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AMMONIUM AND NITRATE FERTILIZATION EFFECTS ON BIOMASS YIELD OF MAIZE (*ZEA MAYS* L.) WITH SPECIAL EMPHASIS ON RHIZODEPOSITION

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A pot experiment was conducted to study the effect of ammonium (NH_4) and nitrate (NO_3) on growth, water relations and rhizodeposition (taking accumulation of sand on the root surface as an indirect measure) of two varieties of maize (C-20 and C-77). Nitrogen was applied as ammonium sulphate or potassium nitrate at 50 or 100 mg L^{-1} of the rooting medium solution and the plants were harvested 15 days after seed sowing when seed reserves were nearly exhausted. It was assumed that form and level of N was the main variable affecting plant growth besides K that was added as potassium nitrate. Data were recorded on root and shoot biomass (fresh and dry weight) and amount and water concentration of sand adhering to the roots. In general, higher level of NH_4-N had a negative effect on plant growth and a positive effect on tissue water concentration. The amount of sand adhering to the roots increased in the presence of lower levels of NH_4 in both the maize varieties. The roots could adhere sand 2-3 times their fresh weight and 30-43 times the dry weight and was assumed to reflect the amount of rhizodeposits. The RAS showed significantly higher water concentration as compared to the bulk sand suggesting the presence of polysaccharides that were assumed to be released from the roots or synthesized by rhizospheric microorganisms. The water concentration of RAS was found to be higher in the presence of NH_4 suggesting a higher rhizodeposition and/or microbial synthesis of polysaccharides. Implications of forms of N in the rooting medium to plant growth are discussed.
Key words: Ammonium, maize, nitrate, rhizodeposition, tissue water concentration

INTRODUCTION

Nitrogen is the major component of atmospheric gases (*ca* 78%) and represents 99.96% of the total that is found on earth, in the sea and around. Of the remaining 0.04%, biosphere contains only 0.005%. In spite of the small proportion found in living beings, N is most often the limiting nutrient for crop production since only a fraction of atmospheric N_2 is made available to the plants through biological nitrogen fixation. Introduction and use of chemical nitrogenous fertilizers has therefore resulted in substantial increases in crop yields.

The forms of N readily assimilable by plants comprise NH_4 and NO_3, the relative availability of which depends on the soil environment and the dynamics of processes involved in N transformations. Although the plants are equipped with the facility to use either or both of the N sources simultaneously, a mixed availability of the two forms is reported to be more beneficial (Bock *et al*., 1991; Gill and Reisenbauer, 1993). While the plants can conserve energy by assimilating NH_4 rather than NO_3, the later is the predominant form of N under arable conditions because of the rapid nitrification of the former. In general, entire NH_4-N is nitrified within the matter of days (Mulvaney *et al*., 1997; Azam *et al*., 2005). However, the rate of assimilation and the effects of the two forms of N on plant growth/functions and rhizospheric microbial activities vary widely. A great deal of work has therefore been reported on different aspects of N cycling processes, N nutrition of plants and plant responses to form of N in terms of rhizodeposition i.e., loss of organic materials from the roots as they grow through soil (Whipps, 1990). For example, NH_4 nutrition is reported to cause higher rhizodeposition (Giordano *et al*., 1994; Mahmood *et al*., 2002; Lodhi *et al*., 2007), while the growth of plants continuously fed with NH_4 may be inhibited (Cramer and Lewis, 1993; Lang and Kaiser, 1994; Marschner, 1999).

One of the aspects of significance to root functioning and nutrient dynamics is the soil aggregation and resultant changes in soil structure as a whole. Carbonaceous compounds released from the plant roots

and those synthesized by rhizospheric microorganisms play a key role in soil aggregation (Kuzyakov and Domanski, 2002; Azam and Farooq, 2005; Kuzyakov, 2006). While both NH_4 and NO_3 are assimilated by microorganisms, the former is more readily assimilable not only because of its reported preference by the microbes (Jansson, 1958) but also because all the microbes may not necessarily synthesize nitrate reductase to enable them assimilate NO_3 (Azam *et al*., 1993). Hence, microbial proliferation in the rhizospehere and synthesis of aggregation enhancing macromolecules will be fairly dependent on the form of available N, while amount of soil adhering to the roots may be a reflection of the root exudation and microbial activity. Therefore, the objectives of this study were to compare the effect of NH_4 and NO_3 on i) growth characteristics of maize with special reference to root development and relative water concentration of root/shoot portions and ii) the amount and water holding capacity of rhizospheric sand that is found adhering to the roots. Sand was used as the rooting medium to avoid interference by compounds other than those released from the plants during seed germination and seedling development. The plants were grown only for 10 days after seed germination when the seed reservoirs were almost exhausted.

