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SHORT COMMUNICATION

Mechanism of nitrogen effect on zinc nutrition of flooded rice*

Summary

Nitrogen application increased Zn contents of flooded rice on two calcareous soils. Urea and $(NH_4)_2SO_4$ being better N carriers than NH_4NO_3 resulted in higher increase. Nitrogen enhanced Zn contents partly through growth promotion but mainly by increasing soil Zn solubility and root efficiency for Zn absorption. Zinc solubility rose by an enigmatic mechanism and not from pH reduction or soluble Zn–HN_3 complex formation as occurs for upland plants. Nitrogen aggravated Zn retention in upland plant roots as immobile Zn-protein complex was not important for rice. Bicarbonate inhibition of Zn uptake by rice from $CO(NH_2)_2$ application or its stimulation by lower redox potential from NH_4NO_3 addition were not involved.

Introduction

Submerged rice (*Oryza sativa* L.) usually responds to N and Zn fertilizers on calcareous soils. Nature of their mutual interaction was never studied critically. Nitrogen often depresses Zn concentration in upland plants causing severe yield reduction[4]. Ozanne[9] suggested this to result from higher Zn retention in roots as immobile Zn-protein complex.

Nitrogen, sometimes, also increases total Zn contents in upland plants[4]. This occurs mainly by growth promotion[4] or soil pH depression[2]. Thus NH_4NO_3 being more efficient for plant growth than other fertilizers, especially on calcareous clayey soils resulted in higher Zn increase[14]. On light textured soils $(NH_4)_2SO_4$ caused more increase due to pH reduction[2]. Nitrogen effect on *per se* Zn uptake or on root efficiency for its absorption is yet unknown.

Nitrogen effect on Zn uptake may strongly differ for flooded rice. It is sown on buffered calcareous clay soils where pH changes from normal doses of acidic N fertilizers may not be involved. Moreover, pH of acidic and alkaline flooded soils usually equilibrate at 7.0[10]. Nitrogen sources may also exhibit differential effect on growth and thus on Zn uptake by rice. By contrast in upland plants, $(NH_4)_2SO_4$ was double efficient for rice than NH_4NO_3 due to severe NO_3–N loss in reduced soils from denitrification[10]. Bicarbonate inhibition of Zn uptake[6] from $CO(NH_2)_2$ application or its stimulation by lower soil redox potential from NH_4NO_3 addition[7] [10] may also be important for rice. The current experiments were conducted to evaluate these hypotheses.

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* No. V in the series Micronutrient availability to cereals from calcareous soils.

Materials and methods

(a) Zinc uptake by rice from soils. Sub-portion of 4.5 kg of two calcareous ($CaCO_3$ 0.5, 2%) clay soils of Pakistan, Gujranwala and Miranpur (pH 8.4, 8.6, organic matter 1%) was filled in plastic pots. Basal fertilizer consisted of 13 ppm P as $KH_2 PO_4$ and treatments included in triplicate were 0, 37, 75 and 150 ppm N as $CO(NH_2)_2$, $(NH_4)_2SO_4$ or NH_4NO_3 in the presence of 0, 5 and 25 ppm Zn, all mixed with soil before planting. Six 20-days nursery seedlings, cv. Basmati 370 rice, were transferred in pots and grown for another 36 days under flooding. After which shoots were harvested, dried, ground and their one g portions digested with HNO_3–$HClO_4$ (4 : 1) mixture. Zinc in the diluted digest was determined by atomic absorption system. Total Zn contents equalled Zn conc. × plant yield.

(b) Kinetics of soil Zn solubility. Sub-samples of Miranpur soil at 4.5 kg in plastic pots received a basal P dose of 13 ppm and various treatments in triplicate at 150 ppm N as $CO(NH_2)_2$, $(NH_4)_2SO_4$, and $(NH_4)_2NO_3$. Pots flooded with 5 cm standing deionized water were placed near those sown under rice during main rice growing months of August and September. Aliquots from 150 ml soil percolate collected by gravity each week from side holes of pots under N gas atmosphere[11] were analysed for pH in an especially designed O-free cell and for HCO_3^- by acid titration. The remaining percolates were preserved against oxidation by adding six drops of conc. H_2SO_4 and later on analysed for Ca Cu, Zn, Mn, and Fe by atomic absorption spectroscopy.

(c) Zinc absorption from solutions by N pretreated and untreated rice seedlings. Earlirose rice seedlings were grown for 10 days in silica sand on solutions of 250 μM $CaSO_4$ or $Ca(NO_3)_2$. Both the treatments had identical plant growth but untreated plants exhibited N deficiency just by 10th day. The seedlings in quadruplicate were then transferred to 300 ml glass tubes containing aerated solutions of 500 μM $CaCl_2$ and 5 μM $^{65}ZnCl_2$ (activity 9000 cpm/ml) at pH 5.6. Absorption time was 48 hours after which the solutions were replaced with $10^{-3}M$ Na_2-EDTA for one hour to eliminate adsorbed root ^{65}Zn. The plants were then washed and radioactivity of its roots and shoots counted in a well-scintillation counter. Zinc absorbed was calculated from specific activity relationship.

