Australian Journal of Basic and Applied Sciences, 5(4): 252-256, 2011
ISSN 1991-8178

Effect of Molybdenum on Nitrogen Fixing Enzymes of Blackgram Using Anabaena Azollae Sp Treated Coir Waste Manure under Drought Stress

[1]Anbuselvi. S, [2]Sathish Kumar.M, [3]Vikram.M and [4]Debi Prasad Pattanaik

[1,2,3]Lecturer, Department of Industrial Biotechnology, Bharath University, Selaiyur, Chennai, India.
[4]Student, Department of Industrial Biotechnology, Bharath University, Selaiyur, Chennai, India.

Abstract: The effect of molybdenum on nitrate reductase, nitrite reductase and nitrogenase activities as well as the growth and mineral nutrition of black gram growing with *Anabaena azollae* sp treated coir waste manure on green house pot experiments under drought stress conditions were evaluated in randomized block design with three replications in the summer of 2008-2009. Plant dry weight and total N-yield appeared to be drastically affected by the severe drought stress. Mo application affected positively black gram growth, total plant nitrogen yield, saccharides, protein, nitrogen, potassium and phosphorus contents both in control and inoculated plants under severe water stress (25%). Its application caused stronger increase in the activity of nitrate and nitrite reductase when compared to nitrogenase activity. Application of Mo led to an increased accumulation of K^+ ions up to 2- fold compared to the respective Mo- untreated plants. Our results indicated that Mo could contribute considerably to the tolerance of water deficits in black gram treated coir waste manure.

Key words: Nitrate reductase, Mo- Molybdenum, nitrogenase, Anabaena azollae sp, Water stress.

INTRODUCTION

Drought stress is one of the main environmental stresses severely affecting nitrogen assimilation in plants (Katerji, 2000). Under normal conditions, nitrate is the principal form of nitrogen available to the plant from soil that is reduced to ammonia via the process of nitrogen fixation which involves sequential participation of two enzymes-nitrate reductase and nitrogenase (Ribaudo, 2001). However, the increasing cost of nitrogen fertilizer and the danger of increasing soil salinity suggest a tendency to the further limitation of nitrogen application in rangelands (Mohammed, M., 1989).

Organic manures have been reported to improve the growth and nitrogen assimilation even under drought stress (Gerald, 2004). These investigations and previous studies have indicated that the positive role of cyanobacteria inoculated organic manures (especially at moderate level of salinity and drought stress) could be associated with its effect on hormonal level and/or an enhancement of root nitrate reductase and nitrogenase activities (El-Komy, 2004).

Cyanobacteria inoculated organic manure treated with legume plants can diminish the adverse effects caused by osmotic stress such as leaf senescence due to water holding capacity of organic manures (Hamdia, 2008).

Molybdenum (Mo), a constituent of nitrogenase (NA) and nitrate reductase (NR), is required for the assimilation of soil nitrates. Mo is a constituent of molecular enzymes which play an important role in many metabolic processes such as purine metabolism, nitrate assimilation and phyto-hormones (Sigel, 2002).

Therefore the function of Mo is closely related to plant nitrogen metabolism and Mo deficiency is manifested as deficiency of plant N (Mendel, 2002; Pollock, 2002). However, lesser Mo content is required for nitrite reduction than nitrogen fixation support (Parker, 1977). Therefore Molybdenum is efficiently concentrated in the nodules of Molybdenum deficient plant (Brodrick, 1991).

Little information is available on nitrogen fixing enzymes like nitrogenase, nitrate reductase activities of drought stressed leguminous plants especially when they are treated with organic manures.

Therefore the aim of the present investigation was to study the effect of Mo on these enzyme activities as well as the growth and mineral nutrition of black gram using an Anabaena azollae treated coir waste manure.

Corresponding Author: M.Sathish Kumar, Lecturer, Department of Industrial Biotechnology, Bharath University, 173, Agaram road, Selaiyur, Chennai, India.
E-mail: Satdna1@gmail.com; Mob: 91- 44- 9840930319.

MATERIALS AND METHODS

Black gram seeds were collected from Tamil Nadu Rice Research Station, Aduthurai. Seeds were transplanted into a pot containing 5kg of sand and fertile clay soil (tanjore) mixture in the ratio of 1:2 (W/W). The second pot containing 5kg of sand and drought soil (Virudhunagar) mixed in the ratio of 1:2.

Log phase culture of *Anabaena azollae* (ML-2) was mixed with coir waste (1:5) and kept for composting. 3g of Anabaena azollae sp coir waste manure was applied in another pot along with above soils (pot 3, pot 4.) The molybdenum at the concentration of 0.01mg per pot 1 (potassium molybdenum) was applied with three replications.

