DOI: 10.1515/cerce-2017-0027
Original Article
Available online: www.uaiasi.ro/CERCET_AGROMOLD/
Print ISSN 0379-5837; Electronic ISSN 2067-1865

Cercetări Agronomice în Moldova
Vol. L , No. 3 (171) / 2017: 71-82

FOLIAR APPLICATION OF SODIUM MOLYBDATE ENHANCED NITROGEN UPTAKE AND TRANSLOCATION IN SOYBEAN PLANTS BY IMPROVING NODULATION PROCESS UNDER SALT STRESS

S. FARHANGI-ABRIZ[1]*, R. FAEGI-ANALOU[2], N. NIKPOUR-RASHIDABAD[1]

*E-mail: Farhanghi@Hotmail.com

Received May 18, 2017. Revised: July 12, 2017. Accepted: July 21, 2017. Published online: Oct. 18, 2017

ABSTRACT. Soil salinity with different harmful effects on plant growth and productivity is one of the main reasons in diminishing biological nitrogen fixation and nitrogen assimilation in legume plants. Molybdate has a key role on nitrogen metabolism of plants and can be has a beneficial effect on it. Thus, this experiment was conducted to evaluate the effects of sodium molybdate spraying (0.2 and 0.4% solutions in water) on nodulation, nitrogen uptake and translocation in soybean plants under different levels of salt stress (0, 5 and 10 dS m^{-1} NaCl, respectively). Salinity reduced the nodulation, root and shoot growth and special flavonoids content in roots, which are have a key role in nodulation includes, daidzein, genistein, coumestrol and glycitein, also diminished nitrogenase, glutamine synthetase (GS), glutamate dehydrogenase (GDH), glutamine oxoglutarate aminotransferase (GOGAT) and nitrate reductase (NR) activities in nodes, nitrogen content of nodes, roots and leaves, nitrogen uptake and translocation by soybean plants. Under salt stress and non-saline condition, sodium molybdate treatments improved the nodulation by increasing flavonoids content of roots, also these treatments enhanced the plant growth and nitrogenase, GS, GDH, GOGAT and NR activities of nodes. Furthermore, nitrogen content of nodes, roots and leaves, nitrogen uptake and translocation by soybean plants improved by sodium molybdate applications. Both of the sodium molybdate doses, exposed the similar effects on improving nodulation and nitrogen metabolism of soybean.

Keywords: flavonoids; nitrogen metabolism; nodulation; salinity; sodium molybdate.

[1] Department of Plant Eco-physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2] Department of Agronomy, Faculty of Agriculture, Islamic Azad University of Roudehen, Tehran, Iran

INTRODUCTION

Salt stress is one of the main limiting factors in agriculture and crop productivity. The adverse effect of salt stress is expressed in whole plant levels (Munns & Tester 2008; Chen *et al*., 2017). A salt concentration in the soil of 4 dS m^{-1} or 40 mM NaCl has an osmotic pressure of about 0.2 MPa, which affects the ability of plants to take up water and nutrients. Previous studies indicated that abiotic stresses, such as salt stress, can have an adverse effect on nodulation and nitrogen metabolism in legume plants (Chakrabati & Mukherji 2003; Flowers *et al*., 2010). High concentration of soil salt has a damaging impact on growth, nodulation and crop yield. In a report, Abd-Alla *et al*. (1998) stated that salt stress reduced the nitrogenase activity in different cultivars of soybean. Comba *et al*. (1998) reported that high level of salt toxicity inhibited the nodulation processes and nitrogen metabolism (by decreasing some key enzymes activity), but these processes did not change under moderate levels of salinity. Similar results were shown in other legumes (Chakrabati & Mukherji 2003; Araújo *et al*., 2015). In upper organs of soybean, salt stress causes a reduction in general growth by decreasing the leaf area and the activation of chlorophyll degradation enzymes such as chlorophyllase and inhibition of chlorophyll synthesis (Munns & Tester, 2008).

