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SIMILARITIES IN RESPONSE OF MAIZE GENOTYPES TO WATER LOGGING AND AMMONIUM TOXICITY[1]

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ABSTRACT: Plant growth, total nitrogen (N), and free ammonia (NH_3) in tissue of different maize genotypes were compared in the presence of either nitrate (NO_3)- or ammonium (NH_4)-forms of N at two oxygen (O_2) levels in solution culture. Shoot and root growth was significantly less with NH_4 than with NO_3 under low O_2 conditions; however, NH_4 toxicity was significantly reduced by supplying maize plants with additional O_2 in the solution culture, and root and shoot growth of NH_4-supplied plants were not significantly different than that of NO_3-treated plants. On the other hand, maize genotypes differed significantly in their response to N forms and O_2. The genotype, Saracura, selected for tolerance to water logging, had the highest tolerance to NH_4 based on plant growth under low O_2 pressure and the concentration of free NH_4 in tissues. This research suggests there may be a common mechanism or an interaction with the mechanism for tolerance to NH_4 and water logging.

INTRODUCTION

Ammonium is a primary form of N fertilizer applied for maize production. All organic forms of N in the plant are obtained from NH_4 or from NO_3 which are subsequently reduced to NH_4 in the plant for utilization. Thus, NH_4 assimilation is an extremely important step in N metabolism by the plant (19). Ammonium in fertile

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neutral soil is converted to NO_3 (nitrification) by soil bacteria so that most of the N is available and taken up by plants as NO_3. In contrast, in acid soils that have a pH below 5.0, or in water-logged soils, nitrifying bacteria are inactive, and N remains available to plants only in the NH_4 form (9,17,21). The use of nitrification inhibitors, such as nitrapyrin to reduce N losses from leaching and denitrification, increase the proportion of NH_4 available for assimilation by plants. Plants adapted to acid soils, such as blueberries and many tree species, or plants adapted to low soil redox potential or water logging such as "paddy" rice, prefer NH_4 rather than NO_3 (4).

The use of NH_4 and nitrification inhibitors could have economic advantages for the production of various agronomic plants compared to the more common NO_3 nutrition (9) since NH_4 is not generally subject to leaching or denitrification from soil and it does not need to be reduced before metabolism into organic materiais in the plant (18). In contrast to these advantages, a high concentration of NH_4 is toxic to many plants even though plants need to assimilate N ultimately in the fully reduced form (6,11,12,13,16).

The O_2 content of the soil atmosphere is of paramount importance for the growth and productivity of crop plants. Few plants can survive under complete anoxia for prolonged periods because of the need for O_2 in aerobic respiration and for various energy-requiring processes, (10). Although few crop plants are subjected to complete anoxia, many are exposed to periods of O_2 deficiency caused by water-saturation or compacted soil. Paddy rice roots have an alternative source of O_2 where gaseous O_2 diffuses from the aerial parts through aerenchyma tissues which are continuous within the roots (2). A mechanism of O_2 secretion (the glycolic acid pathway of respiration) also may account for the strong oxidizing power of rice roots. A shift in respiratory metabolism from the aerobic to anaerobic pathway is one of the main effects of O_2 deficiency as a result of water logging (10). Water logged-tolerant species, like rice, have a much greater ability to synthesize amino acids under flooded conditions than most intolerant plant species. This mechanism is likely linked to the high NH_4-assimilation efficiency of rice compared to other plants (13,16). Thus, plants under various environmental stresses (acidic soils, water logging, drought) have similar biochemical responses to NH_4 toxicity (7,19). The effect of O_2 on NH_4 assimilation has not been reported. The objective of this study was to evaluate the interacting effect of O_2 and N-form on growth, total N, and free NH_4 accumulation in tissues of several maize genotypes.

