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Effect of molybdenum and nitrogen on *Phaseolus vulgaris* L., *Cicer arietinum* L. and *Lens culinaris* M. seedlings grown under salt stress

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Abstract

The leguminous constitute an important food diet component especially in developing countries, they represent 90% of the global consumption, and they are very rich in proteins and relatively adapted to the Mediterranean climate. Soil nutrient deficiencies and salinity are the main factors which reduce plant production in the semi-arid and arid areas around the world. The plant material used is composed by three species from *Fabaceae*; *Phaseolus vulgaris* L., *Cicer arietinum* L. and *Lens culinaris* M. seedlings that were grown in pots under different concentrations of NaCl (3 g/l, 6 g/l, 9 g/l), molybdenum (0,2 ppm) added as ammonium molybdate and nitrogen added as potassium nitrate (0,02 g/l). The fresh shoot, chlorophyll content and nitrate reductase activity were analyzed in order to estimate the effect of molybdenum (Mo) and nitrogen (N) on salt stressed plants. The effect of both molybdenum and nitrogen on lentil has not been well studied and especially for Algerian legumes. This work shows the importance of molybdenum and nitrogen added to irrigation water to avoid the negative effect of sodium chloride and to enhance legume species tolerance to salt stress.

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Introduction

The availability of good quality of water is one of the major limiting factors for plant growth as irrigation water may often contain salts and ions that can have negative impacts on the plant growth and development. Salt water in the root zone induces osmotic changes and directly affect nutrient uptake as Na^+ reducing K^+ uptake or by Cl^- reducing NO_3^- uptake (Cornillon and Palloix 1997, Halperin *et al.*, 2003).

Soil salinity, resulting from natural processes or from crop irrigation with saline water, occurs in many arid to semi-arid regions of the world. (Lauchli and Epstein, 1990). In the Maghreb more than 30 % of irrigation waters are loaded in salt, and lead over time to an accumulation of toxins both in the rhizosphere and in the different parts of the plant. These toxins generate damage to cellular ultrastructures by contributing to the reduction of growth and yields of sensitive varieties. (Rahmoune *et al.*, 2008). In Algeria, 4/5 of the surface is desert and the 1/5 left is an arid and semi-arid region (Abdelguerfi and Ramdane, 2003).

The leguminous constitute an important food diet component especially in developing countries, they represent 90% of the global consumption (Hassan, 2006), they are very rich in proteins and complete often cereals in nutritional quality and they are relatively adapted to the Mediterranean climate. (Abdelguerfi and Ramdane, 2003).

Chickpea seeds are rich in proteins and minerals (Jain, 1977), common bean is a good source of energy, with high protein content, dietary fiber, complex carbohydrates and also provides folic acid (Edje *et al.*, 1980) and lentil is a major grain and widely distributed legume crop grown under a broad range of climates in many developing countries (Turk *et al.*, 2004)

The assimilatory nitrate reduction pathway is a vital biological process, as it is one of the principal routes by which inorganic nitrogen is incorporated into

organic compounds in higher plants, algae, and fungi. After uptake into cells by nitrate transporters, the first and rate-limiting step in this pathway is the reduction of nitrate to nitrite catalyzed by NAD(P)H:nitrate reductase (NR), a molybdenum cofactor (Moco) enzyme (Eckardt, 2005). Unavailability of Mo is lethal for the organism. But even if Mo is available for the cell, it seems to be biologically inactive until it becomes complexed to form Moco thus gaining biological activity. (Mendel and Bittner, 2006).

Mo and N may secondarily influence the growth and yield in legumes (Munns 1970; Bell *et al.*, 1989), Therefore, the molybdenum deficiency may show overall reductions in plant growth, poor pod and/or grain development as well as exposing the plant to pest damage (Graham and Stangoulis, 2005).

In literature there is a lack in studies about effect of both molybdenum and nitrogen on lentil and especially Algerian legumes submitted to salt stress, so we oriented our research in this way in order to evaluate the variations that occur on yield and on nitrate reductase activity in three leguminous species *Phaseolus vulgaris* L., *Cicer arietinum* L., and *Lens culinaris* M. the most cultivated food legumes species in Algeria (Abdelguerfi and Ramdane, 2003), in response to salt stress and with adding both molybdenum and nitrogen in water irrigation.