Nitrogen (N) deficit is one of the most important yield-limiting factors for crop production in the world. This element applied in the highest quantity for most annual crops (Huber & Thompson, 2007). It shows an essential role in various physiological and biochemical processes in crops. Nitrogen is the main element of many important organic compounds, such as proteins and nucleic acids. It is a key component of the chlorophyll molecule, which has an important role in plant photosynthesis. Nitrogen is also a structural constituent of cell walls and enzymes (Fageria & Baligar, 2005). The rhizobia bacteria fix nitrogen in a mutual association with the leguminous plants, such as soybean. Successful conductivity between plant roots and rhizobia is an important process, which that has affected the nitrogen supply for the plants. Some of the root flavonoids (depending on the plant and rhizobia) have a main role in this symbiosis between the bacteria and plant roots (Antunes *et al*., 2006). Enzymatic activities in nodes and plant tissues are the other important factors for a superior nitrogen fixation and nitrogen metabolism of legumes. Some of the key enzymes, such as nitrogenase, glutamine synthetase (GS), glutamate dehydrogenase (GDH), glutamine oxoglutarate aminotransferase (GOGAT) and nitrate reductase (NR), has a key role in nitrogen metabolism in legume plants. The nitrogenase enzyme has a critical role in nitrogen fixation process with the synthesis of the

molecular nitrogen (N_2) to ammonium ($NH4^+$) in nodes (Chakrabarti & Mukherji, 2003). GS is the other important enzyme in nitrogen metabolism with the multifunctional roles in plants, and improving the activity of this enzyme can endorse plant nitrogen metabolism and translocation (Becker *et al*., 2000; Wang *et al*., 2003). The next important enzyme is the NR, this enzyme is a melodic-enzyme that converted nitrate (NO^{-3}) to nitrite (NO^{-2}). This act is very critical for the protein metabolism in most of the plant species, because nitrate is the dominant form of nitrogen in farm soils (Srivastava, 1980). Previous studies indicated that under salt stress, nitrogen uptake and nitrogen metabolism reduced (Bai *et al*., 2017; Ahanger & Agarwal 2017).

The significance of molybdenum (Mo) in plant nutrition is well-known (Fageria, 1992; Marschner, 1995; Mengel *et al*., 2001). Molybdenum is required by crops in very small amounts (Choi *et al*., 2007). Nevertheless, it has vital roles in crops via molybdate-enzymes (Stiefel, 1993). One of the important roles of molybdenum in plant metabolism is related to reduces of nitrate form of nitrogen to nitrite form. In legume plantsv, such as soybean, Mo is a key member in nodulation process for symbiotic nitrogen fixation. Therefore, Mo absence in a legume crop may be displayed as an N deficiency. Mo is a component of some enzymes, including nitrate reductase and nitrogenase (Taiz & Zeiger, 1998). This ion helps plants in several processes, such as protein synthesis and N metabolism. In soybean, Mo deficiency decreased the plant growth, seed yield, nodulation, the total N content of plant and protein contents of the seeds (Sinclair & Shiraiwa 1993).

The soybean is an annual legume that has many industrial, human, and agricultural uses. The productivity of this crop is the limits by soil and water salinization, up to date, there has been no study concerning the interaction effects of sodium molybdate and salt stress on the nodulation and nitrogen metabolism of soybean, Therefore, this study was conducted to assess these factors under salt stress.

MATERIAL AND METHOD

This experiment was conducted on 5 March 2016 in a glass greenhouse with a factorial design on the basis of randomized complete block with four replications. Three levels of salt stress (non-saline, 5 and 10 dS m^{-1} of NaCl) and three doses of sodium molybdate (contains 39% molybdenum) applications (non-sodium molybdate, 0.2, and 0.4 % sodium molybdate solution in water) were used for the analysis of nitrogen metabolism and nodulation of soybean plants under salt stress (*Glycine max* cv. M7). Salinity levels were chosen according to the range of the salinity tolerance of soybean plants (Grieve *et al*., 2012). Initially, the soil filled to the pots (20 cm radius and 30 cm height) with contained 2.5 kg soil, some properties of the uses soil are shown in *Table 1*. Than six seeds of soybean after inoculation

with *Bradyrhizobium japonicum* (10^8 bacteria per gram) were sown in each plastic pot. The pots were kept in a glass greenhouse under controlled conditions with 25/23ºC day/night temperature, 55-60% relative humidity, 150 W m^{-2} light intensifies and 13 h photoperiod. Pots were irrigated every day with tap water in the growth period to preserve the soil water content near field capacity. After exposes of the first trifoliate leaf, NaCl was added to irrigation water supplied to saline treatments. During the experiment, electric conductivity of each pot was measured *via* a digital conductivity meter (Inolab Model, Weilheim, Germany). Conductivity was preserved in a favorable level by adding water or concentrated NaCl to the pots. The sodium molybdate treatments were sprayed on plants at vegetative [V1 (First Trifoliate) and V3 (Third Trifoliate)] and full flowering stages (R2) (Pedersen *et al*., 2014) in accordance with the treatments.

