Nitrogen assimilation and nitrate reductase activity in tomato seedlings

II. Effect of increasing calcium doses in the presence of high magnesium concentrations on nitrate reduction and protein content

A. SUDER-MORAW AND J. BUCZEK

Department of Plant Physiology, Institute of Botany, Wrocław University, Wrocław

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ABSTRACT

A 3:30 Ca:Mg ratio in the nutrient solution produces in tomato seedlings symptoms of Ca^{2+} deficit owing to excessive accumulation of Mg^{2+} ions and the depressed Ca^{2+} accumulation. As a result of this a decrease in dry weight increment and protein content is observed together with inhibition of nitrate reductase activity. A doubled Ca^{2+} dose in the nutrient solution, that is a change in the Ca:Mg ratio to 6:30 abolishes the external symptoms of Ca^{2+} deficit and reduces Mg^{2+} accumulation, that of Ca^{2+} ions remaining unchanged. At the same time an enhanced activity of nitrate reductase appears, reaching values close to those in control plants. Tomato seedlings grown on a 3-fold increased Ca^{2+} dose (Ca:Mg = 9:30) did not differ at all from the control ones. An increase in calcium concentration in the nutrient solution, the high magnesium dose remaining unchanged, causes enhanced K^+ accumulation, and this may affect nitrate absorption and reduction. It would seem that Ca^{2+} deficit in plant tissues induced by excessive Mg^{2+} accumulation with unsuitable Ca:Mg ratio in the nutrient solution in cause of disorders in NO_3^- nitrogen assimilation.

INTRODUCTION

Assimilation of NO_3^- nitrogen by higher plants depends on a number of mineral components in the nutrient solution. Beside nitrates (Beever and Hageman, 1969) and phosphorus (Spencer, 1959), K^+ ions play a role here (Oji and Iizawa, 1969) as well as Ca^{2+} and Mg^{2+} ions. Paulsen and Harper (1968) and Harper and Paulsen (1969) found a depression of NO_3^- absorption and of RN activity in wheat seedlings with diminishing calcium content in the nutrient solution. Morgan et al.

(1972) observed a stimulating effect of Ca^{2+} as compared with that of Mg^{2+} on accumulation of NO_3^- by *Lolium perenne* roots and an inverse relation in the shoots. Suder-Moraw and Buczek (1977) in their investigations on the influence of Mg^{2+} excess in the nutrient solution on nitrogen metabolism in tomato seedlings demonstrated that an unsuitable Ca:Mg ratio in the nitrate solution produces symptoms of Ca^{2+} deficit in the tissues and inhibits NR activity. It was, therefore, suggested that the Mg^{2+} excess in the nutrient solution as compared with Ca^{2+} induces a physiological deficit of calcium in the tissues resulting in nitrate assimilation inhibition.

The aim of the present study was the investigation of increasing Ca^{2+} concentrations in a nutrient solution with high Mg^{2+} content on the growth, yield and accumulation of cations and nitrate reductase activity.

MATERIAL AND METHODS

The experiments were made with tomato (*Lycopersicum esculentum* L. cultivar Karzelek puławski) seedlings grown on nutrient solution containing nitrate as nitrogen source. The nitrate solution in some combinations was enriched with Ca^{2+} in the form of CaCl_2 . The Ca:Mg ratio in the particular nutrient media was: 3:1 (control), 3:30, 6:30 and 9:30. The composition of the nutrient solution and preparation and culture of the plants are described in detail in the preceding paper (Suder-Moraw and Buczek, 1977). After 3 weeks of growth of the plants K, Ca, and Mg content was determined as well as particular nitrogen fractions, activity was measured and the growth and dry weight increments were determined. The analytical methods applied are described in the above mentioned paper.

Nitrate reductase activity *in vitro* (Suder-Moraw and Buczek, 1977) was determined as μmoles of NO_2^- per 1 mg protein/hour. Nitrate reductase activity *in vivo* was determined according to Klepper et al. (1971). From the leaves of 2-week-old seedlings growing on nutrient solution with a 3:1 Ca:Mg ratio discs 7 mm in diameter were cut out. These disc in the amount of about 200 mg were infiltrated with 0.1 M KNO_3 with the addition of the tested salts in the concentrations given in Table 4. Then, after washing with bidistilled water they were placed in Erlenmayer flasks containing 5 ml of 0.1 M KNO_3 in 0.1 M K-phosphate buffer pH 7.5. These manipulations were performed at about $0^\circ\text{--}3^\circ\text{C}$. The flasks were placed on a water bath of 37°C in darkness. At 3-min intervals 0.2 ml of the solution was taken from each flask for determination of NO_2^- formed in the enzymatic reaction. The NO_2^- amount was determined colorimetrically by reading absorption at 540 nm (Buczek, 1976). The enzyme activity was expressed in terms of nmoles of NO_2^- per 1 g of fresh tissue weight.

RESULTS

Growth and yield of seedlings

A doubled Ca^{2+} dose in a nutrient solution with Ca:Mg ratio 3:30 partly abolished the inhibitory effect of large Mg^{2+} amounts on the growth of shoots and roots, whereas a 3-fold Ca^{2+} dose completely neutralized the differences in growth between the experimental and control seedlings (Table 1). The yield of the plants (expressed as dry weight) growing in the nutrient solution with Ca:Mg ratio 6:30 reached at this time 81 per cent of that of the control plants, while in the nutrient solution with Ca:Mg ratio 3:30 it was barely 22 per cent of the dry weight of the control plants. A 3-fold dose of Ca^{2+} in the solution suppressed completely the noxious influence of Mg^{2+} excess on dry weight increment. The yield of plants in this combination was equal to that of the controls (Table 1).

