EFFECT OF MOLYBDENUM ON NITROGENASE AND NITRATE REDUCTASE ACTIVITIES OF WHEAT INOCULATED WITH *AZOSPIRILLUM BRASILENSE* GROWN UNDER DROUGHT STRESS

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Received: July 29, 2004

Summary. The effect of Mo on nitrate reductase and nitrogenase activities as well as the growth and mineral nutrition of wheat inoculated with *Azospirillum brasilense* in greenhouse pot experiments under drought stress conditions was evaluated. Plant dry weight and total N-yield appeared to be drastically affected by the severe drought stress. This inhibition was associated with a decline in nitrate reductase (NR) and nitrogenase (NA) activities of both shoot and root systems. Under severe water stress (35%) low nitrogen-fixing activity and low *Azospirillium* content were obtained. Mo application affected positively wheat growth, total plant N-yield, saccharides, protein, potassium and magnesium contents both in control and inoculated plants under severe water stress (35%). Nitrate reductase (NR) and nitrogenase (NA) activities in inoculated and uninoculated plants in the absence of Mo declined as a result of water stress injury. Mo application caused a stronger increase in the activity of nitrate reductase 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 wheat inoculated with *Azospirillum*.

Abbreviations: Mo- Molybdenum; NR- Nitrate reductase; NA- Nitrogenase; SMC - Soil moisture content; AZ- Azospirillum brasilense

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INTRODUCTION

Adverse environmental conditions like soil salinity, water stress, and heavy metals pollutions, severely affect N assimilation in plants (Al-Rawahy et al., 1992; Katerji et al., 2000). Therefore, the application of N fertilizers to growing plants under such conditions may increase their tolerance (Cordovilla et al., 1994; Van Hoorn et al., 2000). However, the increasing cost of N fertilizers and the danger of increasing soil salinity suggest a tendency to further limitation of N application in rangelands (Mohammed et al., 1989). Hence, the importance of biological fixation of nitrogen has been increasing during the past years (Katerji et al., 2000; Ribaudo et al., 2001).

Azospirillum has been reported to improve growth and nitrogen assimilation of cereals even under drought stress (Ishac et al., 1988; Bashan and Holguin, 1997; Hamdia and El-Komy, 1998; Hamdia et al., 2000; El-Komy et al., 2003). These investigations and previous studies have indicated that the positive role of *Azospirillum* (especially at moderate levels of salinity and drought stress) could be associated with its effect on hormonal level and/or an enhancement of root nitrate reductase (NR) and nitrogenase (NA) activities (Boddy and Dobereiner, 1988; Ribaudo et al., 1998). The best performance of *Azospirillum* under field conditions is usually expressed under not optimal conditions for plant growth (limited fertilization and water), which occur mainly in semiarid agriculture. Semiarid agriculture is frequently associated with increased soil salinization (Matsumoto et al., 1994). Despite of the above marked effect of drought stress on growth, survival and expression of nif gene promotion, *Azospirillum* inoculation can diminish the adverse effects caused by osmotic stress such as reduction of leaf senescence and cleoptile height as well as fresh and dry weight (Jofre et al., 1998; Alvarez et al., 1996). Recently our research group established that *Azospirillum* inoculation under salinity and drought stress enhanced growth, NR and mineral uptake as compared to nontreated plants (Hamdia and El-Komy, 1998; Hamdia et al., 2000; El-Komy et al., 2003).

Molybdenum (Mo), a micronutrient element essential for nearly all organisms, deserves special attention as a major requirement for plant growth. Mo is a constituent of nitrogenase (NA) and nitrate reductase (NR), required for the assimilation of soil nitrates. Therefore, the function of Mo is closely related to plant nitrogen metabolism, and Mo deficiency is manifested as deficiency of plant N (Mendel and Hansch, 2002; Pollock et al., 2002). If a symbiosis is being established, unusual proliferation of nodules is often observed when legumes are deficient in Mo, presumably due to the N deficiency (Marschner, 1995). However, lesser Mo content is required for nitrate reduction than for N_2-fixation support (Parker and Harris, 1977). Therefore, Mo is efficiently concentrated in the nodules of Mo-deficient plant (Brodrick and Giller, 1991).

