nitrification inhibition compared to water or DMPP control. DMPP, as a standard synthetic nitrification inhibitor, always resulted in very low amounts of produced nitrite in all nitrification incubation tests, indicating high efficiency regarding control of nitrification (Figures 1-7).

Collected root exudates in distilled water when dried in freeze drier and extracted with methanol and further with DMSO (Figure 6), showed significant NI activity only in $NH_4^+$ grown plants under low light intensity but not in nitrate or higher light intensities. Nevertheless, there was a

general trend of nitrification reduction with younger plants rather than older plants, lower light intensities rather than higher, as well as lower concentrations of nitrogen in nutrient solution.

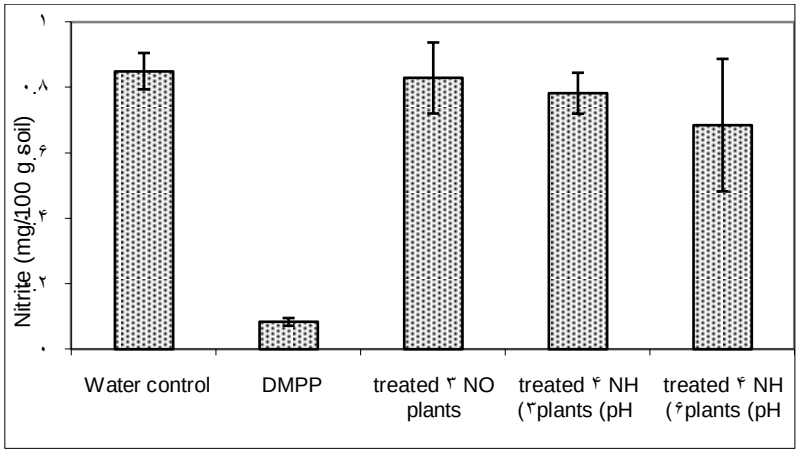


**Figure 1.** Nitrification inhibition effect of root exudates collected in 0.5 l distilled water for 24 hours period. Plants were grown with $NH_4^+$, $NO_3^-$, or buffered-$NH_4^+$, with the concentration of 2 mM N. PH of collected root exudates was ~ 3.5 for $NH_4^+$ and ~ 7 for $NO_3^-$ grown plants. Data are average of four replicates ± SD.