Table 1 - Some physio-chemical properties of the experimental soil

Soil	Values
Texture	Silty loam
pH	7.4
EC (dSm^{-1})	1.38
Organic carbon (g kg^{-1})	13.1
Total N (%)	0.08
P (mg kg^{-1})	37
K (mg kg^{-1})	157
Cation exchange capacity (cmol kg^{-1})	17.8

Node numbers, node, shoot and root dry weights of soybean plants were measured 64 days after sowing at full flowering stage (four days after the last spraying). The dry weights of tissues were determined after oven drying at 80°C for 48 h.

From each pot (at 64 days after sowing), all root systems were carefully washed out of the soil, then, roots were freeze-dried, sectioned with scissors and finely ground in a mortar and pestle for analysis of flavonoids. Concentrations of flavonoids were determined using a high-performance liquid chromatography (HPLC, Model Waters 600 E, Waters Inc., USA) (Franke *et al*., 1995). The reagents were bought from Sigma Chemical Co. (Missouri, USA).

Nitrogen content of nodes, roots and leaves of soybean plants were measured at 64 days after sowing (at full flowering stage). The plant tissues were washed with deionized water, and then dried in an oven for 48 h, at 70ºC. Dried tissues were powdered and then the contents of nitrogen in different parts were assayed by a CHNS elemental analyzer (Elementar-group, Hanau, Germany). Nitrogen uptake was determined as mg total nitrogen/g root dry weight and nitrogen translocation was calculated as mg nitrogen content of leaves /mg nitrogen content of roots.

On full flowering stage (64 days after sowing date) assaying enzymes activity was done from nodes, roots and leaves of soybean plants. In nodes nitrogenase activity was assayed by the method of Turner & Gibson (1980). Acetylene is converted to ethylene by nitrogenase activity. The manufactured ethylene with nitrogenase was analyzed by a gas chromatograph (Agilent 7820A,

equipped with a Column - Porapak Q) with flame Ionization detector (FID). The oven/column temperature was set in 60°C, intector temperature 65°C and detector temperature is 85°C. Helium used as a carrier gas with a flow rate of 30 ml min^{-1}. The nitrogenase activity was stated by ethylene creation per node fresh weight in an hour. The activity of GS is measured by the method of Sawhney & Singhv (1985). A 50 mg of nodes were homogenized in an extraction buffer (0.1 M potassium phosphate buffer with pH 7.8, 10 mM, KCI, 0.4M sucrose, DTT, 10 mM EDTA, 1 mM $MgCl_2$), prepared samples were measured at 540 nm (100 Conc UV Visible Spectrophotometer, Varian, California, USA). GS activity was calculated with the standard curve of commercial y-glutamyl-hydroxmate with 100-800 µg range. For the determining GDH activity, about 50 mg of fresh tissues of nodes were homogenized with 4 ml of extraction buffer (0.05 M Tris-HCL with pH 7.5, 0.01 M β-mercaptoethanol and 0.4 M sucrose) then prepared samples were centrifuged at 20000 g for 30 min, then 1 ml Upper layer of each sample was added to the reaction mixture (1.6 ml of 0.1 M Tris-HCI buffer with pH 7.5, 0.1 ml of NH_4CI 3M, 0.1 ml of 2-oxoglutarate (0.33 M) and 0.2 ml of NADH). The absorbance was read at 340 nm. For the assaying GOGAT activity we used the same extraction buffer for GDH. The reaction mixture was made with 0.7 ml of 0.1M Tris-HCI buffer (pH 7.5), 0.1 ml of 0.33M 2-oxoglutarte, 1 ml of glutamine (pH 7.0), 0.2 ml of 10-3M NADH. 1 ml of extraction enzyme was added to the reaction buffer and then, absorbance was read at 340 nm (Duke *et al*., 1976). The NR activity was Assayed spectrophotometrically at 540 nm (Jaworski, 1971).

The data were analyzed by MSTATC software, and the means were compared by Duncan multiple range test at $p \leq 0.05$. The figures were drawn by the Microsoft Excel 2016.

RESULTS

Node number and node weight per plant, significantly reduced by enhancing salt stress. Foliar application of sodium molybdate under all levels of saline and non-saline conditions improved the number and weight of the nodes in soybean root. Both of the doses of sodium molybdate applications, exposed the similar effect on nodes number and weight (*Fig. 1*).

Increasing salt stress, decreased the root and shoot dry weight of soybean plant. Application of sodium molybdate enhanced the root and shoot dry weight (*Fig. 1*). This improvement effect on root growth is more noticeable under non-saline than in saline conditions. The results clearly showed that two doses of sodium molybdate statistically showed the same effect on root and shoot growth.