Little information is available on nitrogenase (NA) and nitrate reductase (NR) activities of drought stressed nonlegumenous plants, especially when they are inoculated with nitrogen fixing *Azospirillum* sp. and subjected to Mo fertilization. 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 wheat plants inoculated with *Azospirillum brasilense* in greenhouse pot experiments under drought stress conditions.

MATERIALS AND METHODS

Azospirillum brasilense st. Z6/12, (nitrate reductase positive strain NR^+), isolated from maize rhizosphere (El-Komy, 1992), was used. Bacterial strain was grown in malate medium supplied with 0.2 g L^{-1} yeast extract for 20 h at 30°C on a shaker at 200 rpm. Cells were harvested at the logarithmic phase by centrifugation, washed twice in sterile demineralized water and then used as inoculum at the amount of 1 $cm^3 = 10^7$ CFU per seed. Wheat seeds (*Triticum aestivum* L.) were surface sterilized by an immersion for 3 minutes in a mixture of 96% ethanol and 95% H_2O_2 (1:1, V/V). Then they were washed with sterile distilled water several times, and germinated in dark on wet sterile filter paper in Petri dishes for 3 days at 30^{o}C. Six germinated seeds were transplanted into a pot containing 5 kg sand and garden clay soil mixture in a ratio of 1:2 (w/w). Seedlings of the first group were inoculated with *Azospirillum brasilense* st Z6/12 (each group composed of 9 pots), and the second pot group was treated with Mo at the concentration of 0.01 mg pot $^{-1}$ (potassium molybdate). Third group was left without inoculation as a control. Fourth group was treated with AZ inoculation and Mo application at the same as the described above concentration. At the beginning of the second week after sowing, plants were thinned down to three per pot and the soil moisture content was adjusted to 100, 70 and 35% of the field capacity.

Soil moisture content was measured according to Singh, (1980). To determine the field capacity, a glass cylinder was filled slowly with soil (after passing through 2 mm sieve), 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 tube capillary is not filled so that the passage of the displaced air is facilitated. De-aerated water was infused onto the cylinder empty top in order to saturate fully the upper 25 cm of the soil leaving about 10 cm of the bottom soil dry. The upper surface of the soil was then sealed with paraffin wax and covered with a 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 to 72 hrs. Samples were taken from the wet zone and then moisture content at field capacity is determined after drying the soil sample in an oven. 35 days after sowing, plants were harvested, dried in an oven at 70° C to constant mass, then weighed and grounded for further analysis.

Density of *Azospirillum* populations was determined in root histospere (crushed roots after being washed and shaken for 2 min in ethanol) at harvesting using the dilution plate method in DN-medium supplemented with Congo Red (0.25 % aqueous solution according to Rodrigues-Caceres, 1982).

Nitrogenase activity was determined for inoculated and noninoculated plants. The acetylene reduction assay (ARA) was applied according to Turner and Gibson (1980), using a gas chromatography ATIUNICAM 610- GLC (UK) equipped with a glass column backed with activated alumina. ARA was performed after 24 hrs incubation period with 10% acetylene. Results were expressed as nmol (C_2H_4) h^{-1} g^{-1} (f. w.). Nitrate reductase activity was determined according to Jaworski (1971). One gram fresh leaf sample tissue was incubated for 30 min in assay medium (PH 7), then was boiled at 100° C for 5 min. The nitrite was then determined colorimerically using sulphanilic acid and α- naphthylamine solution at 520 nm with a 55B Perking Elemer spectrophotometer (UK). Total N content in plant dry shoots was determined after Kjeldahl digestion, and the total N yield was calculated according to Rennie (1980).