MATERIALS AND METHODS

Acid washed sand was used in the experiment. Four hundred g portions of the sand were filled in 500-ml capacity plastic containers and brought to 15% moisture with deionized water. Each pot was sown to 5 seeds of two maize (*Zea mays* L.) varieites i.e. C-20 and C-77. The stand was thinned to 3 seedlings 3 days after seed germination. After 5 days of germination, the pots were irrigated either with i) deionized water, ii) a solution of ammonium sulphate or iii) a solution of potassium nitrate. The solutions were prepared such that the final concentration of N in the sand solution was either 50 or 100 mg L^{-1}.
One week after exposing the plant roots to ammonium (NH_4) or nitrate (NO_3) nutrition, moisture level of the sand was brought to 15% with deionized water and sand+plants carefully removed from the pots as a whole. The shoot portion was separated, weighed fresh and then dried to a constant weight at 65 ^oC. Bulk of the sand was dislodged from the roots by gentle hand tapping taking care that no root breakage occurred. Roots along with the adhering sand were weighed as such and the weight designated as (a).

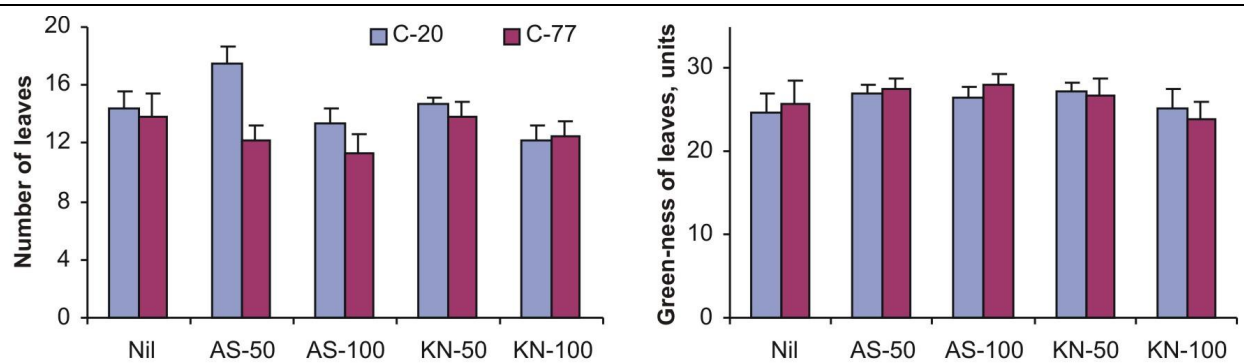

Figure 1. Number and greenness of leaves as affected by form and amount of N in the rooting medium. The bars also show standard deviation of means

The root-adhering sand (RAS) was then washed away with a pre-weighed quantity of water followed by repeated rinsing to completely remove the sand. The sand-water suspension was oven-dried at 105 ^oC and the dry weight of (RAS) determined. The weight of wet sand was calculated and designated as (b). Fresh weight of roots was determined as the difference of (a) and (b). This exercise eliminated the possibility of getting inflated values of root fresh weight during washing. The washed roots were blotted and then dried to a constant weight at 65 ^oC. Tissue water concentration was determined as: [(fresh weight – dry weight)/dry weight] and expressed as g g^{-1} dry matter.

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

RESULTS AND DISCUSSION

Figure 1 presents the data on number and greenness of leaves. Maximum number of leaves pot^{-1} was found in C-20 receiving 50 mg NH_4-N L^{-1}. In general, however, the form and amount of N had no significant effect on number of leaves. Greenness of leaves was only slighty improved by the applied N, but the effect was statistically non-significant. C-20 showed higher biomass accumulation than C-77; average root + shoot biomass for all treatments being 0.67 and 0.59 g pot^{-1}, respectively (Table 1). The biomass was relatively equally distributed amongst root and shoot portions leading to a root/shoot ratio around 1 in both maize varieties. Higher level of NH_4-N (100 mg L^{-1}) had in general a significant negative effect on shoot and root biomass; the effect of NO_3-N was significantly positive in C-20 and negative in C-77. In some previous studies also, high mineral N content of the soil had a negative effect on the growth and biomass accumulation of maize (Lodhi and Azam, 2002), while under hydroponic conditions (Lodhi *et al*., 2006) and in soil (Lodhi and Azam, 1998), enhanced availability of NH_4-N was found to be highly inhibitory for growth of wheat. Such effects could be attributed to a reduction in root growth and consequently reduced volume of soil or any other rooting medium (sand in the present study) being explored for essential nutrient elements. In the present study, root growth was retarded to some extent by NO_3 but not by NH_4 and was reflected on overall biomass of plants.

