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Influence of nitrate and sodium chloride on concentration and internal distribution of mineral elements in broad bean (*Vicia faba* L.) and chickpea (*Cicer arietinum* L.)

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Abstract

The objective of the research was to evaluate the effect of nitrate and salt as limiting factors for the recycling of nutrient solutions in a hydroponic system. A broad bean (*V. faba*) and two chickpea (*C. arietinum*) cultivars (desi early) and kabuli (late flowering) type), were subjected in a factorial designed experiment to three levels of nitrate, and NaCl in the nutrient solution. The treatments were administered in a recycled nutrient solution. In the hydroponics system employed recycled nutrient solution was supplied freshly every 45 minutes. Nutrient solutions were fully refreshed every 2 weeks or when the initial volume declined by about 10 % due to transpiration.

Increasing nitrate levels increased the vegetative yield of both chickpea cultivars in each salt level. Salt always decreased chickpea yields. At only 12 mM NaCl in the nutrient solution seed yield of the kabuli type was reduced by a factor of 8 while in the desi type yield was reduced only by a factor of about 2 to 3. Increasing nitrate in the solution counteracted the salt effect on both vegetative and seed production by up to a factor of 3. In contrast to chickpea the vegetative and seed yield of broad bean was less sensitive to salinity. The distribution of mineral nutrients between leaves and stems was depending on the nitrate and NaCl concentration in the nutrient: Phosphorus and manganese accumulated in the leaves relative to the stem with increasing nitrate supply but magnesium and sodium accumulated more in stems than in leaves. In contrast increasing salt in the solution decreased magnesium and calcium in the leaves relatively to the stems. Increasing nitrate or salt in the solution decreased the number of live nodules on broad bean roots. Increasing nitrate concentration in the solution decreased molybdenum and phosphorus content and uptake in both legumes. The strongest effect in the experiment was observed with nitrate on molybdenum concentration and total uptake, which was reduced due to increasing nitrate supply by a factor of five.

The proposed explanation for these results is that the increase in the rhizosphere pH due to nitrate uptake reduced the concentration of the species $H_2MoO_4^-$ (pK_1=4.2) and $H_2PO_4^-$ (pK_1=7.2) close to the root surface.

Key words: antagonism, broad bean, calcium, chickpea, hydroponics, magnesium, manganese, molybdenum, nitrate, phosphorus, potassium, salinity, sodium, synergism

Zusammenfassung

Einfluss von Nitrat und Natriumchlorid auf Konzentration und Verteilung von mineralischen Nährstoffen in Pferdebohnen (*Vicia faba* L.) und Kichererbsen (*Cicer arietinum* L.)

Ziel der Untersuchungen war es, den Effekt von Nitrat und Salz für die Wiederverwendung gebrauchter Nährlösungen zu quantifizieren. Hierzu wurden Pferdebohnen (*Vicia faba* L., Landsorte) und Kichererbsen (*Cicer arietinum* L., 2 Sorten: "Desi-Typ" (frühblühend) und "Kabuli-Typ" (spätblühend) in einem faktoriellen Experiment mit drei Nitrat- und drei Kochsalz-Konzentrationen in einer wiederverwendeten Nährlösung gezogen. In der Hydrokultur wurde die Nährlösung alle 45 Minuten umgewälzt. Alle 2 Wochen oder wenn mehr als 10 % des Ausgangsvolumens durch Transpiration verbraucht waren, wurde die Nährlösung komplett ausgetauscht.

Steigendes Nitratangebot erhöhte den vegetativen Ertrag sowohl bei Bohnen als auch bei Kicherebsen in allen Salzstufen. Salz verringerte stets den Ertrag von Kichererbsen. Schon bei nur 12 mM NaCl in der Nährlösung ging bei dem „Kabuli“-Typ der Kornertrag um das 8 fache, bei dem „Desi“-Typ um das 2-3 fache zurück. Steigende Nitratgehalte der Nährlösung reduzierten den Effekt des Salzes auf die vegetativen und Kornerträge bis auf ein Drittel. Im Gegensatz dazu waren die Erträge der Bohnen erheblich unempfindlicher gegen Salzstress.