Results and discussion

Nitrogen exhibited no effect on Zn concentration except in 150 ppm N as $(NH_4)_2SO_4$ and 25 ppm Zn treatment on Miranpur soil where Zn concentration in rice shoots increased ($P < 0.01$, Table 1) These results failed to support N depression of Zn concentration in upland plants causing severe yield decline[4].

Nitrogen enhanced total Zn contents in rice on both soils ($P < 0.05$, 0.01, Table 1, 2). Growth promotion appears at least partly responsible. Thus, Zn contents in plant paralled with plant yield exhibiting high correlation coefficient both on Gujranwala and Miranpur soils (r 0.81, 0.78 $P < 0.01$). Urea and $(NH_4)_2SO_4$ increasing rice growth more than NH_4NO_3 ($P < 0.1$) also

TABLE 1

Effect of zinc and rates and sources of nitrogen on dry matter yield and on concentration and total Zn contents of rice shoots on Miranpur soil

N application	Dry matter yield (g/pot)			Zn concentration (ppm)			Total Zn contents (μg/pot)		
(ppm)	Zn application (ppm)			Zn application (ppm)			Zn application (ppm)		
	0	5	25	0	5	25	0	5	25
Urea									
0	3.0	3.2	3.7	23.5	28.2	45.0	70.0	89.7	167.5
37	5.7	5.7	7.7	24.3	30.5	47.3	140.7	174.8	365.2
75	9.2	7.0	9.6	24.2	29.2	49.0	222.2	201.6	472.3
150	10.0	7.4	10.9	23.2	30.0	43.0	231.0	221.8	469.6
Ammonium sulphate									
0	3.0	3.2	3.7	23.5	28.2	45.0	70.0	89.7	167.5
37	5.2	5.8	4.3	23.2	29.5	46.0	149.6	111.6	197.8
75	7.3	5.7	7.4	24.7	29.5	49.8	180.5	166.8	367.8
150	8.0	9.4	9.3	23 5	41.7	61.5	188.3	389.3	572.8
Ammonium nitrate									
0	3.0	3.2	3.7	23.5	28.2	45.0	70.0	89.7	167.5
37	4.0	4.3	3.7	22.3	28.6	46.0	93.4	121.9	171.6
75	5.1	4.5	4.2	23.5	29.2	47.8	120.7	132.4	199.6
150	7.1	6.8	6.0	22.3	28.3	52.7	157.6	191.7	317.5

TABLE 2

Effects of rates and sources of nitrogen on dry matter yield and on concentration and total Zn contents of rice shoots on Gujranwala soil

N application (ppm)	Dry matter yield (g/pot)	Zn conc. in plants (ppm)	Total Zn contents in plants (μg/pot)
Urea			
0	1.7	22.1	37.2
37	3.4	18.5	63.6
75	5.9	17.6	102.7
150	6.4	17.1	108.2
Ammonium sulphate			
0	1.7	22.1	37.2
37	3.4	19.7	67.1
75	5.2	19.2	99.8
150	7.3	20.0	145.9
Ammonium nitrate			
0	1.7	22.1	37.2
37	2.9	20.9	57.4
75	3.2	21.1	67.7
150	4.2	19.6	82.1

resulted in higher Zn increase. These results supported an early report on rice[1] but conflict with those on upland crops showing NH_4NO_3 to enhance Zn content through plant growth more than other fertilizers[14]. Indeed NH_4NO_3 is more efficiently utilized in drained calcareous clay soils but is much less effective for flooded rice due to severe NO_3–N loss from denitrification in reduced soil stratum[10].

Nitrogen appears to increase Zn uptake in rice mainly by enhancing soil Zn solubility. This occurred at all incubation periods ($P < 0.05$, 0.01, Table 3). Mechanism is not clear. Soil pH did not change and was, thus, not responsible. These results contradicted earlier reports showing acidic N carriers as $(NH_4)_2SO_4$ to enhance Zn contents of upland plants from pH depression, mainly on light soils. The current soils are highly buffered calcareous clay soils.