Soluble saccharides were determined according to the anthrone - sulphuric acid method (Fales, 1951) and soluble proteins were measured according to Lowry et al. (1951). Potassium was determined in water plant extract according to the flame photometric method (Williams and Twine, 1960). Calcium and magnesium were determined according to the versene titration method (Schwarzenbach and Biedermann, 1948).

Statistical Analysis

Experimental data were subjected to one-way variance analysis and the means differences were evaluated by the least significant, L.S.D. (Steel and Torrie, 1960). Then, between data, analysis of correlation coefficient was performed. The statistical method used was according to that of the Statgraphics Software Statistical Package 5.0.

RESULTS

Table 1 shows shoot and root dry weight, total N-yield and water content under decreased soil moisture conditions. Water content and total N decreased under the effect of soil water percentages changes. They decreased in nonioculated control plants, as well. However, plant *Azospirillium* sp. inoculation significantly enhanced these growth parameters. Data also showed further stimulatory effect on growth parameters by Mo application either as a single or combined with *Azospirillium*, as compared to the respective untreated plants. The photosynthetic pigments, chlorophylls and caroteniod contents decreased with decreasing soil moisture content (data not shown). *Azospirillium* inoculation as a single or combined with Mo, eliminated this water stress adverse effect on pigment content.

Table 1. Effect of Mo and/ or *Azospirillum* inoculation at different soil moisture contents on dry weight, total N-yield and water content of shoot and root of 35 day-old wheat plants.

Treatment	Soil moisture content	Shoot		Root		Water content	
	%	DW [g plant^{-1}]	N-yield	DW [g plant^{-1}]	N-yield [mg(N)plant]	Shoot	Root
Control	100	0.71	8.5	1.6	16.7	2.9	4.9
	70	0.66	7.8	1.3	12.3	2.8	5.3
	35	0.36	4.2	0.26	2.6	1.4	1.6
Azospirillum	100	1.0	11.0	2.9	25.9	4.6	8.2
	70	0.94	10.3	2.6	23.7	3.9	6.6
	35	0.46	5.1	0.58	5.1	1.9	1.0
Control + Molybdenum	100	0.771	6.9	1.9	18.9	6.1	6.0
	70	1.2	12.3	1.8	16.4	3.4	6.0
	35	0.29	4.5	0.60	5.1	1.6	1.8
Azospirillum+ Molybdenum	100	1.1	13.1	3.7	36.3	5.2	8.8
	70	1.0	11.1	3.1	24.8	4.4	7.8
	35	0.57	6.5	0.77	7.7	2.2	1.9
L.S.D. 5%		0.4	2.0	0.3	3.2,	1.6	1.9

Table 2. Effect of Mo and/or *Azospirillum* inoculation at different soil moisture contents on soluble saccharides and soluble proteins contents in shoots and roots of 35 day-old wheat plants.

Treatment	Soil moisture content	Saccharides [mg g^{-1} DW]		Protein [mg g^{-1} DW]	
	%	Shoot	Root	Shoot	Root
Control	100	34.6	13.6	45.6	31.8
	70	33.5	17.4	42.9	36.7
	35	24.6	16.3	39.2	36.9
Azospirilhim	100	32.6	17.5	46.3	33.4
	70	33.9	18.2	46.3	39.2
	35	34.1	17.8	54.2	28.6
Control+ Molybdenum	100	30.6	21.1	55.1	30.2
	70	29.7	22.1	55.5	29.5
	35	21.8	25.0	54.6	27.6
Azospirillum+ Molybdenum	100	36.2	26.5	46.5	37.1
	70	32.0	24.7	50.2	40.1
	35	29.3	30.7	47.6	30.5
L.S.D. 5%		2.5	3.0	4.5	4.3

Saccharides and soluble protein contents decreased considerably in the shoot of plants grown under 35% of field water capacity as compared to the plants grown under 100% or 70% 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. *Azospirillium* inoculation significantly increased the content of saccharides and proteins, of both shoots and roots of wheat plants. In general, Mo application significantly enhanced shoot and root saccharides and protein contents, either in inoculated and in uninoculated plants at all soil moisture levels.