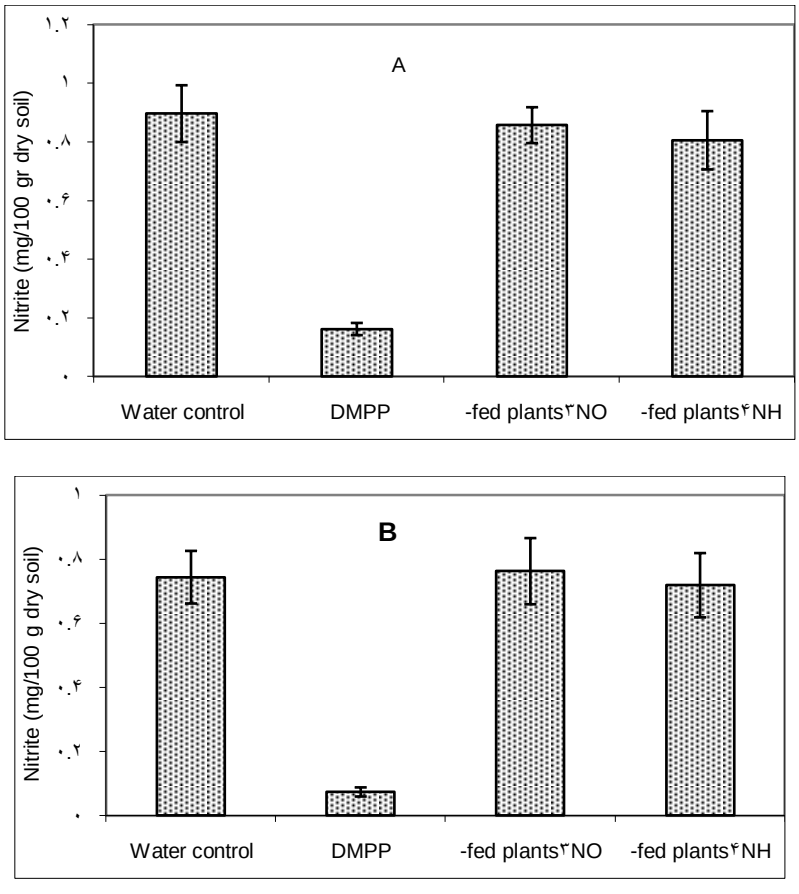


**Figure 2**. Nitrification inhibition effect of root exudates of 3-weeks old (A) and 7-weeks old (B) plants grown under 2 mM N-$NO_3^-$ or $NH_4^+$. The pH of nutrient solution for both ammonium and nitrate medium was adjusted to 5 using MES and $H_2SO_4$ or KOH. PH of medium after collection was ~ 3.5 for $NH_4^+$ and ~ 7 for $NO_3^-$ treated plants. Data are average of four replicates ± SD.

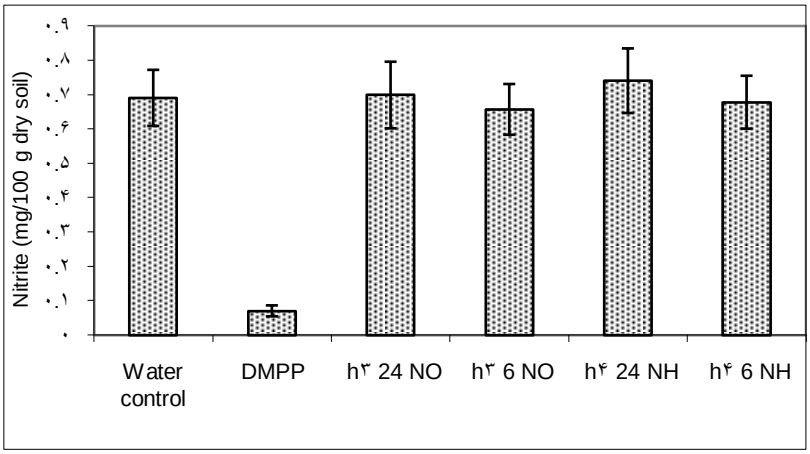


**Figure 3.** Nitrification inhibition effect of root exudates collected in distilled water for 6 or 24 hours. Plants were grown in nutrient solution with ammonium or nitrate (without pH adjustment during growing period). PH of medium after collection was ~ 3.5 for $NH_4^+$ and ~ 7 for $NO_3^-$ plants. Data are the average of four replicates ± SD.

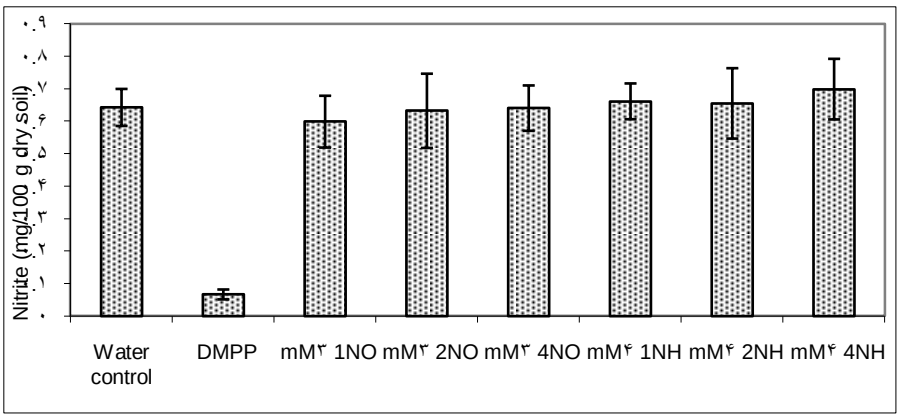


**Figure 4.** Nitrification inhibition of root exudates collected for 24 hours in distilled water. Plants were grown with different N concentrations as $NO_3^-$ or $NH_4^+$. PH of collected root exudates was ~ 3.5 for $NH_4^+$ and ~ 7 for $NO_3^-$ plants. Data are average of four replicates ± SD.

