

## Influence of Phosphorus and Molybdenum on Ammonia Assimilating Enzymes in *Sesbania Rostrata*



### Biotechnology

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### ABSTRACT

A pot culture study was carried out to evaluate the influence of phosphorus and molybdenum on the activities of ammonia assimilating enzymes, glutamate dehydrogenase, glutamate synthase and glutamine synthetase in completely randomized block design with four replications for each treatment. The treatment consists of the addition of two levels of phosphorus and molybdenum, both individually and in combination. The activity of glutamate dehydrogenase was highly influenced by the application of higher level of phosphorus in both the root and shoot of *S.rostrata*. The application of molybdenum at the lower concentration was effective in improving the glutamate synthase activity in the root, whereas the higher concentration of phosphorus was effective in the shoot. The glutamine synthetase activity in the root was elevated several fold by the dual application of phosphorus and molybdenum at both the levels, whereas the individual application of phosphorus at higher level was effective in the shoot. Thus the application of phosphorus and molybdenum improved the activities of the ammonia assimilating enzymes in the green manure *S.rostrata*.

### Introduction

Nitrogen, an element vital for plant growth, constitutes 78% of earth's atmosphere (Sivasankar and Bheemappa, 1996). In spite of its abundance, it is one of the most limiting factors for crop growth and nitrogen fertilizers represent one of the major costs in crop production (Latha *et al.*, 1997). The combined use of organic manure and green manure with chemical fertilizers is essential for sustaining soil fertility and crop production (Sarkar *et al.*, 1996). Legumes possess the capacity to fix nitrogen in symbiotic association with *Rhizobium*. *Azorhizobium -S. rostrata* is the best known symbiotic system, which accumulates more nitrogen due to its stem nodules (Torres *et al.*, 1995). Another advantage of stem nodulating legumes is their ability to fix atmospheric nitrogen in the presence of combined nitrogen (Becker and George, 1995). *Rhizobial* growth is further associated with synthesis of various enzymes required for nitrogen fixation process (Mengel, 1994).

The glutamate plays a central role in ammonia assimilation. All the amino moieties in the cell are derived either from glutamate or glutamine. The enzymes involved in glutamate/glutamine production from ammonia such as glutamate dehydrogenase (GDH), glutamate synthase (GOGAT) and glutamine synthetase (GS) are known as ammonia assimilating enzymes. Glutamate is produced by the action of the enzymes GOGAT, GDH and GS. GDH is the enzyme primarily involved in ammonium assimilation during the initial stages of growth where by  $\text{NH}_4^+$  is incorporated into glutamate through reductive amination of 2-oxoglutarate. The GS/GOGAT pathway becomes the principle pathway of reassimilation in the aerial parts of the plant, at the latter stages of growth (Pandey and Venubabu, 1988). An attempt has been made to assess the influence of phosphorus and molybdenum on the ammonia assimilating enzymes in the root and shoot of *S. rostrata*.

### Materials and Methods

The study was carried out as pot culture, following completely randomized block design with four replications for each treatment. Each of the pot used for the experiment was filled with 10kg of soil enriched with farmyard manure at the rate of 10t/ha and applied two levels of phosphorus in the form of single super phosphate (50kg and 100kg/ha) and molybdenum in the form of sodium molybdate (5kg and 10kg/ha). Along with individual treatments of the above minerals, dual combinations have also been carried out. *S.rostrata* seeds, inoculated with *Azorhizobium Caulinodans* were sown in each pot. The plants were harvested at the end of the 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> day of growth and analyzed for the ammonia assimilating enzymes such as glutamate dehydrogenase (Doherty,1970), glutamate synthase (Van de Castele *et al.*, 1975) and glutamine synthetase (Atkins

*et al.*, 1984).

### Results and Discussion

#### Glutamate dehydrogenase (GDH)

Table 1 depicts the GDH activity in the root and shoot of *S. rostrata* of mineral treated and untreated plants.

**Table 1. Glutamate dehydrogenase activity in the Root and Shoot of *S.rostrata***

Treatments	Activity of Glutamate dehydrogenase#					
	Root			Shoot		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T <sub>1</sub> (Control)	4.66	7.35	11.48	1.11	0.97	0.69
T <sub>2</sub> (50kg P/ha)	5.47	12.36	38.24	2.78	1.72	0.93
T <sub>3</sub> (5kg Mo/ha)	8.89	14.13	16.63	2.61	2.24	1.08
T <sub>4</sub> (50kg P/ha + 5kg Mo/ha)	7.97	13.37	15.17	4.54	3.39	2.36
T <sub>5</sub> (100kg P/ha)	13.94	17.26	44.35	16.92	4.57	2.63
T <sub>6</sub> (10kg Mo/ha)	13.63	17.30	17.85	5.26	4.27	2.02
T <sub>7</sub> (100kgP/ha + 10kg Mo/ha)	8.85	12.11	17.82	3.60	2.38	0.18
CD (0.05)	3.28			5.10		

#### # Nanomoles of NADH oxidized /min/mg protein

The GDH activity in the root increased with the growth period for all the treatments. Among the treatments, T<sub>5</sub> (100kg P/ha) and T<sub>6</sub> (10kg Mo/ha) were elevated the GDH activity similarly at the initial stages (30<sup>th</sup> & 45<sup>th</sup>DAS) of growth but the individual application of phosphorus at both the levels were found to be superior at the latter stage (60<sup>th</sup> DAS) of growth by recording the maximum activity.