In inoculated wheat roots, nitrogenase activity (NA) was inhibited. At 35% soil moisture content, it recorded 30% of the value at 100% soil moisture content. However, the inhibitory effect on nitrogenase activity was not detected at 70% soil moisture content. Data also show that Mo application positively affected nitrogenase activity, as compared to the untreated plants. Uninoculated plants showed an increase in NA activity by Mo application (Table 3) compared to the respective control plants. Total *Azospirillum* content decreased by increasing the water stress, and Mo application did not enhanced total *Azospirillum* content significantly (Table 3).

Table 3. Effect of Mo and/ or *Azospirillum* inoculation at different soil moisture contents on nitrate reductase (NR); nitrogenase (NA) activity of shoots and roots and total bacterial content in the histospere (log number) of 35 day-old wheat plants.

Treatment	Soil moisture content	Nitrate reductase	[ug mol $(N0_2)\ g^{-1}\ h^{-1}$	Nitrogenase [nmol g^{-1} FW	Total content
	%	Shoot	Root		
Control	100	1.7	1.79	6.1	1.4
	70	1.65	1.56	7.4	1.1
	35	1.5	1.2	4.4	0.6
Azospirillum	100	1.85	1.89	24.1	4.9
	70	1.7	1.75	23.0	5.1
	35	1.55	1.80	6.7	2.6
Control + molybdenum.	100	4.1	4.5	10.5	1.6
	70	4.3	4.7	9.8	1.3
	35	3.3	3.2	7.6	0.8
Azospirillum Molybdenum	100	3.1	4.4	25.7	5.3
	70	3.7	4.0	27.2	5.2
	35	3.2	3.9	9.2	2.5
L.S.D. 5%		0.16	0.1	2.4	0.15

Results in Table 3 indicate that drought stress injury effect on nitrate reductase in shoots and roots of control plants started at moderate soil moisture content (70%) and became inhibitory at lower soil moisture content (35%). Inoculation of wheat plants with *Azospirillium* led to an increase of NR activity in roots as compared to the control. Mo application led to a further increase in shoot and root NR activity in both uninoculated and inoculated plants.

In the control, potassium and calcium contents in shoots and roots increased as the soil moisture content decreased (Table 4). Treatment with *Azospirillium* and/or Mo application generally promoted the accumulation of K$^+$ and Mg^{++} in both shoots and roots, whereas Ca^{++} accumulation was rather unchanged by these treatments.

Table 4. Effect of Mo and/ or *Azospirillum* inoculation at different soil moisture contents on minerals content of shoot and root of 35 day-old wheat plants.

Treatment	Spil moisture content	Shoot [ug g^{-1} DW]			Root [ug g$^{-'}$DW]		
	%	K$^+$	Ca^{++}	Mg^{++}	K$^+$	Ca^{++}	
	100	24	10	4.2	7.9	4.8	3.1
Control	70	26	10	4.8	8.4	5.6	3.0
	35	26	12	4.8	8.5	8.0	3.6
	100	28	10	6.0	8.1	8.0	3.4
Azospirillum	70	29	10	6.6	9.7	6.0	3.2
	35	26	10	7.2	9.9	8.0	3.2
	100	24	10.0	11.5	17.5	4.0	5.0
Control	70	24	10.4	9.0	16.0	3.6	5.0
+ molydenum	35	23	8.0	5.0	15.7	3.6	3.9
	100	30	9.6	9.5	17.2	6.8	4.0
Azospirillum	70	31	10.0	9.5	17.9	4.0	4.5
+ molydenum.	35	32	12.0	5.5	14.3	3.2	3.5
L.S.D. 5%		1.2	1.9	2.1	1.7	2.0	1.2

DISCUSSION

Progressive decrease in soil moisture content up to 35% of the field capacity induced changes in the nitrogen metabolism of wheat. Plant dry weight and total N-yield appeared to be inhibited drastically by the decrease of field water capacity. This inhibition was associated with a decline in nitrate reductase (NR) and nitrogenase (NA) activities in both shoots and roots of wheat plants. Similar results have been reported by several investigations (Masepohi, et al., 1993; Peuke et al., 1996; Abd El-Backi et al., 2000; El-Komy et al., 2003). These authors reported that NR activity was affected negatively by the increase of salt and drought stresses intensity. Moreover, Lisnic and Toma (2000) indicated that the denitrification process and nitrate reductase (NR) activity were inhibited under drought stress. Consequently, soil nitrate content increased leading to the inhibition of plant growth.