Materials and methods

Preparation of plant material

The plant material used is composed by three species from *Fabaceae* ; *Phaseolus vulgaris* L., *Cicer arietinum* L., and *Lens culinaris* M. seedlings, they were grown in pots under different concentrations of NaCl (S0:0 g/l, S1:3 g/l, S2:6 g/l, S3:9 g/l), and with adding molybdenum (M+: 0,2 ppm, M-: 0 ppm) as Ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ and nitrogen as nitrate potassium (KNO_3) (N+: 0,02 g/l, N-: 0 g/l).

The treatments of sodium chloride (NaCl), molybdenum (Mo) and nitrogen (N) began after 3 weeks of sowing the seeds and when they are all in the same development stage (the first leaves) and

lasted 5 weeks and then the seedlings are harvested.

Fresh shoot biomass

At the end of the 5 weeks treatment the fresh plants are harvested and then the areal parts biomass was measured with a balance

Chlorophyll content

The measurement of chlorophyll content (Chl) was realized according to McKinney, (1941), and it represents chlorophyll a plus chlorophyll b, the unit is ($\mu\text{g/g FM}$): microgram by gram of fresh material.

Nitrate reductase activity

The assay of estimation of in vivo nitrate reductase activity (NRA) on leaves were conducted according to a modified method of Ferrari and Varner (1981) and of Jaworski (1971) in Naman *et al.*, (1997), the unit is

$\mu\text{mol/h/g}$: micromole per hour by gram of fresh material.

Statistical analysis

The data obtained were assessed by one way anova, tables (1), (2) and (3) were obtained using the LSD of Fischer at the confidence level of 95%, using minitab system (se: standard error).

Results and discussion

Several biochemical processes are affected by salinity, particularly nitrate assimilation. Nitrate is the most significant source of nitrogen for crop plants, and frequently limits plant growth (Meloni *et al.*, 2004), the important molybdoenzymes, nitrate reductase (NR, EC 1.6.6.1) is catalyzing the initial step of the assimilatory nitrate reduction; (Koshiba *et al.*, 1996 in Zimmer and Mendel, 1999).

Table 1. Fresh shoot biomass (FSB) of *Phaseolus vulgaris* (P.v), *Cicer arietinum* (C.a) and *Lens culinaris* (L.c) (Grp: statistical groups).

FSB (g)	P.v	se	Grp	C.a	se	Grp	L.c	se	Grp
N-M-S0	1.365	± 0.02	gh	0.79	± 0.05	de	0.055	± 0.04	j
N-M-S3	1.26	± 0.03	gh	1.58	± 0.15	a	0.25	± 0.07	f
N-M-S6	3.05	± 0.01	bc	0.98	± 0.07	bcde	0.27	± 0.06	ef
N-M-S9	2.135	± 0.19	ef	1.277	± 0.22	abcd	0.36	± 0.04	d
N-M+S0	1.31	± 0.31	gh	1.435	± 0.08	ab	0.085	± 0.01	hi
N-M+S3	2.73	± 0.51	cde	1.177	± 0.24	abcd	0.78	± 0.08	a
N-M+S6	3.64	± 0.16	ab	1.413	± 0.02	abc	0.43	± 0.04	c
N-M+S9	2.91	± 0.23	bcd	1.157	± 0.07	abcd	0.28	± 0.04	e
N+M-S0	1.71	± 0.34	fg	1.135	± 0.035	abcd	0.09	± 0.01	hi
N+M-S3	0.817	± 0.06	h	0.817	± 0.03	cde	0.11	± 0.02	h
N+M-S6	4.17	± 0.32	a	1.31	± 0.04	abcd	0.38	± 0.02	d
N+M-S9	2.34	± 0.21	cdef	1.2	± 0.07	abcd	0.25	± 0.03	f
N+M+S0	2.3	± 0.45	def	0.49	± 0.88	e	0.065	± 0.02	ij
N+M+S3	4.115	± 0.45	a	1.31	± 0.07	abvd	0.16	± 0.01	g
N+M+S6	2.725	± 0.75	cde	1.13	± 0.12	abcd	0.56	± 0.06	b
N+M+S9	2.93	± 0.96	bed	0.74	± 0.07	de	0.1	± 0.02	h

Out of several nutrients provided to plants, nitrogen is a major and essential nutrient for better plant growth and yield. It is considered as most important nutrient for the crop to activate the metabolic activity and transformation of energy chlorophyll and protein synthesis and can constitute 40-50 of protoplasm of plant cell on dry weight basis and can be a limiting

factor under such condition (De, 1993, in Kaneez *et al.*, 2013)

Fresh shoot biomass

The important fresh biomass value for lentil is 0.78 g correspond to N-M+S3 treated plants; with molybdenum adding, which is more than 10 times

higher than the control plants that represent the lowest value.