MATERIALS AND METHODS

Four genotypes of maize (BR 201 double hybrid, BR 107, Saracura, and the simple hybrid 115 20x22) were selected for this study based on their differential tolerance of water-logged conditions. Seed was germinated in moist filter paper and seedlings were then grown for 15 days in an aerated modified Hoagland solution at either 0.7 or 7.0 ppm O_2 in 8-L plastic trays. The modified Hoagland solution contained 5 mM N as either NO_3 [from $CaNO_3)_2$] or NH_4 [from $(NH_4)_2SO_4$) with $CaCl_2$ replacing an equivalent amount of calcium (Ca) in the $Ca(NO_3)_2$ solution] (15). The modified Hoagland solution also contained: 2.0 mM K_2SO_4, 2.0 mM $MgSO_4$, 1.0 mM KH_2PO_4, 25 μM H_3BO_3, 2.0 μM $MnSO_4$, 4.0 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM $NaMoO_4$, 50 μM KCl, and 50 μM Fe-DTPA. The pH was adjusted to pH 5.7 with $CaCO_3$. All experiments were carried out at 24-28°C and 14 h photoperiod at 1,150 μE/m^2/s (photosynthetically active radiation) in a greenhouse with four replications and 12 plants per replication.

Plants were washed briefly at harvest with distilled water to remove adhering nutrient solution, blotted dry, and weighed. A 1.0 g shoot sample from each treatment was placed in 10 mL methanol for determination of free NH_3. The remaining tissue sample was dried in a forced air oven at 70°C and ground to pass a 20-mesh screen in a Wiley mill prior to digesting 100 mg (dry weight) in 5 mL $H_2SO_4/CuSO_4$/ sodium selenate reagent (14) and alkaline steam distilling with 6N NaOH for total N determination. Ammonia in the distillate was trapped in 0.5 mL 2.5N HCl and quantified by the phenol hypochlorite reaction (15,22).

Free NH_3 was determined by phase separating the methanol extract in 5 mL chloroform and 6 mL distilled water. The upper aqueous phase was evaporated to dryness, redissolved in 2 mL water, and assayed for NH_3 by the phenol-hypochlorite reaction (22). Shoot and root fresh weight and dry weight, and root length (20) also were determined from a separate experiment under the same conditions and treatments, but without adjusting the solution pH.

RESULTS

Shoot growth of all four maize genotypes was depressed in the low O_2 solution regardless of N form; however, shoot growth was 64% and 61% smaller with NH_4 than NO_3 at both levels of O_2 (Table 1), respectively. The maize genotype, Saracura, selected for tolerance to water logging, appeared to have the greatest tolerance to NH_4 based on shoot growth at the low level of O_2. Similar effects on

Table 1. Shoot and root dry weights and root length of maize genotypes grown with either NH_4 or NO_3 at two O_2 concentrations in solution culture.

	Solution buffered with $CaCO_3$				Non-buffered Solution Culture			
	7.0 ppm O_2		0.7 ppm O_2		7.0 ppm O_2		0.7 ppm O_2	
Genotype	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4
Shoot Weight:								
	g/12 plants							
BR 201	7.13	6.54	6.74	4.72	5.50	3.99	4.38	3.07
BR 107	6.78	6.45	5.75	4.90	5.45	4.03	4.25	2.49
Saracura	8.08	7.55	7.18	6.12	7.04	4.35	4.86	3.81
HS 20x22	8.05	6.05	6.36	4.12	6.88	3.58	5.14	2.01
Mean	7.51	6.64	6.50	4.96	6.22	3.98	4.66	2.84
LSD (0.05) = 0.64					LSD (0.05) = 0.48			
Root Weight:								
	g/12 plants							
BR 201	3.50	3.16	2.97	2.37	2.66	2.02	2.09	1.72
BR 107	3.55	2.75	3.59	2.44	2.85	2.23	2.10	1.73
Saracura	3.75	3.25	3.36	3.37	3.19	3.01	2.80	2.40
HS 20x22	3.43	2.75	2.82	2.25	2.59	1.92	2.00	1.63
Mean	3.56	2.97	3.18	2.60	2.82	2.29	2.24	1.87
LSD (0.05) = 0.27					LSD (0.05) = 0.20			
Root Length:								
	m/12 plants							
BR 201	132	78	86	63	101	63	68	45
BR 107	167	81	93	62	134	66	78	44
Saracura	156	92	88	76	133	85	73	54
HS 20x22	112	73	81	57	84	58	68	41
Mean	141	81	87	64	113	68	73	46
LSD (0.05) = 22					LSD (0.05) = 17			

root growth and dry weight also were observed (Table 1). Effects of simulated water logging (low O_2 conditions) and NH_4 were more pronounced when the pH was not adjusted with $CaCO_3$. Shoot dry weight was 25% lower with NO_3 and 29% with NH_4 under low O_2 compared with the high O_2 condition. The combined effect of NH_4 and low O_2 was a shoot dry weight that was only 45% of the NO_3 high O_2 treatment (Table 1). Root length was reduced more by reduced O_2 than shoot growth.