Flavonoids content of soybean root considerably decreased by increasing salt stress. Glycitein content up to 5 dS m^{-1} did not change significantly, but further increasing in salt stress reduced that. The contents of daidzein, genistein, coumestrol and glycitein in soybean roots, improved by sodium molybdate applications. Increasing doses of sodium molybdate from 0.2% to 0.4% did not rise the

flavonoids content in soybean roots (*Table 2*).

All of the enzymes activity in soybean nodes includes: nitrogenase, GDH, GS, GOGAT and NR considerably decreased under salt stress. In all of the enzymes, the lowest activities were observed under 10 dS m^{-1} salinity. Foliar applications of sodium molybdate improved the enzymatic activities of nitrogen fixation and nitrogen metabolism in soybean nodes (*Table 3*). Increasing doses of sodium molybdate, significantly improved the GDH, GS and GOGAT activities in soybean nodes, but this increment in doses of sodium molybdate application, did not significantly alter the nitrogenase and NR activities.

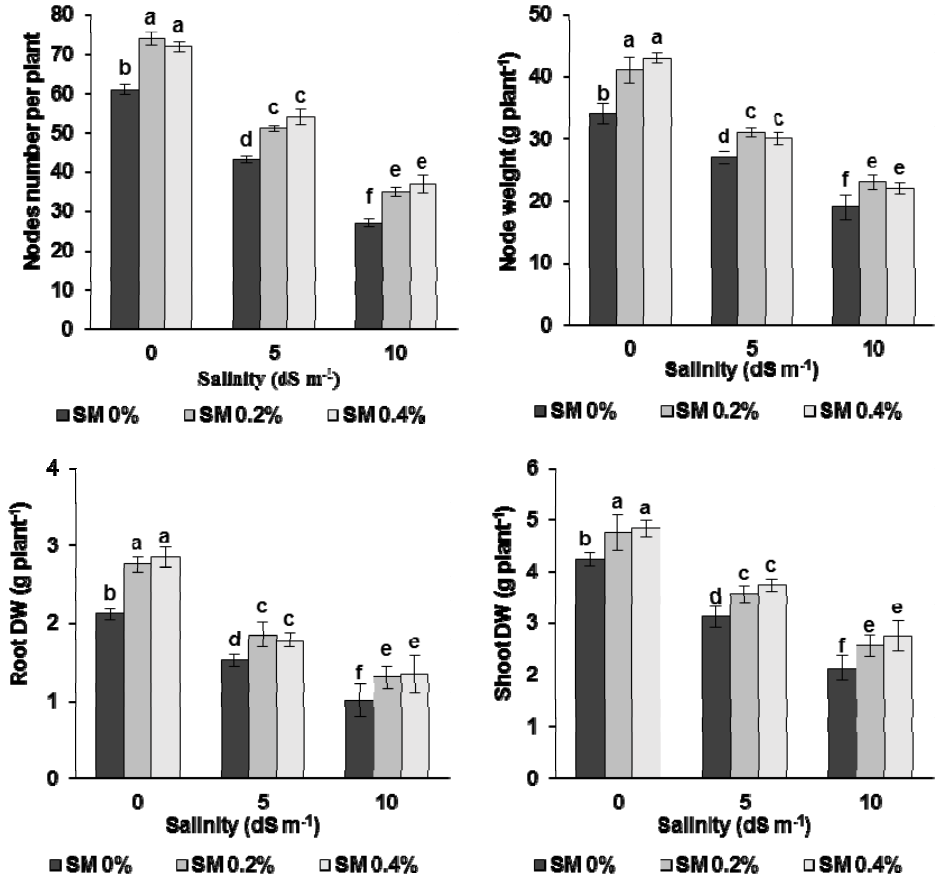

Figure 1 - Nodes number, nodes weights, root and shoot dry weights of soybean plants under different levels of salt stress and sodium molybdate applications. SM: Sodium molybdate; DW: Dry weight. Different letters in each column indicate significant difference at $p \leq 0.05$.

