Rhizobium-induced changes on nitrate reductase activity in rhizosphere and phyllosphere

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A field experiment was conducted to determine *Rhizobium*-induced changes in nitrate reductase activity in rhizosphere of clusterbean and moth bean. Effective inoculation with *Rhizobium* decreased nitrate reductase in rhizosphere by reducing nitrate reductase activity in plants.

NITRATE reductase (NR) activity in legumes is altered by *Rhizobium* innoculation[1,2]. Since (i) most legumes exude NR through roots into the soil[3], (ii) NR activity in soil leads to denitrification[4] and (iii) legumes are essential components of sustainable cropping systems, this study was carried out to determine whether *Rhizobium* inoculation changes in NR activity in rhizosphere and whether these changes are due to their effect on plant NR. Results were expected to help in identifying the *Rhizobium* strain that apart from benefiting legume also leads to low NR enrichment in soil and reduces the risk of denitrification. Dehydrogenase activity[5] (an indicator of soil microbial activity) was also estimated as microorganisms are often the source of soil NR[4].

Two legumes, viz. clusterbean (*Cyamopsis tetragonoloba* (L.) Taub. var. Maru guar) and moth bean (*Vigna aconitifolia* (Jacq.) var. Maru moth) were sown in kharif season with three replicates in a randomized block design in the sandy soil of Jodhpur (typic camborthid with 87.4% sand, 8.7% silt, 4.3% clay, 0.23% organic carbon and pH 8.1). Clusterbean seeds were inoculated with six strains of *Rhizobium*, viz. DRG 3, TAL 1436, TAL 1536, Nif 27 A_2, TAL 1109 and 3Hoa$_9$ and of moth bean with JMT 2D besides TAL 1436, TAL 1536, Nif 27 A_2, TAL 1109 and 3Hoa$_9$ before sowing. The cultures were lignite-based with viable counts of 10^7–10^8 cells per gram. Uninoculated seeds of both crops were sown in two plots. In one, the crop was fertilized with 40 kg N ha^{-1} but in the control, the crop was grown without fertilizers. Crops were grown as rainfed and recommended agronomic practices for each crop were followed.

Ten plants of each crop were carefully uprooted at flowering stage and the soil adhering to the root system of each plant was separated by gentle tapping. This soil was referred to as rhizosphere soil. For non-rhizosphere soil, 0–30 cm of the surface soil sample was collected from the boundary of the plots where plants were not allowed to grow. Soil samples were stored in the polyethylene bags at 10 ± 2°C and were processed for the estimation of nitrate reductase[4], and dehydrogenase[5] on the same day. Second and third fully-expanded leaves from the same plants of each replicate were cut into small pieces, pooled and 100 mg fresh weight of each sample was used for nitrate reductase estimation[6] in triplicate sets. Root samples were also pooled in a similar fashion prior to the estimation of nitrate reductase. Nitrogenase activity of plants was assayed by acetylene reduction using a Aimil-Nucon gas chromatograph[7]. The results were expressed on oven dry basis.

Nitrate reductase activity in the rhizosphere of both crops was significantly higher than in non-rhizosphere and that in clusterbean rhizosphere was comparatively higher than moth bean rhizosphere due to its exudation from roots[3] (Table 1). NR activity was same in non-rhizosphere soil of both crops due to adsorption of root exudates in a smaller zone. Inoculation with DRG 3, TAL 1436 and TAL 1536 suppressed NR activity in rhizosphere of clusterbean and inoculation with JMT 2D, TAL 1436 and TAL 1536 suppressed it in rhizosphere of moth bean. Inoculation with other strains did not significantly change NR activity (data not presented). Minimum NR activity in clusterbean and moth bean rhizosphere was observed after inoculation with TAL 1536 and TAL 1436 respectively. Application of N fertilizer increased NR activity in soil.

Changes in NR activity of leaves and roots due to *Rhizobium* inoculation or fertilizer application also followed the trends discussed for rhizosphere NR.

