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ROOT-INDUCED CHANGES IN POTENTIAL NITRIFICATION AND NITRATE REDUCTASE ACTIVITY OF THE RHIZOSPHERIC SOIL OF WHEAT (*TRITICUM AESTIVUM* L) AND CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

A pot experiment was conducted to study the root-induced changes in potential nitrification (PN) and nitrate reductase activity (NRA) in the rhizosphere of 4 varieties each of wheat and chickpea using unplanted soil as reference. The two crop types were significantly different in gathering biomass over 21 days of growth; chickpea being twice more active when the values were averaged for 4 varieties. Wheat varieties had in general inhibitory and chickpea varieties a stimulatory effect on PN and NRA of the rhizospheric soil. On an average, NRA of the rhizospheric soil of wheat varieties decreased by 50% compared to unplanted soil i.e., non-rhizospheric or bulk soil. In contrast to wheat, chickpea varieties caused 5-30 times increase in NRA as compared to unplanted soil. When data for different varieties within a crop type were averaged, PN and NRA were 2 and 45 times higher in chickpea as compared to wheat. The two parameters were significantly correlated ($r = 0.97$, $n = 9$) suggesting the dependence of NR on *In situ* formation of NO_3^-. However, ratio of NRA/PN suggested chickpea varieties to be more efficient in inducing NO_3^- reduction than nitrification. In wheat varieties, NRA was not induced although NO_3^- was being formed at rates comparable to that in unplanted soil and in soil planted to two of the chickpea varieties. Significance of differential root-induced changes in PN and NRA to nitrogen nutrition of the two plant types is discussed.

Introduction

Bacterial processes of nitrification and denitrification are the most important sources of N_2O in soil and the atmosphere (Granli & Bockman, 1994). The two processes are supported directly or indirectly by the availability of C. Nitrification, that is mainly autotrophic (Wrage *et al*., 2001), is strongly influenced by CO_2 (Azam *et al*., 2005), while denitrification is driven by easily oxidizable C sources (Beauchamp *et al*., 1989). Nitrogen fixation is also reported to be enhanced at elevated CO_2 (Azam *et al*., 2005). Since plants are the predominant source of both organic C released in soil as rhizodeposits (Gregory & Atwell, 1991; Kuzyakov & Domanski, 2000) as well as CO_2 resulting from rhizorespiration (Kuzyakov & Domanski, 2002; Azam & Farooq, 2005; Kuzyakov, 2006), they will exert a significant influence on the processes of nitrification and denitrification by affecting the activities of the relevant sections of microbial population i.e., nitrifiers and denitrifiers. This influence will differ with the plant type as rhizodeposition and rhizorespiration vary between species both qualitatively and quantitatively (Grayston *et al*., 1996). While denitrification may serve as a conduit for excessive amounts of NO_3^- in legumes (NO_3 is inhibitory to N_2 fixation), inhibition of nitrification could be advantageous for the non-legumes, particularly the cereals that are

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known to perform better in the presence of both NH_4^+ and NO_3^- (Gentry & Below, 1993). Experiment were carried out i) to compare four varieties each of wheat and chickpea in inducing changes in nitrification and denitrification activities of the rhizospheric soil using potential nitrification and nitrate reductase (assimilatory/dissimilatory) activity as a measure of the two microbial processes, respectively, and ii) to visualize the implications of root-induced changes in potential nitrification and nitrate reductase activity for nitrogen nutrition of the two plant types i.e., leguminous (chickpea) and non-leguminous (wheat). Unplanted soil was used as a reference for root-induced changes in soil microbial processes.