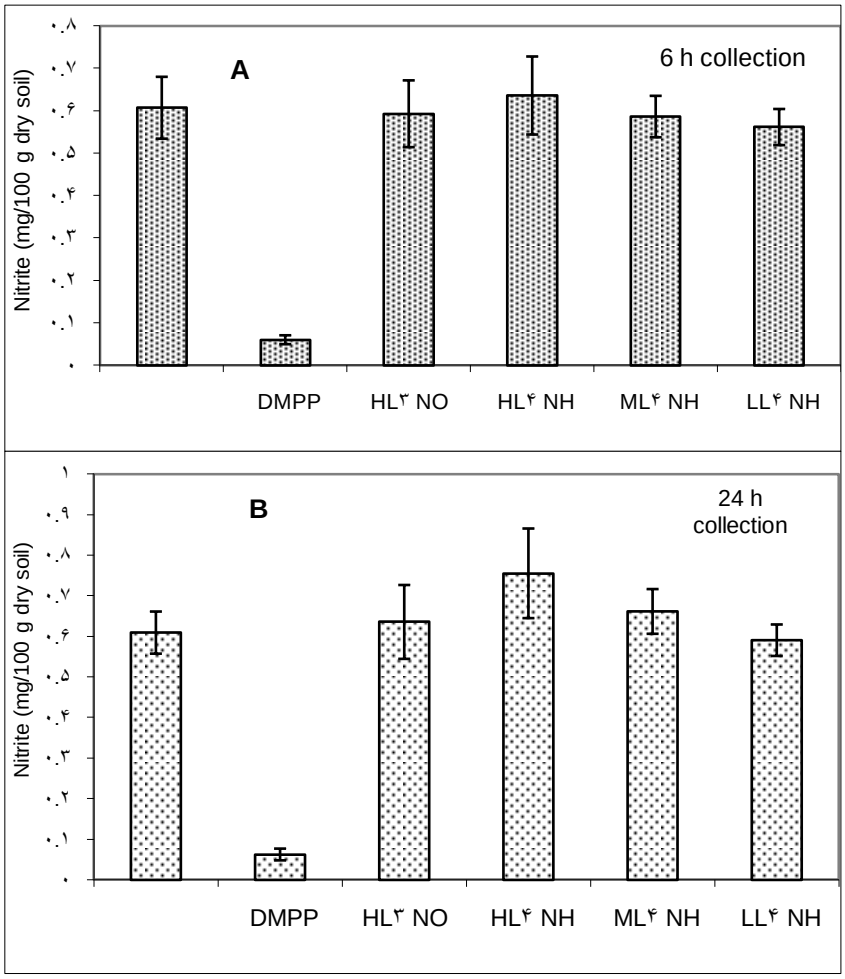


**Figure 5.** Nitrification inhibition of root exudates collected in distilled water of 6 hours period (A) or 24 hours period (B). Plants were grown in low (LL), middle (ML) and high (HL) light intensities with $NH_4^+$ (2 mM N), compared to $NO_3^-$ (2 mM N) grown plants in high light intensity. Data are average of four replicates ± SD.