The GDH activity in the shoot was found to be higher in the treatment that received the higher concentration of phosphorus. Unlike the trend in the root, a decrease in the GDH activity has been noticed in the shoot with the increasing growth pe-

riod. Also the enzyme activity in the root was found to be higher than that of the shoot for all the treatments. In the early stages of growth, GDH plays a fundamental role in the reassimilation of ammonia released from the plant whereas at the latter stages of growth, the GS/GOGAT pathway becomes the principle pathway of reassimilation in the aerial parts of the plant (Loyola Vargas *et al.*, 1988).

### Glutamate Synthase (GOGAT)

As indicated in Table 2, the GOGAT activity of the experimental *S. rostrata* plants was found to be significantly higher ( $P < 0.05$ ) in all the stages of growth in comparison with the control. The application of Mo at the lower concentration was effective in improving the GOGAT activity in the root, whereas the higher concentration of phosphorus was effective in the shoot. The GOGAT activity in the root was also found to be higher than in the shoot, indicating that the ammonia assimilation occurs predominantly in the root. A study by Yamaya *et al.* (1995) had shown that the level of GOGAT activity increased 10 fold in the root than in the shoot of rice seedlings.

**Table 2. Glutamate synthase activity in the Root and Shoot of *S.rostrata***

Treatments	Activity of Glutamate synthase#					
	Root			Shoot		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T <sub>1</sub> (Control)	1.97	4.38	10.22	0.89	2.33	4.09
T <sub>2</sub> (50kg P/ha)	5.06	10.21	15.74	3.03	5.90	7.95
T <sub>3</sub> (5kg Mo/ha)	7.34	12.53	36.38	2.65	4.42	5.61
T <sub>4</sub> (50kg P/ha + 5kg Mo/ha)	7.29	14.73	21.64	4.77	6.70	8.42
T <sub>5</sub> (100kg P/ha)	9.49	17.00	24.29	7.54	10.12	19.81
T <sub>6</sub> (10kg Mo/ha)	6.85	15.26	20.55	4.95	6.24	8.16
T <sub>7</sub> (100kgP/ha + 10kg Mo/ha)	3.85	13.53	17.65	3.90	4.13	5.68
CD (0.05)	1.78			2.93		

### # Nanomoles of NADH oxidized /min/mg protein

### Glutamine Synthetase (GS)

The influence of minerals on the activity of GS in the root and shoot of *S. rostrata* is presented in Table 3.

**Table 3. Glutamine synthetase activity in the Root and Shoot of *S.rostrata***

Treatments	Activity of Glutamine synthetase#					
	Root			Shoot		
	Days after sowing			Days after sowing		
	30	45	60	30	45	60
T <sub>1</sub> (Control)	16.95	43.22	75.56	22.77	37.72	45.32
T <sub>2</sub> (50kg P/ha)	35.92	67.63	107.27	32.09	59.38	109.36
T <sub>3</sub> (5kg Mo/ha)	51.55	73.68	111.69	68.92	85.02	129.32
T <sub>4</sub> (50kg P/ha + 5kg Mo/ha)	180.38	230.70	357.70	55.97	78.26	147.50
T <sub>5</sub> (100kg P/ha)	151.75	224.67	271.08	47.86	98.12	170.21
T <sub>6</sub> (10kg Mo/ha)	122.24	182.05	221.57	30.65	93.81	144.14
T <sub>7</sub> (100kgP/ha + 10kg Mo/ha)	175.55	284.17	316.67	54.72	82.58	135.24
CD (0.05)	19.19			12.25		

### # Nanomoles of $\gamma$ -glutamyl hydroxamate produced /min/mg protein

The dual application of P & Mo at both the levels effectively elevated the GS activity in the root. Along with the lower level of Mo, the same trend was noticed in GS activity of shoot on the 30<sup>th</sup> day of growth, whereas the individual application of P & Mo at higher concentration was found to be effective on the 45<sup>th</sup> as well as on the 60<sup>th</sup> day of growth. Similar to the activities of GDH and GOGAT, the GS activity was also more in the root than in the shoot of *S. rostrata*, indicating that the root is the major site of ammonia assimilation (Srivastava and Singh, 1987). Also the GS activity in *S. rostrata* plants was higher than the GDH and GOGAT activities. This clearly shows that the GS is an important enzyme for the ammonia assimilation in *S.rostrata*. GS catalyses the formation of glutamine from glutamate i.e. the newly fixed N<sub>2</sub> is incorporated in to glutamine by GS, which is then transported to the shoot.

### Conclusion

The present study revealed that the application of phosphorus and molybdenum at both higher and lower levels enhanced the activities of ammonia assimilating enzymes. Thus the effective utilization of N<sub>2</sub>-fixing leguminous green manure crops like *S.rostrata* can make a substantial addition of nitrogen to the crop. In addition, it can provide organic matter to the wetland rice soils. Therefore *Sesbania* cultivation has a high potential as green manure for paddy fields in the tropics. The farmers will benefit not only by reducing the investment on chemical fertilizers by applying *S.rostrata* as green manure in the rice field, but also by the increased yield of rice.

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