Soil moisture content was measured of pot 5, pot 6 & pot7 according to Singh (1980). To determine the field capacity, a glass cylinder was filled with soil, leaving about 10 cm of the cylinder top unfilled. No air pockets should be leaved inside. Then, a small diameter glass tube was placed at the center of the soil ensuring that the passage of the displaced air was facilitated. De–aerated water was infused onto the cylinder empty top in order to saturate fully the upper 25cm of the bottom soil dry. The upper surface of the soil was then sealed with paraffin wax and covered with watch glass to check evaporation from the soil surface. The protruding glass tube was plugged with cotton wool to reduce evaporation losses.

Duplicate cylinders were allowed to stand for 48-72 hours. Samples were taken from the wet zone and then moisture content at field capacity was determined after drying the soil sample in an oven. 40 days after sowing, plants were harvested, dried in an oven at 70^{o}C to constant mass, then weighed and grounded for further analysis.

Nitrogenase activity was determined by Gas chromatography (Turner, 2003) equipped with flame ionization detector. The acetylene reduction was expressed as nmol(C_2H_4) h^{-1} g^{-1}(FW). Nitrate reductase activity was determined calorimetrically according to Jaworski (Jaworski, 1971) by adding sulphanilamide and N 1-naphthyl) ethylenediamine dihydrochloride. Nitrate reductase activity was also determined calorimetrically using methyl viologen and sodium bicarbonate at 520nm.

Soluble saccharides were determined according to the anthrone –sulphuric acid method (Fales, 1951) and soluble proteins were measured according to Lowry *et al.* (1951)Phosphorus and potassium were determined in plant extract according to flame photometer.

RESULTS AND DISCUSSION

Morphological parameters like shoot and root dry weight, total N-yield under different soil moisture were discussed in Table 1.They decreased in content in control plants. However, *Anabaena azollae* sp treated coir waste manure significantly enhanced these growth parameters in both fertile soil treated plants. This was due to symbiotic association between coir waste manure and nutrients in black gram plant. Data also showed further stimulatory effect on growth parameters by Mo application either as single or combined with coir waste manure, as compared to the respective untreated plants. Soil moisture content decreased progressively up to 25% of the field capacity & induced changes in the nitrogen metabolism of black gram. Plant dry weight and total N- yield appeared to be inhibited drastically by drought stress.

Saccharides and soluble protein contents decreased considerably in the shoot of plants grown under 25% of field water capacity as compared to the plants grown under 100% or 50% of field water capacity (Table 2). On the other hand, soluble saccharides and protein contents in roots increased with the decrease of soil moisture content. *Anabaena azollae* inoculated coir waste significantly increased the content of black gram plants in fertile and drought soil. In general, application of Molybdenum significantly enhanced shoot and root saccharides and protein contents either in control and coir waste treated plants.

Table 3 shows nitrogen fixing enzymes- Nitrate reductase , Nitrite reductase and Nitrogenase activity of black gram treated with coir waste manure. Maximum amount of Nitrate reductase and Nitrite reductase were found to be higher in fertile treated plants. Uninoculated plants showed on increase in Nitrate reductase activity by Mo application (Table 3) compared to the respective control plants. El.komy *et al* (2003) reported that nitrate reductase was affected negatively by the increase of salt and drought stress intensity. Moreover Lisnic and Toma (Lisnic, 2000) indicated that the denitrification process and nitrite reductase activity were inhibited under drought stress. *Anabaena azollae* treated coir waste manure used as biofertilizer, helps to produce growth promoting substances as reported by Sreelakshmi (2002).

Coir waste manure treated plants in both fertile and drought soil showed higher nitrogenase activity under 50% soil moisture content. Data also showed that Mo-application to control plants positively affected nitrogenase activity, as compared to the untreated plants.

Molybdenum is an element indispensable in the process of nitrogen fixation and plant metabolism (Vieira, 1992; Xiong, 2001; Sagi, 2002). Mo application positively affected black gram growth and total plant N-yield in fertile soil using *Anabaena azollae* treated coir waste manure under low soil moisture content (25%). This enhances the metabolic pools required for the synthesis of saccharides and protein.

In control, potassium, nitrogen and phosphorus contents of black gram increased at the soil moisture stress (50%) (Table 4). Mo enhanced absorption of a large amount of N, P and K from the soil to the root. This could be linked to the higher accumulation of proteins and /or the increased absorbing zones as indicated by the increase in root fresh and dry weights. Our results showed that Mo application has led to 2-fold increase in the accumulation of K^+ ions compared to the respective control plants.