Tissue water concentration plays a significant role in maintaining optimum physiologically activities of the plants especially under stress situations (Netondo, 2004; Azam *et al*., 2006). In the present experiment, however, the plants were grown in sand medium with sufficient water supply and hence any differences observed were attributed to the effect of form and concentration of N in the rooting medium. The two varieties differed in maintaining tissue water concentration; C-20 being better in shoot and the C-77 in root water concentration. Water concentration of both shoot and root portions of C-20 was significantly higher in the presence of NH_4, while C-77 showed higher water concentration in the shoot portion only. As a whole, NH_4-N had a more positive effect on tissue water concentration than NO_3-N in both the maize varieties.

Table 1. Dry matter and H_2O concentration of root and shoot portions of two maize varieties grown in soil without (Nil) or with 50 or 100 mg N kg^{-1} as ammonium sulphate (AS) of potassium nitrate (KN).

N source	Dry matter, g pot^{-1}			H_2O conc. g g^{-1} dry matter	
	Shoot	Root	Total	Shoot	Root
C-20					
Nil	0.27bc	0.33b	0.59ef	8.10a	12.85d
AS-50	0.36a	0.39a	0.75a	8.53a	14.32b
AS-100	0.25c	0.30b	0.56f	8.28a	13.24cd
KN-50	0.34a	0.32b	0.66bc	8.22a	13.73bc
KN-100	0.35a	0.33b	0.68b	8.00ab	14.53b
C-77					
Nil	0.33a	0.33b	0.66bc	7.05bc	12.76d
AS-50	0.33a	0.28b	0.62de	8.28a	16.68a
AS-100	0.28bc	0.29b	0.57f	7.65ab	14.89b
KN-50	0.29b	0.32b	0.61de	7.57ab	14.47b
KN-100	0.31b	0.28c	0.59ef	7.44bc	14.25b
LSD (p=0.05)	*0.04*	*0.03*	*0.04*	*0.72*	*0.81*

Values sharing a similar letter in a column are not significantly different at 5% level of probability according to Duncan's

Root adhering sand (RAS) ranged between 9 and 14 g pot^{-1} and was consistently higher in C-20 as compared to C-77 in all the treatments (average for all treatments being 12.0 and 10.4, respectively), but was maximum at lower level of NH_4 (Figure 2). The capacity of the roots to retain sand on their surfaces ranged between 2 and 2.7 g g^{-1} fresh weight and 31 and 42 g g^{-1} dry weight of root; generally being higher for C-20 and NH_4-fed plants in all cases. A significant positive correlation (r = 0.84, n = 10) was obtained between RAS and root mass suggesting a relatively symmetrical release of binding materials by the roots. Rhizodeposition from plant roots is well documented as a function of both rhizospheric and atmospheric factors (Høgh-Jensen and Schjoerring, 2001; Shaw and Burns, 2005). Annual plants grown under arable conditions are reported to transport 30-50% of the photosynthetic C below-ground during their life cycle (Swinnen *et al*., 1994; Domanski *et al*., 2001). In fact, almost all organic C found in soil is primarily plant-derived in the form of root/shoot residues and root exudates (Kuzyakov and Domanski, 2000, 2002). Translocation of recent photoassimilates is rapid with ^{14}C detected belowground within 30 minutes of pulse application; maximum rate of translocation (17.8%) being observed during the first 3 hrs. that increased to 33.7% at elevated CO_2 (Rattray *et al*., 1995).