Under severe drought stress (35%) low nitrogen-fixing activity and low *Azospirillum* sp quantity was obtained. Therefore, it can be suggested that the stimulatory effect of bacterial inoculation on wheat growth was maintained by factors different from nitrogen fixation. One of the main mechanisms of the recorded wheat growth promotion is directly related to the capability of *Azospirillum* sp. to produce growth promoting substances (IAA, IBA) as well as its nitrate reductase activities, as reported by Yield Failik et al. (1994); Okon and Labandera-Gonzales, (1994); Hamdia and El-Komy (1998).

Molybdenum is an element indispensable in the process of nitrogen fixation and plant metabolism in general, because of its effect on the nitrogenase and nitrate reductase activities (Vieira et al., 1992; Xiong, et al, 2001, Sagi et al., 2002, Pollock, et al., 2002 and Mendel and Hansch, 2002). Mo application positively affected wheat growth and total plant N-yield in noninoculated or inoculated plants with *Azospirillum* under low soil moisture content (35%). This could be attributed to the increase of the metabolic pools required for the synthesis of saccharides, along with the enhanced photosynthetic apparatus (pigmentation). This stimulatory effect of Mo application was associated with the increased Mg^{++} content, which was accompanied with significant elevation of nitrate reductase activity.

Mo is an essential component of the molecular Mo enzymes (Hill, 1996), which play an important role in many key metabolic processes such as purine metabolism, nitrate assimilation, and phytohormones synthesis (Stallmeyer et al., 1999; Hale et al., 2001, Seo and Koshiba, 2002 and Sigel and Sigel, 2002). Our results showed that Mo application has led to 2-fold increase in the accumulation of K^+ ions compared to the respective control plants.

Mo enhanced absorption of a large amount of K^+ from the soil to the root (about 2-fold as compared to the respective Mo-untreated plants). 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. Root growth improved plant water status under drought conditions (Caldwell, 1976). Shabala et al. (2000) suggested that K^+ 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. Furthermore, K^+ and Cl^- elements were suggested to be the potential targets of osmosensing in the stomatal guard cells (Liu and Luan, 1998). Mo increased the shoot K^+ cation transport, particularly under severe drought, which increased shoots osmoregulation, which in turn operated water flow and growth improvement (Marschner, 1995). Hamdia and Shaddad (1997) suggested that K^+ content is a suitable index for water stress tolerance of glycophytes. Thus, Mo could increase the drought tolerance of these plants by increasing K^+ accumulation and/ or decreasing K^+ leakage, which could be linked to the improvement of the hydraulic conductance of the membrane (Cramer et al., 1985).

A decline in nitrate reductase (NR) and nitrogenase (NA) activities of *Azospirillium* inoculated or uninoculated plants in the absence of Mo was indicated as a result of the water stress injury. Mo application had stronger stimulatory effect on NR than NA activity. This observation suggests closer relation of Mo to NR than to NA. There is evidence, which indicates that Mo application not only increases the specific NR activity, but also enhances the extended period of high NR activity and further inclusion of ammonia into organic compounds (through glutamine synthetase activity) at a higher level compared to the control (Amane et al., 1994; Vieira et al., 1995, and Pollock et al., 2002). The increased NA activity in uninoculated by Mo plants could be explained by the increased efficiency of native soil nitrogen fixing bacteria (Jongruaysup et al., 1993 and 1997; Lisnic and Toma, 2000).