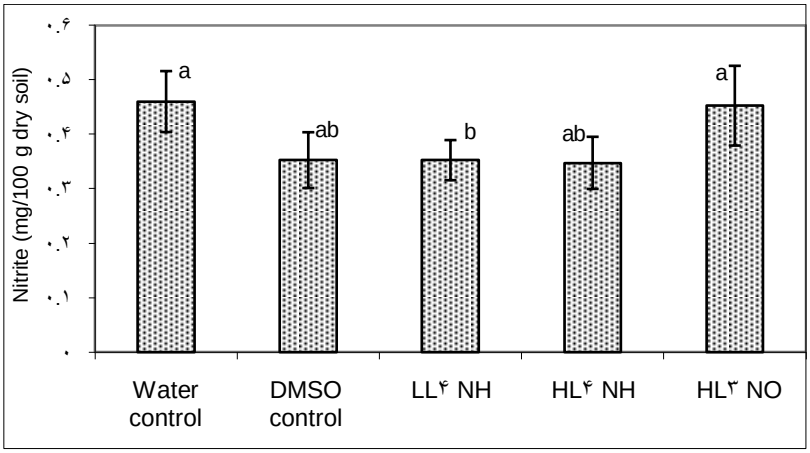


**Figure 6.** Nitrification inhibitory effects of freeze dried root exudates (collected in distilled water) of plants pre-treated with $NH_4^+$ or $NO_3^-$ in high or low light intensity, which have been extracted finally with 0.6% DMSO (on basis of final volume). Data are average of four replicates ±SD. Duncan test was conducted for mean values at P= 0.05.

**B) Collecting Root Exudates in $NH_4Cl$ Medium**

Effects of collected root exudates in 500 ml of 1 mM $NH_4Cl$ solution for plants grown in $NH_4^+$ or $NO_3^-$ under different collecting periods are presented in (Figure 7). Significant inhibition of nitrification compared to control was seen for $NH_4^+$ grown plants, although this was for the 24 hours collection period rather than the 6 hours collection period. There was no significant inhibitory effect for nitrate grown plants. However, root exudates of nitrate grown plants showed considerable inhibition after the 24 hours collection period, which was not statically significant. Electric conductivity (EC) of root washings (Figure 8) showed higher conductivity when ammonium is presents particularly in the collecting medium. Ammonium grown plants showed higher conductivity rather than nitrate grown plants.

## Discussion

It is almost three decades that the role of phytosidrophores has been well established mainly in iron uptake of plants. Their collection procedure is still used for collecting root exudates for different purposes. In these experiments, plant roots were transferred 2 hours after the light period to a distilled water medium for collection of root exudates (Römheld & Marschner 1986). In contrast to (Subbarao *et al*., 2005, 2006a & 2007a) which collected root exudates only in 1 mM $NH_4Cl$ or $KNO_3$, in this study we used both 1 mM $NH_4Cl$ (or $KNO_3$), and distilled water as the collecting medium for root exudates. In Figures 1-6, root exudates collected in distilled water from plants grown under different N forms, N concentrations in nutrient solution, plant age, light intensities, as well as collecting periods showed no significant inhibitory effect on nitrification. However, root exudates of ammonium grown plants under low light intensity which were collected in distilled water and dried using freeze drier instead of rotary evaporator, showed significance differences compared to control (Figure 6). This might be due to avoiding some degradation of NI compounds during routine concentration procedure via rotary evaporator. This, in turn, indicates that the release procedure of natural nitrification inhibitors (NNIs) may not be an active process, since collecting in distilled water due to the osmotic effect, results in higher exudation (Neumann & Römheld, 2000). Exposure of plant roots to external solutions of very low ionic strength is likely to increase exudation rates due to an increased transmembrane concentration gradient of solutes (Neumann & Römheld, 2000). The highest inhibitory effect occurred when 1 mM $NH_4Cl$ was used in the collecting medium (Figure 7). This inhibition was a function of the collection period and N-form. Similar results were obtained by others (Subbarao *et al.*, 2005-2008; Ishikawa *et al.*, 1999; 2003; Gopalakrishnan *et al.*, 2007).