Table 2 - Means± standard error of root flavonoids (mg g^{-1} dry weight) of soybean plants under salt stress and sodium molybdate applications

Treatments	Daidzein	Genistein	Coumestrol	Glycitein
Salinity				
Non-saline	35.54±0.88a	0.070±0.018a	2.20±0.13a	2.25±0.21a
5 dSm^{-1}	27.38±0.43b	0.060±0.011b	1.30±0.24b	2.23±0.18a
10 dSm^{-1}	21.54±0.67c	0.039±0.009c	1.10±0.15c	1.68±0.08c
Sodium molybdate				
SM 0.0%	24.25±0.94b	0.041±0.026b	1.36±0.08b	1.73±0.18b
SM 0.2%	28.97±0.22a	0.061±0.012a	1.64±0.28a	2.21±0.14a
SM 0.4%	29.24±0.89a	0.077±0.016a	1.58±0.19a	2.23±0.15a

SM: Sodium molybdate. Different letters in each column indicate significant difference at $p \leq 0.05$.

Table 3 - Means± standard error of different enzymes activities in soybean nodes under salt stress and sodium molybdate treatments

Treatments	Nitrogenase	GDH	GS	GOGAT	NR
Salinity					
Non-saline	0.088±0.012a	2.54±0.18a	2.43±0.27a	0.121±0.014a	1.48±0.11a
5 dSm^{-1}	0.066±0.019b	1.63±0.15b	2.03±0.18b	0.063±0.018b	0.86±0.13b
10 dSm^{-1}	0.022±0.009c	1.02±0.15c	1.61±0.15c	0.041±0.011c	0.43±0.04c
Sodium molybdate					
SM 0.0%	0.041±0.016b	1.16±0.03c	1.25±0.23c	0.062±0.004c	0.77±0.13b
SM 0.2%	0.081±0.011a	1.78±0.18b	2.34±0.11b	0.088±0.011b	0.97±0.10a
SM 0.4%	0.077±0.016a	1.98±0.15a	2.66±0.10a	0.092±0.023a	1.03±0.14a

SM: Sodium molybdate; Nitrogenase (nmol C_2H_2 g^{-1}FW H^{-1}); GDH: Glutamate dehydrogenase (nmol NADH g^{-1} FW min^{-1}); GS: Glutamine synthetase (nmole y-glutamylhydroxmate g^{-1} FW min^{-1}); GOGAT: Glutamine oxoglutarate aminotransferase (nmol NADH g^{-1} FW min^{-1}) and NR: Nitrate reductase (µmoles nitrite g^{-1} FW H^{-1}). Different letters in each column indicate significant difference at $p \leq 0.05$.

Table 4 - Means± standard error of nitrogen content in different parts of soybean, nitrogen uptake and nitrogen translocation under salt stress and sodium molybdate treatments

Treatments	N_2 Nodes	N_2 Roots	N_2 Leaves	N_2 Uptake	N_2 Translocation
Salinity					
Non-saline	35.61±1.34a	29.63±1.23a	43.78±0.39a	29.30±0.56a	1.48±0.15 a
5 dSm^{-1}	27.43±0.95b	25.03±0.47b	32.21±0.96b	30.12±0.81a	1.28±0.07 b
10 dSm^{-1}	19.13±1.11c	18.73±0.37c	24.23±1.02c	27.62±0.50b	1.29±0.05 b
Sodium molybdate					
SM 0.0%	24.53±1.13c	21.24±0.37b	27.85±0.07b	32.72±0.56b	1.30±0.18b
SM 0.2%	29.64±0.88b	27.75±0.75a	36.23±0.04a	36.45±0.47a	1.30±0.13a
SM 0.4%	33.26±0.94a	28.15±0.40a	35.87±0.16a	35.86±0.82a	1.27±0.06a

SM: Sodium molybdate; N_2 - mg Nitrogen g^{-1} DW, N_2 uptake: mg total nitrogen g root dry $weight^{-1}$ and N_2 - Translocation: mg nitrogen of leaves mg $^{-1}$ mg nitrogen of roots. Different letters in each column indicate significant difference at $p \leq 0.05$.

The content of nitrogen in nodes, roots and leaves of soybean plants considerably decreased under salt stress (*Table 4*). Sodium molybdate treatments raised the nitrogen contents in nodes, roots and leaves of soybean. In soybean nodes, with increment of sodium molybdate doses, nitrogen content increased, nevertheless this increment in doses of sodium molybdate did not change the nitrogen content in roots and leaves of soybean plants.

Enhancing salinity to 5 dS m^{-1} did not change the nitrogen uptake by soybean roots, but further increment of salinity stress from 5 to 10 dS m^{-1} significantly decreased that. Nitrogen translocation from roots to leaves reduced under 5 and 10 dS m^{-1}, there was no difference between the two levels of salt stress. Foliar applications of sodium molybdate, improved the nitrogen uptake and translocation by soybean plants, two doses of sodium molybdate applications exposed similar effects on nitrogen uptake and translocations (*Table 4*).