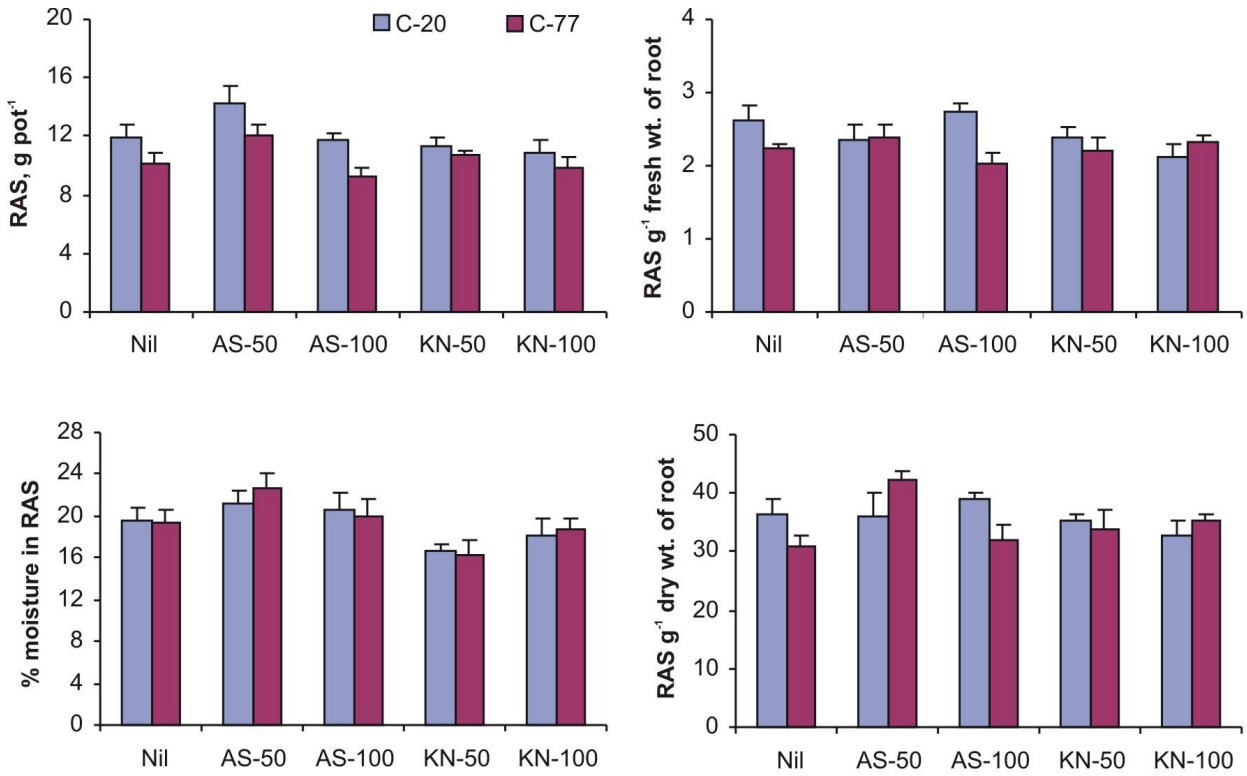

Figure 2. Characteristics of root adhering sand as affected by form and amount of N in the rooting medium. Bars also show standard deviation of means.

The exudates may be mucilaginous in nature and thus have the ability to bind soil (or sand in this study) particles leading to the formation of micro- and then macro-aggregates. In the present study, significant amounts of RAS were found adhering to the roots suggesting the availability of aggregation enhancing macromolecules. Relatively higher amounts of RAS in NH_4-fed plants could be explained on two accounts i.e., i) higher rhizodeposition in NH_4- than NO_3-fed plants (Giordano *et al*., 1994; Mahmood *et al*., 2002; Lodhi *et al*., 2007), and ii) higher activity of microorganisms responsible for the synthesis of polysaccharides effective in binding sand particles; microbial preference for NH_4 over NO_3 has been reported (Jansson, 1958; Azam *et al*., 1993). Some of our unpublished studies showed higher soil aggregation in soil fertilized with NH_4^+- compared to NO_3^-. Lewis *et al*. (1989) reported that NH_4^+-fed wheat plants allocated 36% more C to the roots than NO_3^--fed plants. This may be necessitated by enhanced carbohydrate/energy demands at the root level for efficient NH_4^+ assimilation. Under hydroponic conditions bacterial abundance at barley roots was found to increase at higher NH_4^+-N levels and could be attributed to increased rhizodeposition as measured by ^{14}C methods (Liljiroth *et al*., 1990 a,b) as well as through higher proliferation of bacteria at the expense of NH_4; rise in the pH in the presence of NH_4 could also lead to higher bacterial population. Martins-Lucao *et al*. (2000) suggested that cracks developed by greater root branching and root initials in response to NH_4^+ were responsible for higher rhizodeposition.

Presence of polysaccharides in the RAS was supported by its higher moisture content. Against 15% moisture of the bulk sand maintained before plant removal, the RAS contained 16-23% moisture; higher moisture content of RAS being observed for NH_4-fed plants, while the average for 2 maize varieties (19.3

and 19.4%) was not significantly different. Synthesis of exopolysaccharides by rhizospheric microorganisms, especially bacteria and their ability to hold water several times their weight is well documented (Ashraf *et al*., 1999). Thus by affecting rhizodeposition, the forms of N may play a significant role in modifying soil aggregation and structure in the rhizospehere. The presence of NH_4-N would seem to increase rhizodeposition thereby improving the soil structure. Simultaneously, however, this form of N could make the cells leaky and hence affect the integrity of root system and consequently the flow of nutrients into the roots and shoot portions. The ultimate effect of consistent supply of NH_4-N on plant growth could be negative. Hence, the reported edge of a mixed N (NH_4+NO_3) supply over either of the source present singly can be ascribed, at least partly, to a differential effect on rhizosdeposition and rhizospheric microbial functions.

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