Shabala *et al* (2000)suggested that Potassium ion is likely to be one of the primary targets in the mechanism of osmotic stress perception in the bean mesophyll cells and it should be targeted to improve osmotolerance. K content is a suitable index for water stress tolerance of glycophytes (Hamdia, 1997). Thus, molybdenum soil treated plants had grown even under low water capacity (25%). This could be linked to the improvement of the hydraulic conductance of the membrane (Cramer, 1985).

Table 1: Effect of Molybdenum and *Anabaena azollae* treated coirwaste inoculation in black gram at different soil moisture contents on dry weight and total N-yield of shoot and root of 60-days old plants.

Treatment	Soil moisture content	Shoot		Root	
		Dryweight (g)	N-yield (g)	Dryweight (g)	N-yield (g)
Control (no manure)	100	0.70	8.5	1.5	16.2
	50	0.66	6.8	1.2	12.0
	25	0.36	4.0	0.26	2.6
Coirwaste treated plant in fertile soil	100	1.0	10.0	3.0	26.8
	50	0.94	9.8	2.4	22.8
	25	0.46	6.2	0.46	4.5
Coirwaste treated plant in drought soil	100	0.84	6.8	1.8	19.0
	50	0.70	5.3	1.32	17.8
	25	0.23	4.6	0.40	3.9
Control + molybdenum	100	0.70	7.4	1.9	18.1
	50	1.02	13.4	1.7	14.6
	25	0.21	4.1	0.52	4.4
Coirwaste treated plant in fertile soil + molybdenum	100	1.2	12.6	3.4	36.8
	50	1.0	10.8	3.0	23.2
	25	0.62	5.8	0.71	7.5
Coirwaste treated plant in drought soil + molybdenum	100	0.86	6.9	2.0	20.6
	50	0.72	5.5	1.5	15.8
	25	0.27	4.9	0.42	4.2
LSD 5%		0.5	2.3	0.4	3.3

Table 2: Effect of Molybdenum and *Anabaena azollae* treated coir waste inoculation in black gram at different soil moisture contents on saccharides and proteins content in shoot and root of 60-days old plants.

Treatment	Soil moisture content	Saccharides (mg %)		Proteins (mg %)	
		Shoot	Root	Shoot	Root
Control (no manure)	100	33.6	17.9	44.7	30.8
	50	30.8	14.7	43.6	37.6
	25	26.7	16.2	38.2	35.4
Fertile soil	100	34.9	17.0	45.8	32.8
	50	33.5	18.7	46.7	39.2
	25	32.8	17.8	54.3	28.6
Drought soil	100	38.4	16.7	44.6	32.8
	50	31.7	17.4	46.7	39.2
	25	30.5	17.2	54.3	28.6
Control + molybdenum	100	38.4	16.7	44.6	30.4
	50	31.7	17.4	45.9	32.8
	25	30.5	17.2	50.8	29.6
Coir waste treated plant in fertile soil + molybdenum	100	30.6	22.8	55.1	30.1
	50	29.7	21.6	55.8	27.6
	25	21.8	25.0	54.6	25.8
Coir waste treated plant in drought soil + molybdenum	100	36.7	26.2	46.4	38.3
	50	32.0	24.3	50.7	40.6
	25	29.5	30.6	46.3	35.4
LSD 5%		2.8	3.2	5.2	4.1

Table 3: Effect of Molybdenum on nitrogen fixing enzymes at soil moisture contents of black gram using *Anabaena azollae* treated coir waste manure.

Treatment	Soil moisture content	Nitrate reductase (U/ml)	Nitrite reductase (U/ml)	Nitrogenase nmol(C_2H_4)h^{-1} g^{-1} (FW)
Control (no manure)	100	1.83	1.55	3.51
	50	1.65	1.42	3.42
	25	1.02	1.16	3.24
Fertile soil	100	2.71	2.62	7.51
	50	2.82	2.73	8.60
	25	2.52	2.54	7.23
Drought soil	100	1.50	1.67	3.81
	50	2.41	1.81	4.27
	25	1.74	1.13	3.63
Control + molybdenum	100	4.70	4.63	1.84
	50	4.27	4.34	1.35
	25	3.59	3.81	0.50
Coir waste treated plant in fertile soil + molybdenum	100	3.36	3.35	6.51
	50	3.82	3.82	5.60
	25	3.12	3.53	4.61
Coir waste treated plant in drought soil + molybdenum	100	0.81	1.24	3.90
	50	1.90	1.54	3.52
	25	1.53	0.63	3.06
LSD 5%		0.11	0.17	0.98

Table 4: Effect of Molybdenum on *Anabaena azollae* inoculated coir waste at different moisture content on N, P & K content of black gram.

