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Effects of mineral nitrogen and *Bradyrhizobium* inoculation on growth and iron nutrition of groundnut*

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Abstract

Experiments were conducted in a glasshouse to determine the effects of the mineral N supplied as ammonium nitrate and *Bradyrhizobium* inoculation on the growth and iron nutrition of nodulating and non-nodulating groundnut (*Arachis hypogaea* L.) lines. In a sterilized sand-vermiculite medium supplied with N-free nutrient solution (pH 7.0), inoculation of nodulating groundnut with *Bradyrhizobium* strain NC 43.3 enhanced dry matter production and O-phenanthroline extractable iron and N contents of the plants. The supply of mineral N at a rate of 100 mg N L^{-1} (as NH_4NO_3) through deionized water (pH 8.5) induced iron chlorosis symptoms in the nodulating groundnuts grown in Vertisols, but these symptoms were not observed at higher N levels (200–400 mg N L^{-1}). The induced chlorosis was only partially corrected by inoculation with *Bradyrhizobium* strains NC 92 and NC 43.3. The iron deficiency chlorosis was, however, corrected by application of higher rates of ammonium nitrate.

Introduction

The N requirement of groundnut (*Arachis hypogaea* L.) is usually met through biologically fixed N_2 [1]. Development of non-nodulating groundnut lines has provided a useful means of measuring the amounts of biologically fixed N_2 [9]. Our earlier work showed that the yield of a non-nodulating groundnut line, even at high rates of N fertilizer supply, was lower than that of nodulating groundnut cultivars [9]. Less N and iron were taken up by the non-nodulating groundnut line relative to the nodulating groundnut cultivar, indicating that these nutrients limited the growth of the non-nodulating groundnut line [16]. It was observed that the form of mineral N, ammonium or nitrate also greatly affects rhizosphere pH, which in turn affects iron mobilization and availability to plants [14].

Our aim was to study the effects of continuous mineral N supply and inoculation with efficient strains of *Bradyrhizobium* on the growth and iron nutrition of nodulating and non-nodulating groundnut lines under controlled conditions. Inoculation with *Bradyrhizobium* and *Pseudomonas* was included because recent work has suggested that these microorganisms improve iron nutrition by synthesis of chelates (siderophores) that keep iron in soluble form [3, 11, 12].

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Materials and methods

Bradyrhizobium strains NC 92 and NC 43.3 were obtained from G.H. Elkan, North Carolina State University, Raleigh, NC, USA. A fluorescent *Pseudomonas* strain, isolated from the ICRISAT farm at Patancheru (near Hyderabad, India) was also used in this study.

Experiment 1

The effects of N supplied through biological N_2 fixation or mineral N on growth and iron nutrition of groundnuts were studied in a sterilized sand-vermiculite medium.

The iron response of a nodulating groundnut cultivar (Robut 33–1) and a non-nodulating line [8] was studied in a factorial randomized block design at five levels (0, 100, 200, 300, and 400 mg L^{-1}) of mineral N supplied as ammonium nitrate with three replications. For the nodulating groundnut there were additional treatments consisting of uninoculated control and *Bradyrhizobium* inoculation with strain NC 43.3.

Groundnut plants (3 plants pot^{-1}) were grown in pots of 17 cm top diameter containing 5 kg of sterilized sand-vermiculite mixture supplied with complete nutrient solution (pH 7.0) as described by Nambiar et al. [7]. The plants were watered daily with the nutrient solution and the pots were flushed with 1 L of sterile deionized water every week to remove any toxins that might have accumulated. Plants were harvested 42 days after sowing (DAS) and dry weights were recorded. Plant samples were analyzed for total N, total iron, and extractable iron.

Experiment 2

The details and design of this experiment were similar to those of Experiment 1 except that instead of a sand-vermiculite mixture, unsterile vertisol was used as the growing medium. Five levels of mineral N as ammonium nitrate (0, 100, 200, 300, and 400 mg L^{-1}) were supplied through deionized water (pH 8.5) and two groundnut cultivars (Robut 33–1 and non-nodulating) were used. No other nutrients other than N were supplied. Preliminary evaluation indicated that the soil was able to satisfactorily meet the plant requirement for other nutrients. As in the first experiment, uninoculated and inoculated treatments (*Bradyrhizobium* strain NC 43.3) were carried out for Robut 33–1 cultivar in three replications. Plants were harvested at 77 DAS and dry weights recorded. Plant samples were analyzed for chlorophyll, total iron, extractable iron, and total N content.

Experiment 3

This experiment evaluated the effects of inoculation with *Bradyrhizobium* strains NC 92 and NC 43.3, and *Pseudomonas* with and without mineral N supply (0 and 100 mg N L^{-1} as ammonium nitrate) on the growth and iron nutrition of a nodulating groundnut (Robut 33–1) grown in a Vertisol. Uninoculated treatments for Robut 33–1 were carried out. Eight replications were arranged in a factorial randomized block design. As in the second experiment, the plants were not supplied any other nutrients except N. Nitrogen was supplied through deionized water (pH 8.5) used for irrigation. The plants were harvested at 85 DAS. Measurements were made on soil pH, dry matter weights, nitrogenase activity, and nodule number. Plant samples were analyzed for total N, total iron, and extractable iron content.