In the current study there was always a negative correlation between nitrogen concentrations in nutrient solution and NI activity of root exudates or shoot homogenates independent of N-forms (data

not presented). However, Subbarao *et al*., (2005-2008) showed that amount of NNI production and release is a function of nitrogen status of plant, as well as $NH_4^+$ rather than $NO_3^-$ nutrition, in which higher nitrogen content of plants leads to more production of NI compounds. In contrast, our data support the idea that nitrogen stress (deficiency) could be the main factor behind the evolution of NNIs as an adaptive mechanism, and they are expressed under N limitation (Ishikawa *et al*., 2003; Lata *et al*., 2004; Subbarao *et al*., 2007b). Subbarao *et al*., (2007c) released a patent indicating some isomers of unsaturated linolenic and linoleic fatty acids show strong NI activity. Nevertheless, active exudation of fatty acids specially unsaturated long chain isomers of linolenic acid is not possible (Neumann & Römheld, 2000). This could be possible only through root damage or passive exclusion of root debris. In addition, no significant NI activity was indicated when root exudates were collected in distilled water, while significant inhibition effects when collected in ammonium chloride, indicates no active release of NNIs. This is further supported by low pH as an indirect effect of $NH_4^+$ uptake in collecting medium, which can damage the root cells membrane, and consequently leaching of NNIs could occur as a passive phenomenon (Figure 8).

The inhibitory effect of exudates when collected in 1mM $NH_4Cl$, might be a secondary response of BH to salt or osmotic conditions in collection medium. Mergulhao *et al*., (2002) showed that BH is relatively sensitive to salinity particularly to chloride in growth medium, with a succulent effect on leaves and roots which is more pronounced on roots (Mergulhao *et al*., 2002; Cazetta and Villela, 2004). The same succulent effect was observed in our pot plants in greenhouse (data not presented). Therefore, the presence of Cl in collecting medium may trigger release of phytoalexins which could have an inhibitory effect on nitrification, which further studies are necessary in order to be clarified. Based on their capabilities, plants can change their biochemical, physiological and morphological characteristics in response to environmental variations. The nature of these changes usually determines a species ability to succeed under temporary or permanent environmental stress. It is quite important that interactions among stress factors that occur parallel in infertile acidic soils must always be considered (Wenzl *et al*., 2003). Nevertheless, the ability of plants to inhibit nitrification is also presented in this work (Figure 7), and as our results showed, under specific conditions plants can have NI activity in their root exudates. However, our findings can not support the idea that BH plants release controlled root exudates which strongly and efficiently suppress nitrification.

## Conclusion

There was no detection of NI in root washings, independent of plant age, light intensity, collection period, N-form and N concentrations, except for the ammonium chloride collecting medium. Therefore,

under proper collection conditions for root exudates, (avoiding membrane damage and potential degradation or chemical modification of exudates compounds due to extended collection times in distilled water), there was no evidence for a controlled release of NI compounds from *Brachiaria* roots, independent of plant age, amount and form of N supply, pH of the growth medium and light intensity during the culture period.

However, NI activity was detectable in root washings when plants were exposed to extended collection times (24 h) in combination with $NH_4^+$ supply (Figure 7), but not with $NO_3^-$ in the collection solution or after short-term collection (6 h). This observation is consistent with the findings of (Subbarao *et al.*, (2005, 2006, 2007 & 2008) but also strongly suggests that the observed release of NI compounds was rather a consequence of membrane damage due to inadequate collection conditions (Figure 8), rather than mediated by controlled exudation from undamaged roots. Supplying only ammonium (1 mM) in distilled water as root washing medium over extended time periods (24 h) will lead to rapid ammonium uptake and medium acidification associated with the risk of $K^+$ and $Ca^{2+}$-leaching, as an important element required for membrane stabilisation. Accordingly, (Cakmak & Marschner, 1988) reported detrimental effects on membrane stability in roots of cotton seedlings due to the lack of $Ca^{2+}$ in the washing medium of roots, which was detectable already after an incubation period of only six hours.