DISCUSSION

Soil salinity is one of the main factors in decreasing nodulation processes and nitrogen metabolism of crops (López-Gómez *et al*., 2016). Reducing node numbers and weights of soybean under different levels of salt stress (*Fig. 1*) is related from adverse effect of salt toxicity on rhizosphere biota and soil pH. Furthermore, our results clearly indicated that salt stress with reduction of special flavonoids in soybean roots (which known as nodulation factors in soybean) has an adverse effect on nodulation processes (*Table 2 and Fig. 1*). Decreasing root and shoot growth under salt stress (*Fig. 1*) is the general reaction of crops to the salt toxicity (Ghoulam *et al*., 2002). One of the common effects of salt toxicity in plants, is the enhancing oxidative damages to the root and shoot cells (Farhangi-Abriz & Torabian 2017). Moreover, salt stress with decreasing nodulation processes and nitrogen metabolism in nodes, reduced the nitrogen supply. Also reduction of root and shoot growth of soybean under salt stress is attributed with reducing nitrogen uptake and translocation by soybean plants (*Table 4*). Successful nodulation with application of sodium molybdate under salt stress (*Fig. 1*) is associated with improving root flavonoids composition (*Table 2*), which have a key role in nodulation processes in soybean plant (Antunes *et al*., 2006). Enhancing root and shoot growth of soybean plants with sodium molybdate under salt stress, resulted from improving nitrogen metabolism in nodes (*Table 3*) and increasing nitrogen content in different parts of the soybean (*Table 4*). Because, increasing nitrogen content in plants, has a positive correlation with their growth. Salt stress by decreasing general plant performance (*Fig. 1*) and nitrogen content (*Table 4*) reduced the flavonoids content in soybean roots (*Table 2*). Because nitrogen

availability is so important factor for the flavonoids synthesis processes (Coronado *et al*., 1995). Sodium molybdate with decreasing the harmful effect of salt stress and improving the nitrogen content in plant tissues, increased the contents of daidzein, genistein, coumestrol and glycitein in roots (*Table 2*).

Reduction of nitrogen content in nodes and plant tissues of soybean under salinity (*Tables 4*) is associated with decreasing nitrogen fixation by the nitrogenase enzyme, the inhabitation of nitrogen metabolism in nodes, diminishing nitrogen uptake by roots and translocation to the upper organs (*Tables 3 and 4)*. Nitrogen availability in plant cells directly related to the GS, GDH and GOGAT activities. Decreasing enzymes activity with increasing salt toxicity are related to adverse effect of salt toxicity in plants, such as oxidative damages and downregulation of some important gens (Kusano *et al*., 2011). Availability of oxygen in nodules is an essential aspect of nitrogenase activity in legume plants. Inhibition of nitrogenase activity with rising salt toxicity is resulted from decreasing the oxygen permeability in root cells and nodule cortex (Serraj *et al*., 1994; Fernandez-Pascual *et al*., 1996). Also, assimilate mobilization to the nodes is inhibited under salt toxicity (Fougère *et al*., 1991; Munns & James 2003) and in this situation, nitrogenase activity was diminished (*Table 4*). Sodium molybdate with providing molybdate under salt stress enhanced the enzymatic activities in nodes (*Table 3*), because molybdate has an essential role in symbiotic nitrogen fixation. This element is a structural substitute of some key enzymes in nitrogen metabolism, such as nitrate reductase and nitrogenase (Taiz & Zeiger 1998a). Improvement of nitrogen uptake by soybean plants in response to sodium molybdate applications is resulted from enriching root cells by nitrogen (*Table 4*) and improvement of nitrogen metabolism and fixation in nodes (*Table 3*).

CONCLUSIONS

In conclusion, salt stress reduced plant growth, nodulation, nitrogen metabolism of nodes, nitrogen uptake and nitrogen translocation of soybean. Foliar spray of sodium molybdate in both of the doses improved nodulation processes by increasing special flavonoids includes daidzein, genistein, coumestrol and glycitein in soybean roots. Also, sodium molybdate treatments increased nitrogenase, GDH, GS, GOGAT and NR activities of nodes. Sodium molybdate enhanced the plant growth, nitrogen content in nodes, roots and leaves by increasing nitrogen uptake and nitrogen translocation. In general, results showed that application of sodium molybdate under salt stress has a great potential in alleviation of salt toxicity by improving nodulation and nitrogen metabolism in soybean.