Nitrat- und Salzangebot in der Nährlösung wirkten sich aber auch auf die Verteilung mineralischer Nährelemente in Blättern und Stängeln der Pflanzen aus: Phosphor und Mangan akkumulierten stärker in Blättern als in Stängeln, Magnesium und Natrium dagegen weniger, wenn mehr Nitrat angeboten wurde. Bei steigendem Salzgehalt in der Nährlösung jedoch war relativ mehr Magnesium und Calcium in Stängeln als in Blättern anzufinden. Steigende Nitrat- und Salzkonzentrationen in der Nährlösung verringerten aber auch die Anzahl an vitalen Knöllchen an den Wurzeln. Steigendes Nitratangebot verringerte die Phosphor- und Molybdänaufnahme beider Leguminosen. Molybdänkonzentrationen und -aufnahme gingen bei steigendem Nitratangebot um bis zu 80 % zurück. Mögliche Erklärung hierfür ist, dass infolge der Nitrataufnahme der pH in der Rhizosphäre ansteigt, was zu einer Verringerung der Konzentrationen $H_2MoO_4^-$ (pK_1=4.2) and $H_2PO_4^-$ (pK_1=7.2) an der Wurzeloberfläche geführt haben könnte.

Schlüsselwörter: Antagonismus, Bohne, Calcium, Hydrokultur, Kalium, Kichererbse, Magnesium, Mangan, Molybdän, Nitrat, Phosphor, Salz, Synergismus

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1 Introduction

The recycling of nutrient solutions in agriculture increases the nitrogen and salt concentrations in irrigation water (Xanthoulis et al., 2002). As salinity is a major limitation in legume production in many areas of the world and recycling of water and nutrient resources is a key issue for sustainable plant production this problem hits especially the worlds largest chickpea grower which is India (Singh, 1992).

Recent work in soybean has shown that salt tolerance of plants seems to be reflected in the pattern of macronutrient accumulation in the leaves (Essa, 2002). Increasing nitrate in the nutrient solution considerably moderated the salinity effects on shoot dry weight of *Vicia faba* (Cordovilla et al., 1995) and chickpea (Rao and Sharma, 1995). Both sources suggest that an exogenous supply of nitrate would improve the vegetative growth of *Vicia faba* plants under salt stress by moderating the suppressive effects of salinity. From research with tomatoes Kafkafi et al. attributed this effect already in 1982 to an antagonism between nitrate and chloride. Another effect of salinity is that increasing sodium in the root medium cause reduction of root elongation (Yermiyahu et al., 1997). This reduction seems to be partially compensated by adding calcium to the growth medium.

Ben Hayeem et al. (1984) showed that the leakage of K^+, NO_3^- and $H_2PO_4^-$ from cells was increased by salinity and was reduced by high Ca^{2+} concentration in the growth medium.

Frechilla et al., (2001) and Speer et al. (1994) found that growth of legumes decreased under moderate salt stress while nitrate-fed plants were less sensitive to salinity than ammonium-fed plants. This different sensitivity was mainly due to a better maintenance of root growth in the nitrate-fed plants (Frechilla et al., 2000). Legumes usually divert a very high proportion (up to 45 %) of photosynthesis products to the root (Pate et al., 1988). But Welfare et al. (2002) demonstrated that in the presence of 30 mM NaCl leave biomass increased predominantly on the expense of the roots. Dry weight production of shoots and roots showed positive responses to increasing NO_3^- levels, being greatest in non inoculated plants receiving 3 or 6 mM NO_3^- (Jessop et al., 1984). But also zero effects of N fertilised to legumes (chickpea) has been reported (Bonfil and Pinthus, 1995). They suggested from their results that the main intrinsic factor limiting the seed yield of chickpeas is the continuation of vegetative growth during the period of seed development, which reduces the amount of assimilates allocated to the seeds.