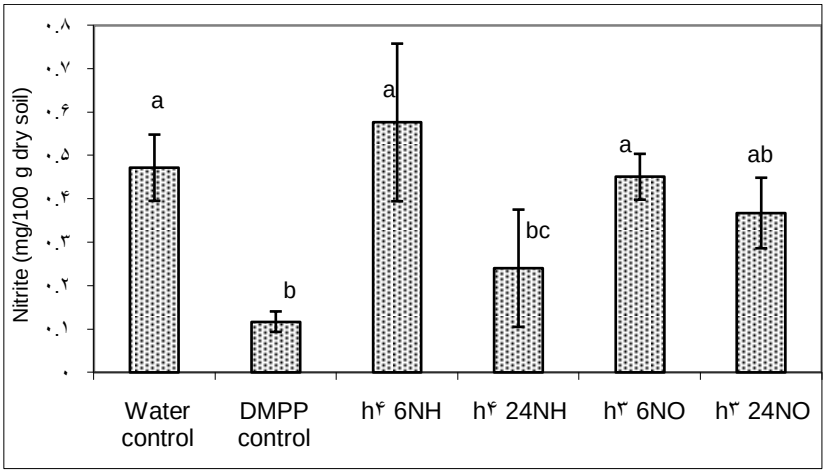


**Figure 7.** Nitrification inhibition effect of root exudates collected in distilled water containing 1 mM $NH_4Cl$ (6 hours versus 24 hours collection). Plants were grown in nutrient solution with 2mM N-$NH_4^+$ or $NO_3^-$. The pH value for ammonium was ~4 and ~3 for 6 and 24 hours, and pH value for nitrate was ~5 and ~4 respectively. Data are average of four replicates ± SD. Duncan test was conducted for mean values at P= 0.05.

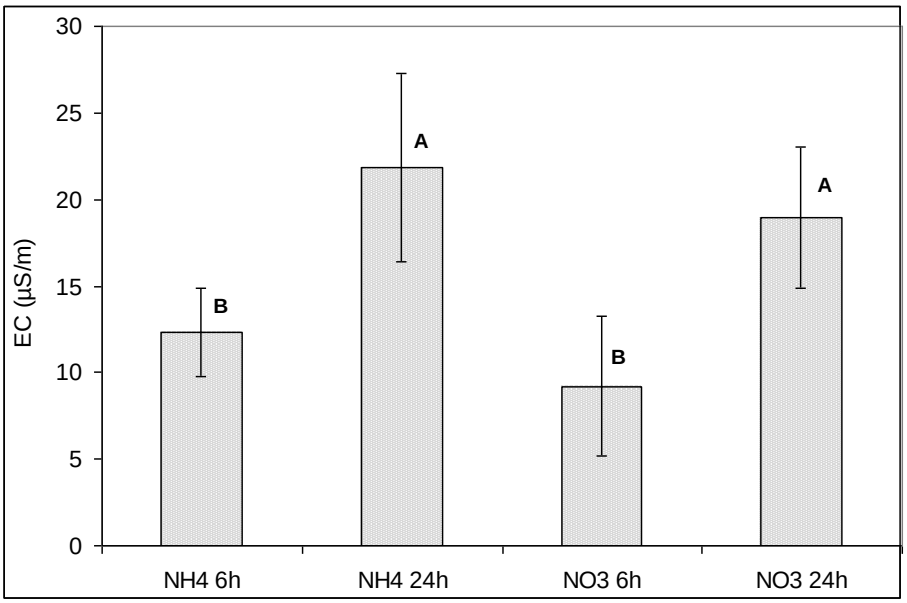


**Figure 8.** Electric conductivity (µS/m) of root washings collected for the 3 hours period in distilled water, just after collection of their root exudates for 6 or 24 hours period in 1 mM $NH_4Cl$. Plants were grown with ammonium, $(NH_4)_2SO_4$ or with nitrate, $Ca(NO_3)_2$. Data are average of four replicates ± SD. Duncan test was conducted for mean values at P= 0.05.

## REFERENCES


Cakmak, I. and Marschner, H. 1988. Increase in membrane permeability and exudation in roots of zinc deficient plants. *Journal of Plant Physiology* **132**: 356-361.

Cazetta, J. O. and Villela, L. C. V. 2004. Nitrate reductase activity in leaves and stems of tanner grass (*Brachiaria radicans* Napper). *Brazilia Scientia Agricola* **61**: 640-648.

CIAT. 1983. Annual report. International center for tropical agriculture, Apartado Aereo 6713, Cali, Colombia, South America.