Acknowledgements. We kindly appreciate the Islamic Azad University of Roudehen (Tehran, Iran) because of providing greenhouse and laboratory.

REFERENCES

Abd-Alla, M.H., Vuong, T.D., & Harper, J.E. (1998). Genotypic differences in dinitrogen fixation response to NaCl stress in intact and grafted soybean. *Crop Sci*., 38(1): 72-77.

Ahanger, M.A., & Agarwal, R.M. (2017). Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. *Plant Physiol.Biochem*., 115: 449-460. doi: 10.1016/j.plaphy.2017.04.017. .

Antunes, P.M., de Varennes, A., Rajcan, I. & Goss, M.J. (2006). Accumulation of specific flavonoids in soybean (*Glycine max* (L.) Merr.) as a function of the early tripartite symbiosis with arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* (Kirchner) Jordan. *Soil Biol.Biochem*., 38: 1234-1242.

Araújo, S.S., Beebe, S., Crespi, M., Delbreil, B., González, E.M., Gruber, V., Lejeune-Henaut, I., Link, W., Monteros, M.J., Prats, E. & Rao, I. (2015). Abiotic stress responses in legumes: strategies used to cope with environmental challenges. *Crit.Rev.Plant Sci*., 34(1-3): 237-280.

Bai, J., Jia, J., Huang, C., Wang, Q., Wang, W., Zhang, G., Cui, B. & Liu, X. (2017). Selective uptake of nitrogen by *Suaeda salsa* under drought and salt stresses and nitrogen fertilization using N^{15}. *Ecol.Eng*., 102: 542-545.

Becker, T.W., Carrayol, E. & Hirel, B. (2000). Glutamine synthetase and glutamate dehydrogenase isoforms in maize leaves: localization, relative proportion and their role in ammonium assimilation or nitrogen transport. *Planta*., 211(6): 800-806.

Chakrabarti, N. & Mukherji, S. (2003). Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. *Biol. Plantarum*, 46(1): 63-66, doi:10.1023/A:1022358016487

Chen, Y., Li, Y., Sun, P., Chen, G. & Xin, J. (2017). Interactive effects of salt and alkali stresses on growth, physiological responses and nutrient (N, P) removal performance of *Ruppia maritima*. *Ecol.Eng*., 104: 177-183, doi 10.1016/j.ecoleng.2017.04.029

Choi, E.Y., Kolesik, P., McNeill, A., Collins, H., Zhang, Q., Huynh, B.L., Graham, R. & Stangoulis, J. (2007). The mechanism of boron tolerance for maintenance of root growth in barley (*Hordeum vulgare* L.). *Plant Cell.Environ*., 30(8): 984-993. DOI:10.1111/ j.1365-3040. 2007.01693.x

Comba, M.E., Benavides, M.P. & Tomaro, M.L. (1998). Effect of salt stress on antioxidant defense system in soybean root nodules. *Funct. Plant Biol*., 25: 665-671.

Coronado, C., Zuanazzi, J.S., Sallaud, C., Quirion, J.C., Esnault, R., Husson, H.P., Kondorosi, A. & Ratet, P. (1995). Alfalfa root flavonoid production is nitrogen regulated. *Plant Physiol*., 108(2): 533-542.

Duke, S.H. & Ham, G.E. (1976). The effect of nitrogen addition on N_2-fixation and on glutamate dehydrogenase and glutamate synthase activities in nodules and roots of soybeans inoculated with various strains of *Rhizobium japonicum*. *Plant Cell.Physiol*., 17: 1037-1044.

Fageria, N.K. (1992). Maximizing crop yields. CRC Press.

Fageria, N.K. & Baligar, V.C. (2005). Enhancing nitrogen use efficiency in crop plants. *Adv.Agron*., 88: 97-185.

Farhangi-Abriz, S. & Torabian, S. (2017). Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicol. Environ. Saf*., 137: 64-70, doi: 10.1016/j.ecoenv.2016.11.029

Fernández-Pascual, M., de Lorenzo, C., de Felipe, M.R., Rajalakshmi, S., Gordon, A.J., Thomas, B.J. & Minchin, F.R. (1996). Possible reasons for relative salt stress tolerance in nodules of white lupin cv. Multilupa. *J.Exp.Bot*., 47(11): 1709-1716, https://doi.org/ 10.1093/ jxb/47.11.1709

Flowers, T.J., Galal, H.K. & Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Funct.Plant Biol*., 37(7): 604-612, https://doi.org/10.1071/FP09269

Fougère, F., Le Rudulier, D. & Streeter, J.G. (1991). Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). *Plant Physiol*., 96: 1228-1236.