High concentration of mineral N (10 mM) in the growth medium, either as nitrate or ammonium or ammonium nitrate, significantly suppressed nodule number, nodule dry weight and total N_2 fixed per plant of nodulated soybeans. Consequently, lower mineral N concentrations, either 1 or 3.75 mM significantly enhanced nodule number, nodule dry weight and total N_2 fixed per plant (Gan et al., 2003). At 0.2 and 2 mM nitrate in nutrient solution of legumes, proton release to the growing medium was negatively correlated with nitrate uptake and nitrate reductase activity (NRA) in shoots, but not with NRA in roots (Fan et al., 2002). Increasing nitrate in the solution reduced the effect of salinity on nitrate reductase activity also in experiments conducted by Sekhon et al. (1987).

The experiments of Mann et al. (2002) revealed changes in ethylene evolution, H_2O_2 scavenging enzymes and membrane integrity in chickpea when chikpeas were exposed to 10, 20 and 40 mmol l^{-1} NO_3^- during vegetative growth. The ethylene production increased in nodules treated with NO_3^-, (Mann et al., 2001). Ethylene evolution due to NO_3^- was interfering with the functioning of roots along with the weakening of the antioxidant defense mechanisms (Nandwal et al., 2000).

The effect of nitrate on P uptake was studied by Nakamaru et al., (2000). Chickpea dry matter yield in the NO_3 plots without P was 75 % of that in the P-amended plots when the soil solution pH of the NO_3 plots was maintained between 7 and 8. They suggested that plant P uptake in the NO_3 plots without P was due to organic acid excretion.

The mineral content of chickpea seeds is to a great extend genetically controlled, but subject to interactions with environmental factors (Abbo et al., 2000; Ibanez et al., 1998 and citations therein). Translocation of minerals from vegetative plant parts to the developing seeds is dependent on uptake efficiency, which in turn is a complex function of physical and chemical features of the growing medium. In this context hydroponics and/or restricted root volumes are very different from common conditions of commercial chickpea cultivation.

The aims of this work were: to evaluate the effects increasing of nitrate supply and salinity levels in a hydroponic system on plant growth, grain yield and nutrient composition of leaves and stems of chickpea and broad bean, and to quantify the effect of nitrate and salt levels in the nutrient solution on the relative distribution of nutrient element accumulation in leaves and stems of chickpea and broad bean plants.

2 Materials and methods

Two chickpea (*Cicer arietinum* L.) and one broad bean *(Vicia faba* L.) cultivars were grown in hydroponic pots containing 15 L Perlite No. 4. The chickpea seeds were germinated on a tissue paper and six seeds were transferred to each hydroponic pot after roots had grown 2 cm long. Six broad beans were placed into each pot without prior germination. Each pot was placed above a 100 L plastic barrel that contained the designed treatment of nutrient solution. The experiment consisted of 5 complete randomised blocks containing 3 levels of nitrate (1, 11, 18 mmol N-NO_3) each in factorial combination with 3 levels of NaCl (0, 12, 36 mmol. NaCl). The two chickpea cultivars were a late flowering cv. Bulgarit, (kabuli type, originally from Bulgaria), with a pea shaped beige seed (270 mg), and an early flowering cultivar, ICC5810, (desi germplasm line from India) with dark seed (150 mg) (Or et al., 1999). The experiment with broad beans employed a local cultivar (Baladi) used by arab farmers in Israel. In the hydroponic system employed (described in detail by Kafkafi et al., 1971) each treatment was irrigated from a barrel reservoir by an airlift system that used air pressure to raise the nutrient solution through a plastic irrigation pipe to the surface of the pot. The excess solution drained back into the container. Irrigating every 45 minutes secured the nutrient solution composition near the roots at all times with minimum variability. The neutral Perlite medium prevented any interaction of the nutrients with the root medium. When 10 % of the solution volume was spend due to transpiration and evaporation, the nutrient solution composition was sampled and the solution replaced with a new solution of the same initial composition. This procedure maintained the designed nutrients concentrations with minimum variations throughout the growth period.

At harvest leaves and stems were separated, weighed, dried and analysed for macro and micro-nutrients after wet combustion. Sulfuric acid/H_2O_2 was used for total organic N, P and K, nitric/perchloric acid ashing for all other cations and micro-nutrients. Mineral elements, except N, were analysed by means of ICP-OES and ("Spectroflame" by Spectro, Kleve, Germany).