TABLE 3

Effect of rates and sources of nitrogen on the solubility kinetics of various ions in the submerged Miranpur soil

N application		Weeks after soil submergence				
Rate (ppm)	Source	I	II	III	IV	VII
pH						
0	–	7.75	7.77	7.70	7.93	7.78
150	$CO(NH_2)_2$	7.73	7.63	7.73	7.87	7.72
150	$(NH_4)_2SO_4$	7.87	7.67	7.60	7.50	7.75
150	NH_4NO_3	7.80	7.80	7.67	7.87	7.80
Zn concentration (ppm)						
0	–	0.50	0.32	0.36	0.28	0.32
150	$CO(NH_2)_2$	0.88	0.30	0.46	0.37	0.41
150	$(NH_4)_2SO_4$	0.53	0.57	0.48	0.46	0.41
150	NH_4NO_3	0.76	0.37	0.45	0.49	0.50
Cu concentration (ppm)						
0	–	0.045	0.052	0.032	0.033	0.035
150	$CO(NH_2)_2$	0.048	0.035	0.043	0.027	0.031
150	$(NH_4)_2SO_4$	0.052	0.053	0.030	0.027	0.030
150	NH_4NO_3	0.050	0.047	0.030	0.030	0.034
Fe concentration (ppm)						
0	–	0.41	0.23	0.21	0.29	0.27
150	$CO(NH_2)_2$	0.26	0.19	0.18	0.17	0.48
150	$(NH_4)_2SO_4$	0.25	0.21	0.13	0.28	0.33
150	NH_4NO_3	0.17	0.20	0.20	0.12	0.46
HCO_3 concentration (meq/l)						
0	–	8.82	16.00	18.15	35.16	23.44
150	$CO(NH_2)_2$	9.25	16.45	18.13	34.68	24.90
150	$(NH_4)_2SO_4$	8.78	14.37	16.28	33.33	20.61
150	NH_4NO_3	6.43	14.32	17.43	36.93	22.88

Nitrogen enhanced Zn solubility also did not occur from soluble Zn–NH_3 complex formation[8] as $CO(NH_2)_2$, $(NH_4)_2SO_4$ and NH_4NO_3 resulted in identical Zn increase (small differences non-significant). Little N effect on Cu and Fe solubility may indicate their little effect on Zn solubility through their competition for specific Zn adsorption sites[20]. Identical soil HCO_3^- and Zn contents from three N carriers indicated HCO_3 inhibition of Zn absorption by rice roots[6] or its precipitation as Zn $(HCO_3)_2$ to be less likely involved. The NH_4NO_3 induced lower soil redox potential increasing Zn solubility[15] also did not operate. Such effect is involved only with continuous high NO_3 doses.

Nitrogen strongly increased Zn absorption by rice. Rates of absorption by N pretreated roots were 40% higher ($P < 0.01$, Table 4). Although not studied here, but more efficient carriers as $(NH_4)_2SO_4$ will be expected to result in higher Zn absorption especially under relatively greater N deficiency. The mechanism of N stimulated Zn absorption is not known. Enhanced cation exchange capacity of roots was earlier considered responsible[3 5]. Recent studies showing Zn absorption a metabolic and not a physical exchange mechanism[12] contradicted this suggestion. Nitrogen may, however, increase the intensity and capacity of protein carriers of plant roots involved in active Zn absorption. Nitrogen did not restrict Zn translocation to rice shoots. Irrespective of N supply to seedlings, 30% of total ^{65}Zn was found in plant tops. These results failed to support Ozanne's hypothesis[9] of N enhanced Zn retention in upland plant roots as immobile Zn-protein complex exhibiting severe Zn deficiency in shoots.

TABLE 4

Effect of nitrogen pretreatment during seedling growth on Zn absorption by rice from solution of $5 \mu M$ $ZnCl_2$ and $500 \mu M$ $CaCl_2$

Pretreatment of seedlings during growth	Zn absorption/g plant material (μ *moles*)			
	Roots	Shoots	Total	% in shoots
$500 \mu M$ $CaSO_4$	0.664	0.346	1.010	34.3
$500 \mu M$ $Ca(NO_3)_2$	0.952	0.423	1.375	30.8
LSD (0.05)	0.101	0.052	0.123	6.8

The present studies indicated differential nature of N stimulation of Zn uptake in flooded rice than in upland crops. Increased plant growth and soil pH depression are mainly responsible in upland crops. Enhanced soil Zn solubility occurring through an enigmatic mechanism and increased efficiency of protein carriers of roots for Zn absorption appear important for rice.

Nitrogen depresses Zn concentration and accentuates its deficiency in upland plant shoots by increasing soil pH from alkaline N carriers[2], by Zn retention in roots as immobile Zn-protein complex[9], or from stimulation of plant growth. The first two processes do not appear important for rice.

Stimulation of plant growth can, perhaps, dilute Zn contents to a deficient level. This did not occur on the present soils containing optimum Zn, slightly deficient N supplies. Consequently growth enhanced only 2 to 3 fold. By contrast, N induced Zn deficiency in upland plants by increasing 10 fold growth on severely N deficient soil[4]. Such soils exist in Pakistan where N may create a serious Zn deficiency problem even for rice. Prior soil Zn evaluation may, therefore enhance N efficiency for rice grain yield. Further studies involving several soils with graded N and Zn contents are needed to test this hypothesis.

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