Statistical results anova for chickpea showed that the difference between the mean values is not too much significant so we cannot define which element has the most important effect on fresh biomass seedlings (Table 1), it seems like neither molybdenum nor nitrogen have any specific effect on the fresh biomass for this species.

For common bean plants, results show a significant difference between the fresh shoot biomass values, the higher ones are 4.170 g and 4.115 g, which are 3times higher than control plants (N-M-So), these seedlings were submitted to these treatments N+M-S6 and N+M+S3 respectively, and which represent the same statistical group (a), these plants were grown on a medium containing nitrogen, 3 g/l and 6 g/l of NaCl.

Table 2. Chlorophyll content (Chl) of *Phaseolus vulgaris* (P.v), *Cicer arietinum* (C.a) and *Lens culinaris* (L.c).

Chl (µg/gFM)	P.v	se	Grp	C.a	se	Grp	L.c	se	Grp
N-M-So	1.106	± 0.021	f	0.351	± 0.004	m	0.119	± 0.003	l
N-M-S3	1.158	± 0.004	e	3.583	± 0.002	c	0.701	± 0.008	g
N-M-S6	1.095	± 0.001	f	3.457	± 0.069	d	0.721	± 0.001	fg
N-M-S9	0.549	± 0.001	l	2.67	± 0.001	f	0.742	± 0.002	ef
N-M+So	1.679	± 0.003	c	1.729	± 0.002	k	0.768	± 0.002	e
N-M+S3	1.073	± 0.002	g	4.08	± 0.004	a	1.844	± 0.003	a
N-M+S6	0.516	± 0.003	m	2.628	± 0.027	g	0.862	± 0.064	d
N-M+S9	0.964	± 0.001	i	1.502	± 0.001	l	0.697	± 0.004	g
N+M-So	1.726	± 0.006	b	3.082	± 0.048	e	1.201	± 0.003	b
N+M-S3	3.857	± 0.006	a	3.857	± 0.006	b	0.468	± 0.005	h
N+M-S6	0.987	± 0.013	h	2.505	± 0.001	h	1.011	± 0.005	c
N+M-S9	0.959	± 0.011	i	2.024	± 0.005	j	0.407	± 0.001	i
N+M+So	1.352	± 0.002	d	0.169	± 0.013	n	0.303	± 0.002	j
N+M+S3	0.969	± 0.004	i	4.07	± 0.004	a	0.413	± 0.001	i
N+M+S6	0.834	± 0.013	j	2.472	± 0.007	h	1.182	± 0.003	b
N+M+S9	0.566	± 0.003	k	2.432	± 0.001	i	0.18	± 0.002	k

Since these three plants have a higher biomass value in presence of sodium chloride salt in irrigation water, it lead us to say that these legumes are not so much sensitive to salt stress and they behave differently for bean it was the combination between molybdenum and nitrogen which gives the important shoot biomass, but for lentil adding only molybdenum enhance shoot biomass.

Chickpea which is known to be sensitive to salt stress (Tejera *et al.*, 2006) in this work chickpea seedlings behave as tolerant when grown under 3g/l of NaCl.

For bean plant biomass, adding nitrogen alone has no significant effect, but it seems that the combination of nitrogen and molybdenum has more positive effect and may adjust a damage that could caused by an

irrigation water moderately saline, abiotic stresses are responsible for yield losses estimated at 50% for most crops (Vincent, 2006). These stresses result in changes in morphological, physiological, biochemical and molecular level that affects negatively plant growth and productivity (Ben Naceur *et al.*, 1998), but the application of N fertilizers to growing plants under soil salinity conditions may increase their tolerance (Cordovilla *et al.*, 1994; Van Hoorn *et al.*, 2000).