3 Results

Total dry matter and seed yield influenced by nitrate and salt concentrations of two chickpea and one broad bean cultivars are presented in table 1.

Increasing nitrate from 1 to 11 mM increased the dry matter and seed yield of all legume tested. Further increase to 18mM nitrate did not resulte in significant increase in dry matter or seed yield in the chickpea but significantly reduced the broad bean seed yield. Increasing salt concentration significantly reduced the seed yield of the chickpea cv. Bulgarit but not of the dry matter yield. In the cv. ICC5810, both dry matter and yield were negatively affected by high salt levels. Although the dry matter of stem and leaves started to decline only at 36 mM NaCl, the seed yield was reduced at all salt concentrations. In contrast none of the salinity effects were observed in the broad bean.

The mineral content of leaves and stems of chickpea cv. Bulgarit is presented in table 2.

Increasing nitrate in the solution significantly increased the Ca content in the leaf blades but at the same time reduced the content of Mg and K. Ca was increased and Mg was significantly decreased by each increase in nitrate level in the solution. The K content was reduced by increasing nitrate from 1 to 11 mM but a further increase to 18 mM had no more effect. Increasing nitrate in the solution significantly reduced the concentrations of Fe, Zn and Mo as well as P. In the case of Mo, 11 mM of nitrate in the solution reduced Mo in the leaf blades by about a factor of five. The same effect of nitrate on Mo was also expressed in the stem. Further increase to 18 mM nitrate

Table 1:
Dry matter and seed yield of broad beans (*Vicia faba* L.) and chickpeas (*Cicer arietinum* L.) as affected by nitrate and salt in the nutrient solution

Treatment	Chickpea				Broad bean		
	cv. Bulgarit dry matter (g per plant)	dry seeds (g per plant)	cv. ICC5810 dry matter (g per plant)	dry seeds (g per plant)	dry matter (g per plant)	fresh seeds (g per plant)	nodules dry weight (g per plant)
Nitrate (mmol L^{-1})							
1	37.4	12.6	41.3	38.5	93.2	557	0.48
11	70.6	23.7	66.4	46.1	181.3	1196	0.06
18	71.2	31.1	72.9	54.5	174.9	1068	0.08
Probability of F	**	*	***	-	***	***	***
NaCl (mmol L^{-1})							
0	69.3	38.0	67.1	69.7	152	897	0.416
18	49.0	8.1	64.5	40.9	161	1018	0.107
36	49.0	10.7	49.4	25.6	136	907	0.111
Probability of F	-	***	**	***	-	-	**

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Table 2:
Mineral element concentrations in chickpea leaves (*Cicer arietinum* L.) cv. Bulgarit as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(mg g^{-1})
1	22.5	7.6	84.4	21.4	364	124	132	57.1	5.9
11	30.1	6.2	69.9	22.8	335	92	109	12.1	3.4
18	41.0	4.7	64.5	18.3	294	77	111	8.6	2.3
Probability of F	***	***	***	-	**	**	-	***	***
NaCl (mmol L^{-1})									
0	36.2	7.9	85.1	5.5	326	114	111	24.8	3.8
12	29.2	5.4	66.5	24.8	336	89	124	24.8	3.6
36	28.2	5.2	67.2	32.2	331	90	117	28.2	4.1
Probability of F	**	***	***	***	-	-	-	-	-

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

had no effect on Mo concentration in the tissue. Only the contents of Na and Mn in the leaf blades were not significantly affected by increasing nitrate concentration in the solution. Increasing NaCl concentration in the solution increased the Na content in the leaves but decreased Ca, Mg and K. Only the first dose of relatively low salt concentration decreased Ca, Mg and K but further increase in salt concentration did not affect the concentration of these cations in the plants. There was no significant effect of increasing salt concentration on the micro nutrients nor on P content in leaves of cv. Bulgarit. In the stems (table 3), Ca increased and K decreased with the increase in nitrate concentration in the solution. However stem Mg and Na concentrations were not significantly influenced by nitrate. Nitrate had no effect on stem Fe and Zn but mainly reduced the contents of Mn, Mo and P in the stem. Increasing NaCl in solution had no effect on Ca and Mg concentration in the stem nor on any of the micronutrients content. Of the 4 major cations, only K decreased and Na increased in the stem due to increasing nutrient solution salinity level. However, K decreased only by 12 mM Na in the solution. Increasing Na to 36 mM did not have any further effect on K content while that of Na still increased.