Materials and Methods

Soil used in the study was collected from the top 0-15 cm of an experimental field at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Air-dried and sieved (<2mm) soil had the following characteristics: pH (1:1, soil:water suspension), 7.6; electrical conductivity, 0.8 d Sm^{-1}; organic carbon (C), 0.6%; total nitrogen (N), 0.09%; NH_4^+-N, 4.2 mg kg^{-1} soil; NO_3^--N, 11.9 mg kg^{-1} soil; sand, 30%; silt, 31%; clay, 39%; and water-holding capacity, 30%. Standard methods were followed for all analyses as described by Lodhi *et al*., (2006). Organic C was determined using a modified wet oxidation method (Azam & Sajjad, 2005).

Four varieties of wheat (*Triticum aestivum* L.) viz., U-2000, Inqlab, Chenab, and WL-1076 and four of chickpea (*Cicer arietinum* L.) viz., 98004, P-2000, 90261, and 97086 were used in the study. Except for the wheat variety WL-1076 that was produced through wide hybridization (Farooq *et al*., 1995), rest of the varieties is the outcome of conventional breeding.

Portions of soil (500 g) contained in plastic pots (9 x 20 cm) were fertilized with a solution of Ammonium sulphate and Potassium dihydrogen phosphate to obtain N, P and K concentration of 25, 25 and 32 mg kg^{-1} soil, respectively. Fifteen seeds of wheat and 8 of chickpea were planted per pot using triplicate pots for each variety. Triplicate pots were left unplanted to serve as control. After germination, the crop stand was thinned to 10 and 5 seedlings pot^{-1} in case of wheat and chickpea, respectively. This plant number was considered to be sufficient for achieving effective root distribution in the entire soil mass and to get good rhizospheric effect. Both planted and unplanted pots were weighed twice daily and the loss in weight made up by adding water to maintain the moisture content of the soil to 15% (w/w) throughout the experiment period. The pots were placed in a netted house with day/night temperature of 20/14ºC, 40-52% relative humidity and photosynthetic photon flux density of 1450-1675 μmol m^{-2} s^{-1}.

After 3 weeks of growth, the entire soil-plant system was removed from the pots as a column by gently tapping the pots. Before this exercise, however, it was ascertained that soil moisture content was *ca* 13-14% which was reasonable for removing root-adhering soil with minimum root breakage. The soil adhering to the roots was removed by gentle tapping of the root system with hand. Total potted soil was considered as rhizospheric, while that from the unplanted pots was taken as non-rhizospheric or bulk soil. In either case, the wet soil was passed through 0.5 mm sieve and visible pieces of roots removed to the maximum extent. Aliquots of the soil were immediately subjected to determination of potential nitrification and nitrate reductase activity as described by Schinner *et al*., (1996). The roots were washed free of remnants of soil using running tap water and after noting the fresh weight, both root and shoot portions were dried to a constant weight at 65ºC.

Significance of differences between treatment means was determined be using the SAS statistical package (Anon., 1998) and coefficient of correlations was calculated with the help of Microsoft Excel software.

Results and Discussion

Figure 1 gives a comparison of the root and shoot mass of different varieties of wheat and chickpea. Root proliferation that is supposed to affect the rhizospheric functions did not seem to be very different in wheat and chickpea. Visual observation of the figure suggests that presumably the root distribution in soil and the volume of soil being influenced was fairly similar. However, fresh weight and water concentration of shoot and root portions was significantly different for the two crop types (Table 1). Varieties of chickpea gathered >3 times greater biomass per pot as compared to those of wheat irrespective of the fact that inter-varietal differences were significant or not. Chickpea roots showed significantly lower dry matter content i.e., *ca* 50% of that determined for wheat varieties, while there was no difference in percent dry matter of shoot of the two crop types. However, water retaining capacity (expressed as the ratio of water held per unit weight dry matter) of both root and shoot portions of chickpea varieties was *ca* 34% better than that of wheat. Water retaining capacity of the chickpea roots was 9.9-11.0 times the dry matter (average for 4 varieties being 10.4 times) as compared to 5.3-11.5 times in wheat varieties (average for 4 varieties being 7.8 times). Ability to maintain higher water content of the cells could possibly be one of the reasons for survival of chickpea under water limiting conditions. Roots of only one wheat variety i.e., WL-1076 were comparable to chickpea varieties and held water equivalent to 11.5 times the dry weight. Reportedly, this variety produced by crossing bread wheat with *Aegilops cylindrica*, a wild grass (Farooq *et al*., 1995), is tolerant to low water availability and salinity (Farooq & Azam, 2001). In the present study, however, no attempt has been made to determine the significance of plant water content to nitrification and nitrate reductase in the rhizosphere.