Sidari *et al.*, (2007) reported that four lentil (*Lens culinaris* M.) genotypes were treated with salt stress (0, 50, 100, 150 or 200 Mm NaCl), increasing NaCl concentration had reduced the germination percentage, the growth parameters and the relative water content. Since grain legumes especially lentil

are salt sensitive, farmers do not consider growing them in a saline environment, though; there is a considerable difference in salt tolerance among crops/accessions (Ahmed, 2009.), but for our lentil we observed that lentil could keep a higher level of shoot biomass at 3 g/l of sodium chloride but with molybdenum

Chlorophyll content

We observe that sodium chloride and molybdenum enhance chlorophyll content for the three species, for bean plants, the important chlorophyll content value was 3,857 µg/g FM (Table 2) registered for seedlings submitted to these treatment N+M-S3 (Table 2) which is 3 times higher than control plants (1.106

µg/g FM), we note that these seedlings were grown on a soil moderately saline and with nitrogen, for chickpea plants, the superior value is 4.070 µg/g FM and 4.080 µg/g FM (10 times higher than control plants with only 0.351 µg/g FM) observed in plants grown on soil containing 3g/l and with the presence of molybdenum. Lentil plants recorded a higher value of chlorophyll content with 1.844 µg/g FM (which is much more than 10 times the control plants), where there is a moderate salt stress (3 g/l) but when molybdenum is present in water irrigation also. We note that for lentil and chickpea adding molybdenum alone enhance considerably chlorophyll content, but for bean it was the nitrogen which gives the important one.

Table 3. Nitrate reductase activity (NRA) of *Phaseolus vulgaris* (P.v), *Cicer arietinum* (C.a) and *Lens culinaris* (L.c)s.

NRA(µmol/h/g)	P.v	se	Grp	C.a	se	Grp	L.c	se	Grp
N-M-So	33.254	± 0.168	m	43.365	± 0.054	i	49.74	± 0.037	a
N-M-S3	46.07	± 0.153	f	43.946	± 0.169	h	37.79	± 0.053	h
N-M-S6	48.102	± 0.032	d	47.132	± 0.037	e	43.82	± 0.077	g
N-M-S9	49.801	± 0.025	a	47.953	± 0.032	c	47.03	± 0.496	c
N-M+So	40.851	± 0.59	k	40.554	± 0.466	j	45.879	± 0.22	d
N-M+S3	43.301	± 0.239	i	46.119	± 0.086	g	44.37	± 0.086	f
N-M+S6	48.414	± 0.032	c	49.57	± 0.042	a	45.45	± 0.075	de
N-M+S9	47.769	± 0.037	e	47.613	± 0.032	d	45.88	± 0.077	d
N+M-So	39.81	± 0.193	l	40.101	± 0.093	k	47.953	± 0.798	b
N+M-S3	43.712	± 0.085	h	43.712	± 0.085	h	43.84	± 0.074	g
N+M-S6	47.628	± 0.054	e	47.599	± 0.077	d	43.46	± 0.085	g
N+M-S9	47.514	± 0.077	e	48.357	± 0.032	b	44.64	± 0.013	f
N+M+So	41.446	± 0.075	j	32.772	± 0.295	l	44.363	± 0.687	f
N+M+S3	44.576	± 0.068	g	46.119	± 0.086	g	45.39	± 0.065	e
N+M+S6	48.881	± 0.08	b	46.813	± 0.112	f	44.77	± 0.064	f
N+M+S9	48.449	± 0.074	c	48.201	± 0.044	bc	43.8	± 0.032	g

Salinity is one of the most important environmental factors limiting production and biological N₂ fixation of chickpea which is considered to be sensitive to salt stress, in arid and semiarid regions. Several studies were carried out on chickpeas (Zurayk *et al.*, 1998, Soussi *et al.*, 1998, Welfare *et al.*, 2002, Singla and Garg 2005, Garg and Singla, 2009), but in this work chickpea seems to tolerate moderate salt stress (3g/l)

in presence of nitrogen and molybdenum, and another work realized on chlorosis, it showed that is due to incapacity of plants to form chlorophyll because of molybdenum deficiency (Gupta, 1997 in Hristoskova *et al.*, 2006), so molybdenum has a role in biosynthesis of chlorophyll, because under molybdenum deficiency, intermediates of chlorophyll biosynthesis decrease in winter wheat cultivar (Yu *et*

al., 2006), so adding molybdenum and nitrogen in a saline soil can avoid the negative effect of salt stress on chlorophyll biosynthesis for chickpea, bean and lentil plants.

