# *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.