In leaves of the chickpea cv. ICC5810, Ca increased and Mg decreased with increasing nitrate in the solution but the content of K and Na were not affected (table 4). The content of all micronutrients tested was reduced by 11 mM of nitrate. Mn and P further declined with the increase of nitrate to 18 mM. Like in the cv. Bulgarit (table 2 and 3), the decline in Mo concentration by a factor of 5 was the biggest of any effect observed (table 4). As far as macroelements are concerned the pattern of the NaCl effects on leaf nutrient concentrations was the same as in cv. Bulgarit. Different were the leaf blade content of Mn and P which were significantly increased with increasing NaCl in the solution. No significant effect occurred on Fe, Zn and Mo concentrations in the stem (table 5). The stem nutrient content differed from the pattern found in cv. Bul-

Table 3:
Mineral element concentrations in chickpea stems (*Cicer arietinum* L.) cv. Bulgarit as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(mg g^{-1})
1	5.9	3.9	72.5	29.3	82	45	28	107.3	5.5
11	7.8	4.5	60.4	30.8	113	51	17	27.6	1.9
18	14.4	4.4	50.6	28.5	89	55	16	20.2	1.1
Probability of F	***	-	***	-	-	-	***	***	***
NaCl (mmol L^{-1})									
0	9.1	4.6	71.4	14.2	103	46	17	45.1	2.9
12	9.9	4.4	58.5	30.3	89	49	23	56.1	2.7
36	9.2	3.8	53.6	44.1	92	56	21	54.0	2.9
Probability of F	-	-	***	***	-	-	-	-	-

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Table 4:
Mineral element concentrations in chickpea leaves (*Cicer arietinum* L.) cv. ICC5810 as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(mg g^{-1})
1	27.7	7.4	78.0	25.6	463	94	254	77.8	5.3
11	36.6	5.6	75.4	30.5	367	75	192	14.3	3.3
18	45.8	4.4	70.5	31.9	337	73	135	10.9	2.5
Probability of F	***	***	-	-	*	*	**	***	***
NaCl (mmol L^{-1})									
0	37.1	7.2	86.0	9.6	385	83.1	149	39.4	3.3
12	39.6	6.1	76.6	29.4	391	79.1	189	39.6	3.7
36	32.5	4.4	62.6	47.4	393	80.9	244	24.3154	4.2
Probability of F	***	***	***	***	-	-	*	-	**

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

garit in its response to nitrate. Increasing nitrate concentration in the solution increased Ca in each nitrate increment in the solution and that of Mg by only the first dose of 11 mM nitrate. There was no significant effect on K, Na and Fe content in the stem. Increasing salt had no effect on the stem Ca and Mg content but significantly increased Na and decreased K content. Stem content of Mn decreased with increasing nitrate and increased with salinity. Mo and P behaved in the same pattern as in cv. Bulgarit in response to nitrate and NaCl.

The results of the mineral concentration in leaf and stem in the broad bean are presented in table 6.

The concentration of Ca increased with nitrate concentrations in the solution. The concentrations of Mg, K and Na showed a maximum value at 11 mM nitrate in the solution, both in leaves and stems. The increased salinity significantly reduced Ca, Mg, and K while Na accumulated in leaves and stems in very high concentrations but without any dramatic effect on plant yield (table 1).