Fig. 1. Comparison of root and shoot of different varieties of wheat (U-2000, Inqlab, Chenab, WL-1076) and chickpea (98004, P-2000, 90261, 97086); varieties arranged from left to right.

Table 1. Fresh biomass and water concentration of root and shoot portions of different varieties of wheat and chickpea.

Crop	Variety	Fresh weight, g pot^{-1}		Percent dry matter	
		Root	Shoot	Root	Shoot
Wheat	U-2000	3.02e*	2.57d	18.00a (5.34e)§	18.50a (1.52g)
	Inqlab	3.72e	3.19c	12.23b (8.76d)	11.90d (2.81c)
	Chenab	3.34e	3.33c	18.03a (5.62e)	15.12b (2.02f)
	WL-1076	4.59d	3.01c	10.17c (11.48a)	12.24cd (2.60d)
	Average	**3.67**	**3.00**	**14.61 (7.80)**	**14.46 (2.24)**
Chickpea	98004	6.10c	3.80b	8.74d (9.87c)	14.55b (2.52d)
	P-2000	6.99b	4.83a	7.94e (10.37bc)	12.74c (3.13b)
	90261	8.73a	4.69a	7.76e (10.98ab)	15.55b (2.33e)
	9708	6.30c	4.88a	6.55f (10.35bc)	11.38d (4.04a)
	Average	**7.01**	**4.55**	**7.55 (10.4)**	**13.56 (3.01)**

§Figures in parentheses show the ratio of water content and dry matter content signifying the water holding capacity of the plant material on dry weight basis.
*Values sharing a similar letter for a parameter in a column are not significantly different from each other at 5% level of probability.

The two crop types differed significantly in root-induced effects on potential nitrification (PN) and nitrate reductase activity (NRA) in the rhizospheric soil (Table 2). Unplanted soil was used as a reference for both the assays. Two of the wheat varieties i.e., Chenab and WL-1076 had a significant inhibitory effect on PN, while the remaining two had no effect. In comparison to wheat, all the chickpea varieties caused a significant increase in PN with two of the varieties showing >2 times greater PN compared to unplanted soil. With reference to unplanted soil, an inhibition of NRA in wheat rhizosphere and enhancement in chickpea rhizosphere was obvious. When data for different varieties within a crop type were averaged, NRA and PN were *ca* 2 and 45 times higher in chickpea as compared to wheat. The two parameters were significantly correlated ($r = 0.97$, $n = 9$) suggesting the dependence of NR on *In situ* formation of NO_3^-. Induction of nitrate reductase by nitrate has also been reported (Caba *et al*., 1995). Thus the plant types that encourage nitrification may induce enhanced NO_3^- reduction as well. However, ratio of NRA/PN suggested chickpea varieties to be more efficient in inducing NO_3^- reduction than nitrification, while in wheat varieties NRA was not induced although NO_3^- was being formed at rates comparable to that in unplanted soil and in soil planted to two of the chickpea varieties. Average ratio for 4 chickpea varieties was *ca* 22 times higher as compared to wheat varieties (0.184 – 0.648 and 0.006 - 0.029, respectively; Table 2) suggesting that in the chickpea rhizosphere, NO_3^- will be much more quickly

eliminated. This could lead to an increase in pH in chickpea rhizosphere and hence improved N_2 fixation (Frame *et al*., 1998). When grown in isolation, elimination of NO_3^- from the chickpea rhizosphere through reduction can be considered advantageous for the process of N_2 fixation that is inhibited more by NO_3^- than NH_4^+ (Marschner, 1995). However, legume species differ in their ability to take up nitrate (NO_3^-) and in the degree to which soil NO_3^- impairs legume nodulation and N_2 fixation (Tang *et al*., 1999). Through a rapid NO_3^- reduction, chickpea roots can ensure optimum working of the N_2 fixation machinery. At the same time N losses via denitrification will be reduced through a decrease in the pool size of NO_3^- (Firestone & Davidson, 1989).