There were significant interactions between N-form and O_2 concentration for the different maize genotypes. Saracura appeared to be the most tolerant genotype to NH_4 and low O_2 while the simple hydrid HS 20x22 appears to be the most sensitive to those stresses. The other genotypes were intermediate in their response to these conditions.

The total N content of shoot tissue of all genotypes was significantiy higher for the NH_4 treatment than NO_3 at both O_2 levels (Table 2), but was highest under low O_2 conditions for all genotypes. There was no significant difference in N uptake by the different genotypes. The NH_4 treatment also resulted in more free NH_3 in the shoot tissue compared with the NO_3-treated plants (Table 2), and the free NH_3 concentration was inversely correlated with shoot growth. Higher levels of free NH_3 were present in the NH_4-treated plants under reduced O_2 than in the presence of high O_2 in the solution culture. Saracura had the least free NH_3 in tissues under NH_4 and O_2 stress, and the best growth.

DISCUSSION

The depressing effects of NH_4 on plant growth observed in this study have been widely reported (1,3,5,6,8,11,23); however, the effect of reduced O_2 on NH_4 assimilation has not been reported even though NH_4 is the predominant, if not the sole form of N available for plant uptake under low O_2 conditions. Under low O_2 conditions, NO_3 is readily denitrified and lost by leaching, while nitrifying bacteria are inactive (9,17,21).

There appears to be an interacting effect of O_2 deficiency and NH_4 nutrition in depressing maize growth, with NO_3 enhancing plant growth under low O_2 conditions with NH_4 toxicity being reduced in the presence of high O_2. In nature, the presence of NH_4 is associated with anoxia under water-logged conditions. Plants adapted to water logging and NH_4, like paddy rice, have an alternative mechanism for high NH_4 assimilation and the synthesis of amino acids under flooded conditions

Table 2. Total N and free NH_3 in shoots of maize genotypes grown with either NH_4 or NO_3 at two O_2 concentrations in solution culture.

	7.0 ppm O_2			0.7 ppm O_2		
Genotype	NO_3	NH_4	Mean	NO_3	NH_4	Mean
TOTAL N:	----------------	----------------	---------------%	----------------	----------------	----------------
BR 201	2.36	2.94	2.65	2.58	3.91	3.24
BR 107	2.46	2.94	2.70	2.50	3.65	3.07
Saracura	2.38	2.61	2.49	2.50	3.65	3.07
HS 20x22	2.36	2.80	2.49	2.51	3.64	2.90
Mean	2.36	2.80	2.58	2.51	3.64	3.07
LSD (0.05) = 0.46						
FREE NH_3:	----------------	---------------nmol g^{-1}	fresh weight---------------	----------------	----------------	----------------
BR 201	730	1784	1257	1098	2240	1669
BR 107	692	1920	1306	1160	2418	1789
Saracura	618	1410	1014	906	1808	1357
HS 20x22	830	2208	1519	1246	3164	2205
Mean	718	1831	1275	1103	2408	1756
LSD (0.05) = 326						

to overcome NH_4 toxicity and O_2 deficiency (2,10). Based on growth and free NH_3 accumulation in tissue under low O_2 or with NH_4, the genotype, Saracura, which is adapted to water-logging conditions, appears to be more efficient in NH_4 assimilation compared to the other maize genotypes tested (13,16).

Increasing the O_2 concentration to 7.0 ppm in this study, enhanced plant growth and decreased free NH_3 in green tissues. This suggests that O_2 in the root system is involved with NH_4 assimilation. The glycolic acid pathway of respiration in roots could provide O_2 as well as carbon (C) skeletons for the incorporation of NH_4 into amino acids; however, another hypothesis is needed to explain the effect of exogenously supplied O_2 on enhanced NH_4 assimilation which was observed in this study. Tolerance to water logging and NH4 assimilation may be related physiologically, although additional research is needed to elucidate the physiological mechanism(s) involved.

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