Analytical methods

Nitrogenase activity of groundnut plants was measured as described by Nambiar and Dart [6]. Chlorophyll content in the leaf tissue was determined by the DMSO extraction method [2]. Ferrous iron in the leaf tissue was estimated by extracting the thoroughly washed fresh tissue of the youngest fully matured leaves with 1.5% O-phenanthroline solution (pH 3.0) termed as 'extractable iron' [4, 13].

Plant samples for total iron analysis were thoroughly washed, dried in an oven at 60°C for 3 days, and ground to pass through a 40-mesh sieve. Total iron content was determined with the atomic absorption spectrophotometer following digestion of the plant samples using the triacid digestion method [15]. Total N analyses were made colorimetrically using a Technicon Autoanalyser II [17] following digestion of the plant samples in a block digester.

Results and discussion

In Experiment 1, without added mineral N, the dry matter production of Robut 33–1 groundnut inoculated with *Bradyrhizobium* strain NC 43.3 was significantly higher than the uninoculated control and the non-nodulating line (Table 1). Dry matter yields increased with the application of 100 mg N L^{-1}, and higher rates of N had no significant effect on the dry matter production of both nodulating and non-nodulating groundnuts.

Without mineral N, the content of extractable iron in the groundnut leaves was significantly higher in the inoculated Robut 33–1 than in the uninoculated Robut 33–1 and the non-nodulating line (Table 1). The data suggested that the nodulating groundnut plants were able to acquire more iron than the uninoculated Robut 33–1 and the non-nodulating line because iron was supplied in equal amounts during the three treatments. The supply of mineral N increased the extractable iron in the leaves at 100 and 200 mg N L^{-1} rates in uninoculated Robut 33–1, while the increase occurred only up to the 100 mg N L^{-1} rate in the non-nodulating line. The uptake of total iron did not show any definite trend with the application of mineral N.

Without added N, the total N uptake $plant^{-1}$ was also significantly higher in the inoculated Robut 33–1 than the uninoculated Robut 33–1 and non-nodulating line. Application of mineral N increased N uptake up to the 100 mg N L^{-1} rate in the inoculated Robut 33–1, while application in the uninoculated control and non-

Table 1 Dry matter yield and content of total and extractable iron and N in groundnut plants as affected by mineral N supply and inoculation with *Bradyrhizobium* strain NC 43.3[a]

Mineral N level (mg L^{-1})	Robut 33-1 inoculated	Robut 33-1 control	Non-nod line
Dry matter yield, g $plant^{-1}$			
0	2.6	1.4	0.9
100	4.2	3.3	2.5
200	4.5	3.9	3.1
300	4.7	4.3	3.5
400	4.4	4.4	3.4
SE ±		0.34	
Extractable iron in youngest leaf tissue, ug g^{-1} fresh			
0	8.3	3.8	3.1
100	9.4	7.7	8.7
200	9.9	9.5	8.8
300	9.2	9.3	10.0
400	10.1	9.3	10.0
SE ±		0.52	
Total iron uptake, ug $plant^{-1}$ dry wt			
0	1056	616	339
100	2269	1740	778
200	1562	2293	1628
300	1150	2515	1186
400	1525	2086	901
SE ±		435.2	
Total N uptake, mg $plant^{-1}$ dry wt			
0	88	27	12
100	153	85	64
200	172	143	117
300	188	163	149
400	181	190	155
SE ±		11.9	

[a] Three groundnut plants pot^{-1} were grown in pots containing a 5 kg sand-vermiculite mixture under sterile conditions for 42 days

nodulating line increased N uptake up to 200 mg N L $^{-1}$ rate (Table 1)

In Experiment 2 (Table 2) the inoculated Robut 33-1 showed severe iron chlorosis deficiency symptoms in the younger leaves when supplied with mineral N at a rate of 100 mg N L $^{-1}$ Without added mineral N however only mild iron chlorosis symptoms were observed The uninoculated Robut 33-1 showed moderate iron chlorosis deficiency symptoms at both the 0 and 100 mg N L $^{-1}$ rates Application of mineral N at 200 mg N L $^{-1}$ or higher rates alleviated the iron deficiency symptoms in both inoculated and uninoculated nodulating cultivars Application of mineral N at 300 mg N L $^{-1}$ significantly increased the contents of extractable

Table 2 Effects of mineral N supply and inoculation with *Bradyrhizobium* strain NC 43 3 on dry matter yield content of chlorophyll iron and total n in groundnut plants and soil pH