It can be concluded that an adequate supply of Mo can maintain efficient wheat-*Azospirillum* association under severe water deficiency. Under experimental conditions, Mo made an important contribution to the tolerance of wheat inoculated with *Azospirillum* under drought.

References

Abd El-Baki, G. K., Siefritz F., Man, H. M., Weiner, H., Kaldenhoff, R. and Kaiser, W. M., 2000. Nitrate reductase in Zea mays L. under salinity. Plant Cell Environ., 23, 515-521.

Al-Rawahy, S. A., Stroehlein, J. L. and M. Pessarrakli, 1992. Dry matter yield and nitrogen-15, Na, Cl, and K content of tomatoes under sodium chloridestress. J. Plant Nutri., 15, 341-358.

Amanne, M. I. V., Vieira, C., Cardoso, A. A. and C. A. A. Araujo, 1994. Resposta de cultivars de feigo (Phaseolus vulgaris L.) as adubacoes nitrogenadae molibdica R. Ceres.,41. 202-216.

Bashan, Y. and G. Holguin, 1997. Azospirillum- Plant relationships: environmental and physiological advances (1990-1996) Can. J. Microbial, 43, 103-121.

Boddy, A. and J. Dobereiener, 1988. Nitrogen fixation associated with grasses and cereals : recent results and perspectives for future research. Plant Soil, 108, 53-65.

Brodrick, S. J. and K. E. Giller, 1991. Root nodules efficient scavengers of molybdenum for N2 fixation. J. Exp. Bot., 42, 679-686.

Caldwell, M. M. 1976. Root extension and water absorption. In: Water and plant life-Ecological studies, (ed O. Lange, 19-Springer-Verlag, Berlin, Pp. 63-85.

Cordovilla, M. P., Ligero, F., C. Lluch, 1994, The effect of salinity on nitrogen fixation and assimilation in Vicia faba. J. Exp. Bot.,45, 1483-1488.

Cramer, G. R., Lauchli, A., and V. S. Polito, 1985. Displacement of Ca2+ by N from the plasmalemma of root cells. A primary response to salt stress. Plant Physiol, 79, 207-211.

El-Komy, M. 1992. Ecological and physiological studies on genus Azospirillum from the rhizosphere of maize and rice plants. Ph.D. Thesis. Institute of Agricultural Microbiology, Russian Acad. Agr. Sci., Sankt Petroburg.

El-Komy, M.H., Hamdia, M. A. and G. K. Abd El-Baki, ,2003 a. Nitrate reductase in wheat plants grown under water stress and inoculated with Azospirillum spp. Biologia Plantarum. 46: 281-287.

El-Komy, M.H., Hamdia, M. A., Hetta, M. A. and A. B. Barakat, 2004 . Possible roles of nitrogen fixation and mineral uptake induced by rhizobacterial inoculation on salt tolerance of maize. Polish J. of Microbiology, 53, 53-60.

Failk, E., Sarig, S. and Y. Okon, 1994. Morphology and physiology of root assiciated with Azospirillum / plant associations. Edited-by Okon. CRC Pres, Bioca Raton. Gla. Pp. 77-85.

Fales, D. R., 1951. The assimilation and degradation of carbohydrates of yeast cells. J. Biol. Chem., 193, 113-118.

Hale, K. L., McGrath, S. P., Lombi, E., Stack, S. M., Terry, N., Pickering, I. J., George, G. N., and E. A. H. Pilon-Smits, 2000. Molybdenum sequestration in Brassica species. A role for anthocyanins. Plant Physiol., 126.,1391-1402.

Hamdia, M. A. and M. H. El-Komy, 1998. Effect of salinity, gibberellic acid and Azospirillum inoculation on growth and nitrogen uptake of Zea mays. Biologia plantarum, 40, 109-120.

Hamdia, M. A. and M. K. Shaddad, 1997. Salt tolerance of soybean cultivars. Biologia Plantarum., 39, 263-269.