Franke, A.A., Custer, L.J., Cerna, C.M. & Narala, K. (1995). Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Exp.Biol.Med*., 208(1): 18-26.

Ghoulam, C., Foursy, A. & Fares, K. (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ.Exp.Bot*., 47: 39-50.

Grieve, C.M., Grattan, S.R. & Maas, E.V. (2012). Plant salt tolerance. Agricultural salinity assessment and management. 2nd Edition, *ASCE Manual and Reports on Engineering Practice*., 71: 405-459.

Huber, D.M. & Thompson, I.A. (2007). Nitrogen and plant disease. Mineral Nutrition and Plant Disease. In: Datnoff, L.E., Elmer, W.H., Huber, D.M. (Eds.) *The American Phytopathological Society Press*, St. Paul M.N, 31-44.

Jaworski, E.G. (1971). Nitrate reductase assay in intact plant tissues. *Biochem.Biophys.Res.Commun*., 43(6): 1274-1279.

Kusano, M., Fukushima, A., Redestig, H. & Saito, K. (2011). Metabolomic approaches toward understanding nitrogen metabolism in plants. *J.Exp.Bot*., 62(4): 1439-1453 https://doi.org/10.1093/jxb/erq417

López-Gómez, M., Hidalgo-Castellanos, J., Lluch, C. & Herrera-Cervera, J.A. (2016). 24-Epibrassinolide ameliorates salt stress effects in the symbiosis *Medicago truncatula-Sinorhizobium meliloti* and regulates the nodulation in cross-talk with polyamines. *Plant Physiol. Biochem*., 108: 212-221, doi: 10.1016/ j.plaphy.2016.07.017

Marschner, H. (1995). Mineral nutrition of higher plants (2nd Edition): Functions of mineral nutrients: Macronutrients, My Science Work, pp. 229-312 https://doi.org/10.1016/B978-012473542-2/50010-9

Mengel, K. (2001). Alternative or complementary role of foliar supply in mineral nutrition. In: *International Symposium on Foliar Nutrition of Perennial Fruit Plants*, 594: 33-47.

Munns, R. & James, R.A. (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil*., 253(1): 201-218, doi: 10.1023/ A: 1024553303144

Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu.Rev.Plant Biol.*, 59: 651-681. doi: 10.1146/ annurev. arplant. 59.032607.092911

Pedersen, P., Kumudini, S., Board, J. & Conley, S. (2014). Soybean growth and development, Ames, IA: Iowa State University Extension, United States.

Sawhney, V. & Singh, A.R. (1985). Effect of applied nitrate on enzymes of ammonia assimilation in nodules of *Cicer arietinum* L. *Plant soil*., 86(2): 241-248, doi:10.1007/BF02182899

Serraj, R., Roy, G. & Drevon, J.J. (1994). Salt stress induces a decrease in the oxygen uptake of soybean nodules and in their permeability to oxygen

diffusion. *Physiol.Plant*., 91: 161-168. DOI: 10.1111/j.1399-3054. 1994.tb00414.x

Sinclair, T.R. & Shiraiwa, T. (1993). Soybean radiation-use efficiency as influenced by nonuniform specific leaf nitrogen distribution and diffuse radiation. *Crop Sci*., 33: 808-812.

Srivastava. H.S. (1980). Regulation of nitrate reductase activity in higher plants. *Phytochemistry*, 19: 725-733.

Stiefel, E.I. (1993). Molybdenum enzymes, cofactors, and chemistry. An Introductory survey. Chapter 1, pp. 1-19 *ACS Symp. Ser.*, Vol. 535, DOI: 10.1021/bk-1993-0535.ch001

Taiz, L. & Zeiger, E. (1998a). Mineral nutrition. In: *Plant Physiol*. (2nd Edition). Sinauer Assoc., Inc., Publishers, Sunderland, MA., pp. 103-124.

Turner, G.L. & Gibson, A.H. (1980). Measurement of nitrogen fixation by indirect means. In: *Methods for Evaluating Biological Nitrogen Fixation* (Ed. Bergersen, F.J). John Wiley and Sons, New York, pp. 111-138.

Wang, Y.F., Jiang, D., Yu, Z.W. & Cao, W.X. (2003b). Effects of nitrogen rates on grain yield and protein content of wheat and its physiological basis. *Sci.Agric.Sin*., 36(5): 513-520 (in Chinese with English abstract).