In the broad bean, similar to the effect on chickpea cultivars, high levels of nitrate in the solution significantly decreased the Mo and P content in the stem and in the leaves by about a factor of 4 to 5 (table 6). The magnitude of the effect of nitrate on these two nutrients is relatively the highest observed among all mineral nutrients. The Fe content in the leaves was neither affected by nitrate nor by salt treatments. Zn in stems was significantly reduced by nitrate but not in the leaf blades (table 6). Mn in stems was reduced by both salt and nitrate treatments but not in the leaves (table 6).

The ratio of the concentration of the nutrients in leaf blades to that in stems as a function of nitrate and salt concentration is presented for the chickpea cultivars and the bean in tables 7 - 9. The results there reveal that the ratio of P concentration in the leaves to that in the stem increased in both legumes with increaseing nitrate in the nutrient solution. Mo leaf /stem ratio decreased in both chickpea cultivars (table 7 and 8). In contrast the ratio for

Table 5:
Mineral element concentrations in chickpea stems (*Cicer arietinum* L.) cv. ICC5810 as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(mg g^{-1})
1	6.3	5.8	78.1	34.7	120	66	44	51.8	3.6
11	11.4	7.11	62.0	37.3	111	35	22	16.4	1.7
18	17.1	6.8	52.8	35.4	90	36	14	12.8	0.7
Probability of F	***	***	-	-	-	*	***	***	***
NaCl (mmol L^{-1})									
0	8.5	6.2	76.3	12.1	82	41	19	29.4	2.0
12	11.5	6.8	65.6	35.5	117	47	29	31.4	1.9
36	13.9	6.6	52.6	58.1	121	50	31	20.4	2.0
Probability of F	-	-	*	***	*	-	*	-	-

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Table 6:
Mineral element concentrations in broadbean leaves and stems (*Vicia faba* L.) as affected by nitrate and salt in the nutrient solution

Leaves	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(µg g^{-1})	(mg g^{-1})
1	26.7	6.2	44.1	36.6	1049.8	65.4	140.8	15.4	10.3
11	31.8	7.5	62.2	61.2	1088.7	48.7	132.0	4.4	2.9
18	38.2	6.9	55.6	41.5	1049.7	57.3	145.6	5.6	2.3
Probability of F	***	-	*	***	-	-	-	***	***
NaCl (mmol L^{-1})									
0	36.0	8.6	78.0	14.7	1049.3	46.4	152.5	8.3	5.9
12	32.1	6.3	53.9	47.3	1072.1	62.4	139.9	7.8	5.5
36	28.6	5.7	30.0	77.3	1066.9	62.6	126.0	9.4	4.1
Probability of F	***	***	***	***	-	-	-	-	-
Stems									
NO_3 (mmol L^{-1})									
1	7.3	1.9	26.0	31.2	153.9	45.7	54.1	37.7	9.5
11	8.1	2.7	38.5	51.5	183.7	35.641.0	9.5	1.8	
18	9.0	2.2	31.4	37.0	142.5	32.433.1	10.1	0.8	
Probability of F	*	*	***	**	-	*	***	***	***
NaCl (mmol L^{-1})									
0	8.9	2.6	48.1	15.4	139.8	38.9	36.1	19.0	3.9
12	7.7	2.2	31.4	42.2	174.6	38.5	41.3	17.8	3.7
36	7.7	2.0	16.4	62.0	165.7	36.2	50.8	20.5	4.5
Probability of F	*	*	***	***	-	-	***	-	-

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Mn increased with increasing nitrate supply to the chickpeas. In the broad beans (table 9) only the ratios of P and Mn were affected by increasing nitrate in the solution.

The leave/stem concentration ratio for Ca and Mg changed in an opposite direction to that of P. Increasing nitrate reduced this ratio in the chickpea cultivars (tables 7 and 8) but had no effect on the ratio in the broad beans (table 9). Increasing salt in the nutrient solution reduced significantly the leaf/stem ratio for Ca and Mg but at the same time the ratio for Na increased. The fact that the