CIAT. 1985. Annual Report. pp. 222–224. International center for tropical agriculture, Apartado Aereo 6713, Cali, Colombia, South America.

Gopalakrishnan, S., Subbarao, G. V., Nakahara, K., Yoshihashi, T., Ito, O., Meada, I., Ono, H. and Yoshida, M. 2007. Nitrification inhibitors from the root tissues of Brachiaria. *Journal of Agriculture and Food Chemistry* **55**: 1385 – 1388.

Ishikawa, T, Watanabe, T. and Minami, K. 1999. Possibility of nitrification suppression by a tropical grass. The effect on multiplication of ammonium-oxidizing bacteria and nitrous oxide efflux. *Japanese Journal of Soil Science and Plant Nutrition* **70**: 762-768.

Ishikawa, T., Subbarao, G. V., Ito, O. and Okada, K. 2003. Suppression of nitrification and nitrous oxide emission by the tropical grass *Brachiaria humidicola*. *Plant and Soil* **255**: 413-419.

Jones, D. L.http://www3.interscience.wiley.com/journal/118760292/ - c1, Hodge, A. and Kuzyakov, Y. 2004. Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* **163**: 459 – 480.

Kandeler, E. 1993. Bodenbiologische Arbeitsmethoden, Springer- Verlag, *Heidelberg, Germany* **163**-167.

Lata, C., Degrange, V., Raynaud, X., Maron, P. A., Lensi, R. and Abbadie, L. 2004. Grass populations control nitrification in savanna soils. *Functional Ecology* **18**: 605-611.

Liljeroth, E. and Baath, E. 1988. Bacteria and fungi on roots of different barley varieties (*Hordeum*

*vulgare* L.). *Biology and Fertility of Soils.* **7**: 53– 57.

Mergulhao, A., Burity, H. A., Tabosa, J. N., Figueiredo, M. and Maia, L. C., 2002. Influence of NaCl on *Brachiaria humidicola* inoculated or not with *Glomus etunicatum*. *Investigacion Agraria Produccion y Proteccion Vegetales* **17**: 219-227.

Nakamura, T., Kanno, T., Miranda, C., Ohwaki, Y. and Macedo, M. 2005. Characterization of nitrogen utilization by *Brachiara* grasses in Brazilian savannas, (Cerrados). *Soil Science and Plant Nutrition* **51**: 973-979.

Neumann, G. and Römheld, V. 2000. The release of root exudates as affected by the plant physiological status, *In*: R. Pinton, Z.Varanini, Z. Nannipieri (eds.) The rhizosphere: biochemistry and organic substances at the soil-plant interface. Marcel Dekker 2000.

Römheld, V. and Marschner, H. 1986. Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. *Plant Physiology* **80**: 175–180.

Souri, M.K. 2008. Characterization of natural and synthetic nitrification inhibitors and their potential use in tomato culture, PhD dissertation, University of Hohenheim, Stuttgart-Germany.

Subbarao, G. V., Ito, O., Wang, H. Y., Nakahara, K., Suenaga, K. and Rondon, M., Rao, I. M., Carlos, L. and Ishitani, M. 2005. Root exudates of *Brachiaria humidicola* inhibt nitrification characterization and quantification of this unique biological phenomenon. Plant nutrition for food security, human health and environmental protection, pp. 444-445.

Subbarao, G. V., Ito, O., Sahrawat, K., Berry, W. L., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M. and Rao, I. 2006. Scope and strategies for regulation of nitrification in agricultural systems-challenges and opportunities. *Critical Reviews in Plant Sciences* **25**: 303-335.

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

Subbarao, G. V., Rondon, M., Ito, O., Ishikawa, T., Rao, I. M., Nakahara, K., Carlos, L. and Berry, W. L. 2007b. Biological nitrification inhibition (BNI)-is it a widespread phenomenon?. *Plant and Soil* **294**: 5-18.