Table 2. Nitrate reductase activity (NRA) and potential nitrification (PN) in the rhizosphere and non-rhizosphere soil of different varieties of wheat and chickpea.

Crop	Variety	PN	NRA	NRA/PN
		NO_2^- formed, µg h^{-1} 5-g^{-1} soil		
Wheat	U-2000	2.57d	0.07e	0.027e
	Inqlab	2.61d	0.01e	0.006f
	Chenab	2.18e	0.05e	0.024e
	WL-1076	2.10e	0.06e	0.029e
	Average	**2.37**	**0.05**	**0.020**
Chickpea	98004	7.77a	3.57a	0.459b
	P-2000	2.87c	0.53d	0.184c
	90261	2.99c	1.94c	0.648a
	97086	6.48b	3.10b	0.478b
	Average	**5.03**	**2.28**	**0.440**
None		**2.60d**	**0.11e**	**0.043d**

*, Values sharing a similar letter are not significantly different from each other at 5% level of probability.

In the present study, some of the chickpea varieties showed significant increase in potential nitrification (Table 2). This phenomenon could benefit the associated cereal crop like wheat which itself does not seem to support nitrification in its rhizosphere (Table 2). Being fairly mobile, NO_3^- will be easily available for uptake by wheat crop, while its reduction is being curtailed at the same time (Table 2). Since availability of both NH_4^+ and NO_3^- compared to either N source alone is more beneficial for wheat (Gentry & Below, 1993), the crop will benefit from the rhizospheric effects of chickpea on nitrification and denitrification. Similarly, wheat plants can help associated leguminous crop by efficiently taking up NO_3^- and thus facilitating N_2 fixation. Some of our unpublished work suggests higher nodulation in chickpea grown in association with wheat, while the latter has higher chlorophyll concentration at the same time suggesting substantial transfer of biological fixed N. Therefore, differential effect of roots of the two crop types on rhizospheric nitrification and nitrate reduction could be of value in mixed cropping. Indeed, wheat-chickpea co-culture may be useful for decreasing denitrification as well as enhancing N_2 fixation. However, this aspect of N transformation processes in the rhizosphere seems to have received little attention in comparison to studies where transfer of biologically fixed N from legume to non-legume crop is fairly elaborately documented (Martin *et al*., 1991).

The results of the present study also show significantly wide varietal differences in root-induced changes in N transformation processes. It was interesting to note that chickpea varieties P-2000 and 90261 were not different in affecting PN, but the latter was 4 times more efficient in reducing NO_3^- as compared to the former. Such variations are useful in the sense that the varieties with a higher NRA could support higher nodulation and N_2 fixation. While, sufficient information is available on rhizobial denitrification (Rosen *et al*., 1996), root-induced changes in NRA of the rhizospheric soil have received little attention *vis-à-vis* nodulation and N_2 fixation. Some of our unpublished work shows that besides inhibiting nodulation, increasing NO_3^- levels in the soil had a significantly depressing effect on root NRA and growth of chickpea plants. This observation suggested that NO_3^--N may not be an easily assimilable form of N for chickpea and probably for other legumes as well. A high NRA in chickpea rhizosphere points to the possibility that a decrease in nodulation and growth of leguminous plants in the presence of higher concentrations of NO_3^- could well be due to allocation of photoassimilates for NO_3^- reduction rather than their use in N_2 fixation which research investigation.

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