Table 7:
Leaf/Stem concentration ratios for mineral elements in chickpeas (*Cicer arietinum* L.) cv. Bulgarit as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
NO_3 (mmol L^{-1})									
1	3.9	2.0	1.2	0.8	4.7	3.5	4.9	2.1	1.1
11	4.1	1.4	1.2	0.6	3.8	1.9	6.4	0.8	1.9
18	3.0	1.1	1.4	0.6	3.6	1.6	7.2	0.6	2.6
Probability of F	0.011	***	0.255	0.100	0.060	*	0.023	<.0001	0.001
NaCl (mmol L^{-1})									
0	4.5	1.9	1.2	0.5	4.3	2.6	6.7	1.2	1.9
12	3.2	1.3	1.2	0.9	4.0	2.2	6.0	1.0	1.8
36	3.2	1.4	1.3	0.7	3.8	2.2	5.8	1.3	1.9
Probability of F	0.001	0.008	0.349	0.002	0.593	0.801	0.542	0.599	0.944

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Table 8:
Leaf/Stem concentration ratios for mineral elements in chickpeas (*Cicer arietinum* L.) cv. ICC5810 as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})									
1	4.6	1.3	1.0	0.9	4.1	1.8	6.8	1.5	1.6
11	3.6	0.8	1.3	0.8	4.0	2.7	11.2	1.1	2.8
18	2.7	0.6	1.3	0.9	3.9	3.7	9.9	0.8	3.6
Probability of F	0.021	<.0001	0.081	0.607	0.908	0.4	0.039	0.1	0.002
NaCl (mmol L^{-1})									
0	4.8	1.3	1.2	0.9	5.1	2.2	10.2	1.2	2.6
12	3.9	0.9	1.3	0.9	3.7	3.5	8.6	1.1	2.3
36	2.6	0.7	1.2	0.8	3.5	2.1	9.3	1.1	2.8
Probability of F	0.002	0.004	0.976	0.929	0.011	0.458	0.413	0.845	0.357

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

Table 9:
Leaf/Stem concentration ratios for mineral elements in broad beans (*Vicia faba* L.) as affected by nitrate and salt in the nutrient solution

Treatments	Ca	Mg	K	Na	Fe	Zn	Mn	Mo	P
Nitrate (mmol L^{-1})									
1	3.7	3.2	1.6	1.1	8.1	1.7	2.7	0.5	1.2
11	3.9	2.7	1.7	1.2	7.0	1.6	3.5	0.5	1.9
18	4.4	3.0	1.7	1.1	8.5	1.9	4.2	0.5	2.9
Probability of F	0.067	0.387	0.154	0.779	0.534	0.783	0.000	0.955	<.0001
NaCl (mmol L^{-1})									
0	4.0	3.0	1.6	1.0	8.1	1.6	4.3	0.4	2.5
12	4.2	3.0	1.7	1.1	7.0	1.9	3.4	0.6	2.1
36	3.8	2.9	1.7	1.3	8.5	1.7	2.6	0.5	1.3
Probability of F	0.304	0.717	0.181	0.006	0.850	0.714	<.0001	0.241	0.003

Probability of F: - > 0.05; * = < 0.05; ** = < 0.001; *** < 0.001

leaf/stem ratio for Na in both chickpea cultivars is less than one suggests that the stem content of Na is always higher than that in the leaf (tables 7 and 8). However in the case of broad bean, (table 9) Na in the leaf blades was higher than in the stem. The relative distribution of all the micronutrients and that of P between leaves and stem due to increased salinity in the solution remained constant in the chickpea cultivars. The exception was Fe: here the leaf/stem concentration ratio declined in the stem of ICC5810 but not in those of cv. Bulgarit and also not in the beans. In the beans (table 9) increasing salinity decreased the leaf/stem ratio only of Mn and P.