Treatment	Soil moisture content	Nitrogen (%)	Phosphorus(%)	Potassium (%)
Control (no manure)	100	2.03	0.15	0.73
	50	2.35	0.18	0.92
	25	1.98	0.12	0.81
Fertile soil	100	2.19	0.22	0.86
	50	2.24	0.24	1.23
	25	2.13	0.19	0.95
Drought soil	100	1.98	0.15	0.62
	50	2.04	0.20	0.79
	25	1.36	0.09	0.37
Control + molybdenum	100	1.87	0.10	1.42
	50	1.96	0.13	2.03
	25	2.01	0.08	1.76
Coir waste treated plant in fertile soil + molybdenum	100	1.82	0.18	1.63
	50	1.92	0.24	1.72
	25	1.63	0.20	1.45
Coir waste treated plant in drought soil + molybdenum	100	2.02	0.15	1.38
	50	1.96	0.21	1.48
	25	1.32	0.12	0.97
LSD 5% (0.05)		NS	NS	NS
CV (%)		7.06	6.20	5.18

Conclusion:

Anabaena azollae treated coir waste manure has good mulching effect, water holding capacity and translocation of nutrients. The above results show positive linear relationship between manure and nature of soil. It can be concluded that an adequate supply of molybdenum can maintain efficient yield in coir waste treated plants grown in drought soil under severe water deficiency.

REFERENCES

Brodrick S.J. and K.E. Giller, 1991. J. Exp. Bot., 42: 679-686.

Cramer, G.R., A. Lauchi and V.S. Polito, 1985. J. Plantarum, 46: 281-287.

El-Komy, M.H., M.A. Hamdia, M.A. Hetta, and A.B. Barakat, 2004. Polish J. of Micribiology, 53: 53-60.

El-Komy, M.H., M.A. Hamdia and G.K. Abd El-Baki, 2003. Biologia Plantarum, 46: 281-287.

Fales, D.R., 1951. J. Biol. Chem., 193: 113-118.

Gerald, 2004. World journal of microbiology, 26: 37-45.

Hamdia, M.A., H.M. El-Komy and N. Barakat, 2008. Xth International Colloquium for the Optimization of Plant Nutrition. April. 8-13. Cairo Sheraton, Cairo-Eqypt, 193.

Hamdia, M.A. and M.K. Shaddad, 1997. Biologia Plantarum, 39: 263-269.

Jaworski, E.G., 1971. Biochem. Biophys. Res Commun. 43: 1274-1279.

Katerji, N., Van J.W. Hoorn, A. Hamdy, M. Mastrorilli, 2000. Agric. Water Manage, 43: 99-109.

Lowry, O.H., N.J. Roserbrough, A.L. Fail and R.J. Randall, 1951. J. Biol. Chem., 193: 265-275.

Lisnic, S. and S. Toma, 2000. Xth International Colloquium for the Optimization of Plant Nutrition, Abstract, pp: 180.

Mohammed, M., W.F. Campbell, M.D. Ruambaugh, 1989. Arid Soil Rehabil, 3: 11-20.

Mendel, R.R. and R. Hansch, 2002. J. Exp. Bot., 53: 1689-1698.

Pollock, V.V., R.C. Conover, M.K. Johnson and M.J. Barber, 2002. Arch. Biochem. Biophys, 2: 237-248.

Parker, D.R. and H.B. Harris, 1977. Agron. J., 69: 551-554.

Ribaudo, C.M., D.P. Rondanini, J.A. Cura, A.A. Fraschina, 2001. Biol Plant., 44: 631-634.

Sigel, 2002. Metal ions in biological systems. New York Marcel Dekker.

Singh, R.A.S., 1980. Soil Physical Analysis, (Department of Argonomy, Banaras Hindu University, Varanasi-2210005), Kalyani Publishers, New Delhi-LUDHIANA.

Sreelakshmi, 2002. Journal of plant science, 7: 37-45.

Sagi, M., C. Scazzocchio and R. Fluhr, 2002. Plant J., 31: 301-317.

Shabala, S., B. Ogla and I. Newman, 2000. Journal of Experimental Botany, 51: 1293-1253.

Turner, G.L.K., A. H. Gibson, F.J. Bergersen, (ed.): 2003. Methods for Evaluating Biological Nitrogen Fixation. John Willey & Sons, New York, pp: 111-138.

Vieira, C., A.O. Nogueira and G.A.A. Araujo, 1992. R. Agric., 67: 117-124.

Xiong, L., M. Ishitani, L. Hojoung and J. Kang Zhu, 2001. The Plant Cell, 13: 2063-2083.