Table 1. Effect of different *Rhizobium* strains on nitrate reductase and dehydrogenase activity in rhizosphere soil and non-rhizosphere soil

Crop/ *Rhizobium* strain	Nitrate reductase µg NO_2 formed g^{-1} d^{-1}		Dehydrogenase pKat g^{-1}	
	R	NR	R	NR
Clusterbean				
Control (Uf)	15.1	1.8	21.8	12.9
Control (F)	18.3	1.9	26.2	12.3
DRG-3	11.0	2.1	25.5	13.3
TAL 1536	9.3	1.7	30.0	14.6
TAL 1436	12.6	1.9	26.1	14.7
LSD (p = 0.05)	1.7	0.7	4.0	2.8
Moth bean				
Control (Uf)	9.4	1.8	18.3	17.7
Control (F)	12.7	1.6	20.3	16.1
JMD-2D	5.1	1.7	16.5	15.4
TAL 1536	6.9	1.8	27.4	15.1
TAL 1436	4.4	1.6	26.4	16.4
LSD (p = 0.05)	1.3	0.6	4.5	2.6

Uf, Unfertillized; F, Fertilized; R, Rhizosphere soil; NR, Non rhizosphere soil; d, day.

Table 2. Effect of different *Rhizobium* strains on nitrogenase, nitrate reductase activity and crop yield

Crop/ *Rhizobium* strain	Nitrogenase (nmol) C_2H_4 plant^{-1} h^{-1}	Nitrate reductase µg NO_2 g^{-1} tissue h^{-1}		Yield q ha^{-1}	
		Leaf	Root	Grain	Straw
Clusterbean					
Control (Uf)	381	17.7	9.7	8.4	37.6
Control (F)	129	19.8	11.3	11.2	48.4
DRG-3	1587	10.3	2.7	10.9	42.7
TAL 1536	1617	9.1	2.7	11.1	46.8
TAL 1436	1516	10.5	3.2	10.8	44.8
LSD (p = 0.05)	161	2.8	1.2	2.1	4.9
Moth bean					
Control (Uf)	281	5.3	3.2	2.2	16.9
Control (F)	97	7.3	4.7	4.2	24.6
JMT 2D	1273	3.2	2.0	3.8	22.2
TAL 1536	1150	4.3	2.3	3.7	21.9
TAL 1436	1808	1.2	1.0	3.3	19.2
LSD (p = 0.05)	131	1.9	1.1	0.9	5.1

Uf, Unfertilized; F, Fertilized.

However, dehydrogenase activity in the rhizosphere of clusterbean inoculated with DRG 3, TAL 1436 and TAL 1536 and moth bean with JMT 2D, TAL 1436 and TAL 1536 followed an opposite trend. N application increased dehydrogenase activity.

Nitrogenase activity in control plants of clusterbean and moth bean was 381 and 281 nmoles C_2H_4 plant^{-1} h^{-1} respectively. It increased significantly after inoculation with DRG 3, TAL 1436 and TAL 1536 strains of *Rhizobium* in clusterbean and JMT 2D, TAL 1436 and TAL 1536 in moth bean respectively (Table 2), suggesting that the *Rhizobium* inoculation was effective. Inoculation with three other strains Nif 27 A_2, TAL 1109 and 3Hoa$_9$ did not significantly influence activity, suggesting that inoculation was not effective (data not presented). Maximum activity was observed in clusterbean and moth bean plants inoculated with TAL 1536 and TAL 1436 respectively. Application of N decreased the nitrogenase activity. Grain and straw yields higher than control were recorded in clusterbean plants innoculated with DRG 3, TAL 1436 and TAL 1536 and moth bean plants innoculated with JMT 2D, TAL 1436 and TAL 1536. However differences among strains were not significant. Other strains of *Rhizobium* showed no effect on yield (data not presented).

Comparison of the changes in nitrogenase and plant NR showed that plants with high nitrogenase activity following *Rhizobium* inoculation had low NR activity as was also reported by Chamber[8]. But the contrast in the rhizosphere activity of NR and dehydrogenase of these plants was surprising as microorganisms are known to be a source of NR in soil. Since crops may also contribute towards NR activity in soil[3], these results suggest that NR in rhizosphere of both crops was mainly of plant origin. This possibility is further strengthened as addition of fertilizer N increased NR activity in rhizosphere of both crops.

These results show that (i) effective inoculation with some strains of *Rhizobium* decreases NR activity in rhizosphere by reducing NR activity in plants, and (ii) among effective *Rhizobium* strains, some lead to lesser NR enrichment in soil in spite of similar effect on crop yield. These strains are likely to be more useful in cropping systems as they may lead to better N utilization by subsequent crop, by reducing the possibility of denitrification.

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Received 6 January 1997; revised accepted 13 May 1997