Nitrate reductase activity

Table 3 shows that NR activity increases as NaCl concentrations in water irrigation increase for both chickpea and bean plants with 49.80 $\mu\text{mol/h/g}$ (N-M-S9) and 49.57 $\mu\text{mol/h/g}$ (N-M+S6) respectively, for lentil, the highest value of NRA is recorded for the control plants 49.74 $\mu\text{mol/h/g}$.

There are currently contradictory reports regarding salinity effects on NR activity and nitrate assimilation. Several study have shown that salinity decreases NR activity (Aslam *et al.*, 1984; Martinez & Cerda, 1989; Gouia *et al.*, 1994 in Silveira *et al.*, (1999)), whereas others have shown opposite results (Misra & Dwivedi, 1990; Sargi *et al.*, 1997 in Silveira *et al.*, (1999)) or have not observed significant effects on NR activity (Bourgeais- Chaillou *et al.*, 1992; Cramer & Lips, 1995 in Silveira *et al.*, (1999)). Most likely, the salinity effects depend on many other factors such as plant species, NO_3^- level, stress intensity, plant age etc. (Silveira *et al.*, 1999).

For our bean and chickpea plant, it seems that the expression of nitrate reductase activity in shoot is higher as the salt stress is the most pronounced which correspond to a work done on cumin, drought stress increased nitrate reductase activity in cumin root and shoot (Sepehr *et al.*, 2012), but for lentil plants it is completely opposite, the NRA level decrease when salt stress increases, several studies have reported that nitrate uptake and nitrate reductase activity (NRA) decrease in plants under salt stress (Rao and Gnaniham 1990, Gouia *et al.*, 1994, in Meloni *et al.*, 2004). Drought tolerance of *S. aculeata* was found to be associated with a smaller reduction in number and mass of root nodules, a high activity of nitrate reductase in leaves and nodules, high accumulation of free proline in roots and nodules, and high glycine betaine content in nodules. (Ashraf and Iram, 2005). Molybdenum (Mo), a constituent of nitrogenase (NA)

and nitrate reductase (NR), is required for the assimilation of soil nitrates. (Bishop and Premakumar, 1992).

Mo application on a leguminous (*Chamaecrista rotundifolia* cv. 'Wynn') grown on red soil, could increase the number of chloroplasts and activities of nitrate reductase in leaf (Weng *et al.*, 2009), salinity induced by 100 mM NaCl in cowpea plants may have induced the decrease on leaf-NR activity (Silveira *et al.*, 1999), 600 mmol.L^{-1} NaCl caused a significant reduction in root and shoot growth, and nitrate reductase activity in leaves and roots were also decreased in *Prosopis alba*. (Meloni *et al.*, 2004), molybdenum application positively affected wheat growth and increased nitrate reductase activity (Abd El-Samad *et al.*, 2005). The supply of molybdenum to *Salicornia* grown in seawater enhances plant biomass accumulation by increasing the activities of nitrate reductase (Ventura *et al.*, 2010). The application of KNO_3 significantly increased leaf area, its fresh and dry weight per plant, and NR activity in leaves irrespective to the growth of plant under non saline or saline conditions (Jabeen and Ahmad, 2011).

These results (Table 1, Table 2, Table 3) indicate that bean, lentil and chickpea plants could be tolerant to a moderate salt stress (3g/l) but behave differently in presence of nitrogen and molybdenum, it corresponds to a previous work that showed that common bean plants are able to maintain an optimum level of morphological parameters and chlorophyll content under mild salt stress of (3g/l) in the presence of molybdenum, (Bouazid and Rahmoune, 2012).

Conclusion

As concluding remarks, the presence of molybdenum and nitrogen together caused the increase of nitrate reductase activity in bean and chickpea plants grown under salt stress. Both molybdenum and nitrogen have a positive effect on yield and nitrate reductase activity for these three plant species that behave differently considering tolerance to salt stress, they could maintain a good yield level at 3 g/l of sodium chloride.

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