Hamdia, M. A., H. M. El-Komy and Barakat, N. (2000): The role of foliar and potassium fertilization and /or p Azospirillium lipoforum .‘ or Bacillus polymexa inoculation in nitrogen fixation and mineral nutrition of maize grown under salt stress. Xth International Colloquium for the Optimization of Plant Nutrition. April. 8-13.Cairo Sheraton, Cairo- Egypt. 193

Hill, R., 1996. The mininuclear molybdenum enzymes. Chem Rev, 2757-2816.

Ishac, Y. Z., El-Haddad, M. E., El-Borollosy, M. A., Eweda, W. E. and M. G. Z. Giris, 1988. Studies on Azospirillum in Egypt. 1- Densities as an effect by some ecological factors. In: Proc. 2 nd AABNF Conference. Cairo, Pp. 450: 468.

Jaworski, E. G., 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res Commun. 43: 1274-1279.

Jongruaysup, S., O, Hara, G. W., Bell, R. W.. and Dell, B., 1993. Effect of molybdenum deficiency on noduole intiation, development and N2 fixation in black gran (Vigna mungo L Hepper) Plant and Soil. 155/156, 345-348.

Jonruaysup, S., Dell ,B., Bell, R. W., O,Hara, G. W. and Bradley, J. S., 1997. Effect of molybdenum and inorganic nitrogen on molybdenum redistribution in black gran (Vigna mungo L.) with particular reference to seed fill. Annals of Botany. 79: 67-74.

Katerji, N., Van Hoorn, J. W., Hamdy, A., M. Mastrorilli, 2000. Salt tolerance classification of crops according to soil salinity and to water stress an index. Agric. Water Manage, 43, 99-109.

Lisnic, S. and S. Toma, 2000. Nitrate reductase activity in soil and soybean plants, effect of trace elements. Xth international Colloquium for the Optimization of Plant Nutrition, Abstrat Pp. 180.

Liu, K. and S. Luan, 1998. Voltage dependent K+ channels as targets for osmosensing in guard cells. Plant Cell, 10, 1957-1970.

Lowry, O. H., Roserbrough, N. J., Fail, A. L. and R. J. Randall, 1951. Protein measurements with folin phenol reagent. J. Biol. Chem., 193, 265-275.

Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press, San Diego. Page. 229-299.

Masepohi, B., Kry, R. and W. Klipp, 1993. The draTG gene region of Rodobactor capsulatus is a requirement for post-translational regulation of both the molybdenum at the alternative nitrogenase. J. Gen Microbiol, 139, 2667-2675.

Mendel, R. R. and R. Hansch, 2002. Molybdoenzymes and molybdenum cofactor in plants. J. Exp. Bot., 53, 1689-1698.

Mohammed, M., Campbell, W. F., M. D.Rumbaugh, 1989. Varation in salt tolerance of alflalfa. Arid Soil Rehabil, 3, 11-20.

Okon, A. and C. A.Labanadera-Gonzales, 1994. Agronomic application of Azospirillum. Ann Evaluation of 20 years worldwide field inoculation. Soil. Biol. Biochem., 26, 1591-1601.

Parker, D. R. and H. B. Harris, 1977. Yield leaf nitrogen of nodulating soybeans as affected by nitrogen and molybdenum. Agron. J., 69, 551-554.

Peuke, A. D., Glaab, J., Kaiser, W. M., W. D. Jeschke, 1996. The uptake and flow of C, N and ions between roots and shoots in Ricinus communis L. IV. Flow and metabolism of inorganic nitrogen and malate depending on nitrogen nutrition and salt treatment. J. Exp. Bot., 47, 377-385.

Pollock, V. V., Conover, R. C., Johnson, M. K, and M. J. Barber, 2002. Bacterial expression of the molybdenum domain of assimilate nitrate reductase: Production of both the functional molybdenum-containing domain and the nonfunctional tungsten analog. Arch. Biochem. Biophys, 2, 237-248.

Rennie, R. J. 1980. 15N- isotop dilution as a measure of nitrogen fixation by *Azospirillum brasilense* associated with maize. Can. J. Bot., 58: 21-24.