4 Discussion

The key result of this work was the large reduction in absolute uptake of Mo caused by an increased nitrate supply in both legumes tested. Mo is a key element in nitrate metabolism in plants due to its role as a coenzyme in the nitrogenase and nitrate reductase (Marschner, 1995). Mo has a special meaning for the growth of chickpeas, as Bhuiyan et al (1998) demonstrated that the inocculation of rhizobium together with Mo produced significantly more nodule, increased nodule and shoot weight as well as straw and seed yield. Nitrate uptake by plants usually induces an increase of pH in the rhizosphere and the extend of this pH changes is clearly different among plant species (Marschner, 1995). According to Marschner (1995) under same conditions the root surface pH of chickpeas after feeding with nitrate is still much lower (3.5) than the one of maize (6.5). Reason for this is the internal nitrate metabolism of different plants and the excretion of specific organic acids that control the pH near the root surface (Imas et al., 1997). Kanan and Ramani (1978) assumed that plants take up Mo mostly as MoO_4^{2-}. The fact that in the experiments reported here increasing nitrate decreased theMo uptake do not support this hypothesis. The pK_1 of H_3MoO_4 is 4.2. At this pH 50 % of the molybdate in solution is in the form of $H_2MoO_4^-$. The pK_2 of phosphoric acid is 7.2 and at that pH 50 % of P in solution will be in $H_2PO_4^-$ form. While with P it is well established that uptake is mainly in the form of $H_2PO_4^-$, (Marschner 1995), very little information is available on the ionic form of the Mo uptake. Stout et al., (1951) showed a steep decline in Mo uptake by tomato when the solution pH was raised from 3 to 5.5. An increase of rhizosphere pH from 4.2 to only 5.2 is sufficient to cause the molybdate anion concentration in the solution to drop from 50 % of total mono-valent Mo to less than 10 % of total Mo in solution. The total decline in Mo content in the chickpea is also by a factor of 5 (tables 2, 3) and by a factor of 4 in the broad bean. Even if the pH is 5.5 near the roots, there is still about 50 % of the phosphorus in the form of $H_2PO_4^-$ allowing enough P to be taken up by the plant. In this context it makes sense that the effect of nitrate on Mo uptake was much stronger than on P uptake.

Relating leaf dry matter production with Mo uptake (low nitrate treatments excluded) results a linear function:

Chickpea leaf dry matter (g) =
12.69 + 0.048 Mo uptake (ug $plant^{-1}$)

According to McCants and Black (1957) such a linear relationship suggests that the Mo uptake was the limiting factor to chickpea leaf yield.

Legumes fix N_2 from the soil air and therefore can survive without external nitrogen fertiliser. However, the use of recycled water that contain both salt and amino nitrogen results in increased soil salinity and by nitrification increase nitrate concentration in the soil solution. As early as 1935 Allison suggested that high nitrate in the solution reduced the sugar content in legumes roots and as a consequence it prevented root nodule development. Hallsworth (1958), allocated the treshold between beneficial and toxic nitrate levels at 4.5 mM in the soil solution.

Ibrikci et al. (2003) reported maximum variation of about 1.4 to 1.8 fold in macronutrient concentrations among 19 diverse accessions of chickpeas. Chickpea is the most salt sensitive species among legumes, but also much more snesitive to salt than cereal crops. (Katerji et al., 2003). The authors related this fact to the continuous flowering of chickpea and the high sensitivity of crops to salinity mainly during flowering period.

If only changes in the dry matter distribution would have been affected (tables 5, 6 and 7), then a constant effect on the distribution of all nutrients in the plant would have been expected and the same trend for all nutrients would also be expected, which was not the case. The Na distribution in young blades of salt tolerant was different from that of salt sensitive cultivars (Wei et al., 2003). Chickpea cv. ICC5810 is relatively salt tolerant as compared with cv. Bulgarit. This observation is in line with the better adaptation of desi chickpea types to water limited environments compared with kabuli cultivars (Liu et al., 2003). The distribution of Na in this and in broad bean plant is not affected by the salt treatments or by the nitrate. The broad bean showed in this experiments to be much more tolerant to salt than the chickpea (table 1). The Na leaf/stem ratio in this plant is hardly affected by increased salinity up to 36 mM NaCl in the solution. When bean was grown in salt concentrations below 50 mM Na was excluded from the leaf blades. Only when exposed to NaCl concentrations of 75 mM a decline in leaf growth was correlated with Na accumulation in bean leaf (Sibole et al., 1998). It is likely that Na distribution between plant organs might be considered as a breeding tool for Na tolerance selection in legumes.

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