Mineral N level (mg l)	Robut 33 1 inoculated	Robut 33 1 control	Non nod line
Dry matter yield g plant			
0	4 8*	4 5**	3 2
100	4 5***	6 5**	6 0
200	5 8	7 6*	6 9
300	6 1	(7	6 3
400	5 7	6 0	5 6
SE		0 47	
Extractable iron in youngest leaf tissue ug g fresh tissue			
0	3 1*	3 0**	4 2
100	2 2***	3 2**	3 7
200	5 1	3 5*	3 9
300	6 4	5 8	3 3
400	4 9	5 6	5 7
SE +		0 73	
Chlorophyll content ug g fresh leaf tissue			
0	580'	550 *	820
100	470'	680 *	730
200	1220	1270*	730
300	1990	1830	880
400	1860	1730	1400
SE +		280 3	
Total N content mg plant dry wt			
0	112*	113**	35
100	124**	177**	151
200	180	219*	210
300	185	201	198
400	185	194	189
SE +		16 3	
Soil pH 1 2 H O			
0	8 7*	8 8**	8 8
100	8 6**	8 6**	8 6
200	8 2	8 1*	8 2
300	8 0	7 9	8 1
400	7 6	7 5	7 6
SE +		0 10	

* Groundnut plants were grown in pots containing 5 kg soil for 77 days

* Plants showing mild iron chlorosis symptoms

** Plants showing moderate iron chlorosis symptoms

*** Plants showing severe iron chlorosis symptoms iron and chlorophyll in the inoculated and uninoculated nodulating cultivars (Table 2) Dry matter yield of the nodulating cultivar was significantly higher than the non-nodulating line without any added mineral N However, with the application of mineral N, the dry matter yields were similar for the nodulating cultivar and the non-nodulating line

The soil pH at harvest of groundnut plants was lower in the treatments receiving 200 300, and 400 mg N L^{-1} than in those receiving no mineral N or 100 mg N L^{-1} (Table 2) The soil pH was more than one unit lower at the higher mineral N supply compared with that obtained in treatment receiving no mineral N This decrease in pH at higher rates of mineral N application might have contributed to higher mobilization of iron by the plants and alleviation of iron deficiency chlorosis [14]

Results from Experiment 3 (Table 3) indicated that mineral N application at 100 mg N L^{-1} induced a higher degree of iron deficiency chlorosis, especially in the plants grown without *Bradyrhizobium* inoculation As in Experiment 2, application of mineral N decreased soil pH measured at harvest Among the bacterial strains evaluated, groundnuts inoculated with NC 92 and NC 43 3 showed only mild iron deficiency chlorosis, while those inoculated with *Pseudomonas* showed moderate iron deficiency chlorosis symptoms The uninoculated plants showed severe iron deficiency chlorosis when the plants

Table 3 Effects of mineral N supply and bacterial inoculation on dry matter yield and other attributes of nodulating groundnut plants and soil pH[a]

Mineral N level mg L^{-1}	NC 92	NC 43 3	Pseudomonas	Uninoculated control	Mean
Dry matter yield g plant^{-1}					
0	12 5	12 9	12 0	11 9	12 3
100	14 1*	14 1*	14 5**	14 2***	14 2
SE ±			0 45		0 23
Extractable iron in youngest leaf tissue ug g^{-1} fresh tissue					
0	2 96	3 33	2 69	2 65	2 91
100	1 68*	1 70*	1 51**	1 35***	1 56
SE ±			0 164		0 082
Chlorophyll content ug g^{-1} fresh leaf tissue					
0	1290	1580	1310	1340	1380
100	820*	730*	810**	720***	770
SE ±			70 1		30 5
Nodule number plant^{-1}					
0	145	236	118	112	153
100	32*	37*	32**	29***	33
SE ±			10 3		5 2
N uptake mg plant^{-1} dry wt					
0	218	236	197	197	212
100	319*	306*	297**	292***	304
SE ±			14 2		7 1
Soil pH 1 2 H_2O					
0	8 6	8 7	8 6	8 6	8 6
100	8 1*	8 1	7 9**	8 0***	8 0
SE ±			0 045		0 022

Groundnut plants were grown in pots containing 5 kg soil (Vertisol) for 85 days
Plants showing mild iron chlorosis symptoms
* Plants showing moderate iron chlorosis symptoms
** Plants showing severe iron chlorosis symptoms

were supplied with 100 mg N L [1] Extractable iron and chlorophyll content were lower in treatment receiving 100 mg N L [1] than in treatment receiving no mineral N A similar trend was obtained with regard to nodulation and N uptake nodules plant [1] and N uptake (Table 3) were decreased by adding mineral N, as observed by Nambiar [5]

Groundnut plants growing on biologically fixed N_2 acquired more iron than uninoculated plants In contrast to an early study [3], inoculation with fluorescent *Pseudomonas* sp did not fully correct iron chlorosis deficiency symptoms In a recent study using 59_{Fe} Nambiar and Sivaramakrishnan [11] showed that NC 92 grown in culture medium produced more siderophore-bound iron than NC 43 3 In the present study however inoculation with both NC 92 and NC 43 3 partially corrected iron chlorosis in groundnuts Application of higher rates of ammonium nitrate also corrected chlorosis The mechanism involving mineral N on iron chlorosis is unclear and requires further study

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