Subbarao, G. V., Nakahara, K., Ishikawa, T., Ito, O., Ono, H., Kameyama, M., Yoshida, M., Rondon, M., Rao, I. M., Lascano, C. and Ishitani, M. 2007c. Nitrification inhibitor and soil improver and fertilizer containing the same, United States Patent 20070028659. http://www.freepatentsonline.com/20070028659.html. (accessed 5 July 2007).

Subbarao, G. V., Nakahara, K., Ishikawa, T., Yoshihashi, T., Ito, O., Ono, H., Ohnishi-Kameyama, M., Yoshida, M., Kawano, N. and Berry, W. L. 2008. Free fatty acids from the pasture grass *Brachiaria humidicola* and one of their methyl esters as inhibitors of nitrification. *Plant and Soil* **313**: 89-99.

Sylvester-Bradley, R., Mosquera, D. and Mendez, J. E. 1988. Inhibition of nitrate accumulation in tropical grassland soils: effect of nitrogen fertilization and soil disturbance. *Journal of Soil Science* **39** : 407–416.

Wang, H. Y., Subbarao, G. V., Ito, O. and Nakahara, K. 2005. Regulation of nitrification inhibitory (NI) activity in the root exudates of *Brachiaria humidicola*. Plant Nutrition for Food Security, Human Health and Environmental Protection, pp. 478-479, China.

Wenzl, P., Mancilla, L. I., Mayer, J. E., Albert, R. and Idupulapati, M. R. 2003. Simulation infertile acid soils with nutrient solutions: The effects on *Brachiaria* species. *Soil Science Society of American Journal* **67**: 1457-1469.

## چکیده

ممانعت از نیتریفیکاسیون در اکوسیستم های طبیعی چندین دهه است که مطرح می باشد. این اثرات ممانعت کنندگی یک ویژگی ژنوتیپ های وحشی می‌باشد تا ارقام تجاری. بسیاری از گیاهان مخصوصاً گراس ها نشان داده شده اند که دارای این خاصیت ممانعت از نیتریفیکاسیون هستند و اخیراً برخی ژنوتیپ‌های *Brachiaria* مخصوصاً *Brachiaria humidicola* توجه زیادی را به سبب نقش آن ها در کاهش میزان نیتریفیکاسیون در خاک از طریق ترشحات ریشه ای جلب نموده است. در این مطالعه طی یک سری آزمایشات کشت هیدروپونیک نشان داده شد که وقتی ترشحات ریشه ای این گیاه در آب مقطر جمع آوری گردید، جدا از فرم و غلظت نیتروژن، شدت نور، سن گیاه و طول مدت جمع آوری ترشحات ریشه، ترشحات ریشه این گیاه هیچ اثر معنی داری بر فرایند نیتریفیکاسیون نداشت. به هر حال وقتی ترشحات ریشه در محیطی حاوی یک میلی مول کلرید آمونیم جمع آوری گردید ، آن به طور معنی داری از نیتریفیکاسیون ممانعت نمود و این اثر ممانعت کنندگی در گیاهان رشد یافته با آمونیم بیشتر از گیاهان رشد یافته با نیترات بود. نمونه های جمع آوری شده ترشحات ریشه وقتی لیوفیلایز شدند، عصاره استخراج شده ترشحات ریشه ای گیاهان روئیده با آمونیم و آن هم تنها تحت ش‌رایط شدت نور کم (و نه نور متوسط یا زیاد)، ممانعت از نیتریفکاسیون را باعث گردید. اندازه گیری هدایت الکتریکی ترشحات ریشه در آب مقطر (بعد از جمع آوری ترشحات ریشه در محلول یک میلی مول کلرید آمونیم ) بیان کننده هدایت الکتریکی بیشتر تحت شرایطی بود که آمونیم در محیط ریشه، مخصوصاً طی مرحله جمع آوری ترشحات ریشه ای، برای مدت طولانی (۲۴ ساعت بجای ۶ ساعت) وجود داشت.


**کلمات کلیدی**: *Brachiaria humidicola*، ترشحات ریشه، آمونیم، نیترات، هدایت الکتریکی