Ribaudo, C. M., Paccusse, A.N., Cura, J. A. and A. A. Frascina, 1998. *Azospirillum* maize association: effects on dry matter yield and nitrate reductase activity. Agr. Trop. Subtrop, 31, 61-70.

Ribaudo, C. M., Rondanini, D. P., Cura, J. A.,, A. A. Fraschina, 2001. Response of zea mays to the inoculation with *Azospirillum* on nitrogen metabolism under greenhouse conditions. Biol Plant., 44, 631-634.

Rodrigues-Caceres, E. A. 1982. Improved medium for isolation of *Azospirillum* spp. Appl. Environ. Microbiol.., 44, 990-991.

Sagi, M., Scazzocchio, C. and R. Fluhr, 2002. The absence of molybdenum cofactor sulfuration is the principle cause of the flacca phenotype in tomato plants, Plant J., 31, 301-317.

Schwarzenbach, G. and W.Biedermann, 1948. Komplexone X. Edrakali-Komplexe von 0,6-dioxyazofarbstoffen.Helv, Chem. Acta, 31, 678-687.

Seo, M. and T. Koshiba, 2002. The complex regulation of ABA biosynthesis in plants. Trends in Plant Science, 7, 41-48.

Shabala, S., Ogla, B. and I. Newman, 2000. Ion-specific mechanisms of osmoregulation in bean mesophyll cells. Journal of Experimental Botany, 51, 1293-1253. Sigel, A. and H.

Sigel, 2002. Molybdenum and tungsten. Their roles in biologicasl processes. Metal ions in biological systems. New York Marcel Dekker.

Singh, R. A. S., 1980. Soil Physical Analysis. Department of Agronomy, Banaras Yindu University, Varanasi-2210005. Kalyani Bublishers, Nerw Delhi- LUDHIAVA.

Stallmeyer, B., Schwarz, G., Schulz, J., Nerlich, A., Kirsh, J. and R. R. Mendel, 1999. The neurotransmitter receptor-anchorinh protein gephrin reconsitutes molybdenum cofactor biosynthesis in bacteria, plants and mammalian cells. Proc Natl Acad Aci. USA, 96, 1333-1338.

Steel, R. G., J. H. Torrie, 1960. Principles and Procedures of Satatistics. McGraw-Hill Book Co., New York. Pag***

Turner, G. L. K., A. H. Gibson, 1980. Measurement of nitrogen fixation by indirect means. In : Bergersen, F. J. (ed.): Methods for Evaluating Biological Nirtogen Fixation. Pp. 111-138. John Willey & Sons, New York.

Van Hoorn, J. W., Katerji, N., Hamdy, A., M. Mastrorilli, 2000. Effect of salinity on yield and nitrogen uptake of four grain legumes and on biological nitrogen contribution from the soil. Agr. Water Manage, 51, 87-98.

Vieira, C., Nogueira, A. O. and G. A. A. Araujo, 1992. Adubacao nitrogenada e molibdica na cultura dofeijo, R. Agric., 67, 117-124.

Vieira, R. F., Cardoso, E. J., Vieira, C. and S. T. Cassini, 1995. Foliar application of molybdenum in common. I. Nitrogenase and nitrate reducase activities in a soil of high fertility. In International Sympsium on Sustainable Agriculture for the Tropics. The role of biological nitrogen fixation. 26th Vov.- ist Dec. 1995- Angradod Reis-Rio de Janeiro Brasil, Pp. 189-190.

Williams, V. and S. Twine, 1960. Flame photometeric method for sodium, potassium, and calcium. In: Paech, K., Tracey, M. V. (eds): Modern Methods of plant Analysis. Vol. V. pp. 3-5. Springer-Veralg, Berlin.

Xiong, L. Ishitani, M. Hojoung, L. and J. Kang Zhu, 2001. The Arabidopsis lOS5/ABA3 locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress responsive gene expression. The Plant Cell, 13, 2063-2083.