Table 1

Mean plant length and mean dry weight of 3-week-old tomato seedlings

Nutrient solution	Shoot mm	Root mm	Seedlings mm	No. of leaves	Leaves mg	Shoot mg	Root mg	Seedling mg
3:1	157	166	323	8	202	122	202	359
3:30	61	44	105	5	52	24	9	85
6:30	124	140	264	7	168	94	29	191
9:30	152	160	312	8	205	110	36	351

Mean from 10 plants

NR activity and nitrogen fractions content

The results of NR activity measurement after 3 weeks of growth of the seedlings are shown in Table 2. As seen, the activity depends on the Ca:Mg ratio in the solution. Mg^{2+} in excess with optimal Ca^{2+} content (Ca:Mg = 3:30) distinctly limited NR activity in the leaves and completely inhibited it in the roots.

Table 2

Nitrate reductase activity in leaves and roots after 3 weeks of growth of tomato seedlings

Nutrient solution	Leaves	Roots
	$\mu\text{moles NO}_2/\text{mg}/\text{hour}$	
3:1	499	26
3:30	32	0
6:30	484	21
9:30	425	23

A two- or threefold Ca^{2+} dose with the same amount of Mg^{2+} prevented depression of NR activity in the leaves and roots. Some slight differences in the enzyme activity between the combinations and the control were nonsignificant.

A high Mg^{2+} dose produced a marked decrease in NO_3^- ion content in all the organs of the seedlings (Table 3). An increase of the Ca^{2+} amount in the nutrient solution, the Mg^{2+} dose remaining the same, enhanced nitrate accumulation to a level close to that in the control seedlings.

Table 3
 NO_3 nitrogen content (in mg) per 100 g dry weight. Mean values from 5 replications

Nutrient solution Ca:Mg	Leaves	Shoots	Roots
	mg	mg	mg
3:1	0.82	3.23	1.68
3:30	0.37	1.32	0.77
6:30	0.60	2.30	1.23
9:30	0.62	2.40	1.10

Experiments *in vivo* on the influence of Ca^{2+} or Mg^{2+} ions or both introduced into the tissues by infiltration on NR activity did not give a conclusive answer. It results from the data in Table 4 that infiltration of the particular ions into the tissues, Ca^{2+} or Mg^{2+} , caused a slight depression of NR activity in the particular periods of measurement. The presence of Mg^{2+} gave a more pronounced depression of enzyme activity. Infiltration of leaf tissues simultaneously with Ca^{2+} and Mg^{2+} in a joint concen-

Table 4

NR activity (*in vivo*) in tomato leaves in dependence on Ca^{2+} and Mg^{2+} ions infiltrating tissues. Infiltrating solution contained 0.1 M KNO_3 with salts of tested ions added. Mean values from 5 replications

Infiltrating solution	Incubation time, minutes			
	30	60	90	120
	$\mu\text{moles NO}_2/\text{g fr. weight}$			
K_2SO_4 — 0.5 M	491	1517	2232	2590
MgSO_4 — 1.0 M	330	1106	1625	1800
KCL 0.1 M	602	1790	2695	3185
MgCl_2 — 0.1 M	568	1348	2100	2500
CaCl_2 — 0.1 M	824	2059	2410	2645
CaCl_2 —0.075 M + MgCl_2 —0.025 M (Ca:Mg=3:1)	1470	5715	8488	10180
CaCl_2 —0.009 M + MgCl_2 —0.09 M (Ca:Mg=3:30)	2539	5228	7647	9200

tration of 0.1 M, but in various proportions, enhanced nitrate reduction in the combination with 3:30 ratio after 30 min of incubation, later, however, the enzyme activity diminished as compared with that in the control (3:1). The results of this experiment are not univocal and do not indicate a distinct influence of both the cations or their ratio on NR activity. A certain tendency to NR activity depression is noticeable, however, under the influence of an excess of Mg^{2+} ions in experiments of short duration *in vivo*.

The results listed in Table 5 indicate that the level of the particular nitrogen fractions in the seedlings depends also on the endogenous Ca:Mg ratio. A high Mg^{2+} dose in the 3:30 combination reduced protein production in the leaves, as shown by the lowered amount of the N-i (insoluble) fraction. At the same time the content of fraction N-s (soluble) was enhanced. A doubled Ca dose (Ca:Mg = 6:30) reduced the unfavourable effect of Mg^{2+} excess on the level of the nitrogen fractions, and a 3-fold increase of the Ca dose in the solution (Ca:Mg = 9:30) completely abolished the noxious influence of excess Mg^{2+} in the solution. Analysis of cations content in the particular seedling organs showed a close relation between the Ca:Mg ratio in the nutrient medium and cation accumulation (Table 6). When the Mg^{2+} concentration in the solution exceeded 10 times the Ca^{2+} content (Ca:Mg = 3:30), Ca^{2+} accumulation diminished by one half and that of Mg^{2+} increased fivefold as compared with the accumulation of these components in the controls (Ca:Mg = 3:1). This dependence was noted in all organs and the Ca:Mg ratios in the tissues were on the average 1:6. A doubled calcium dose in the nutrient medium changed the internal relations between the tested cations in the tissues to 1:4 and 1:5 for roots and shoots, respectively. This change occurred mainly owing to the distinct decrease in Mg^{2+} accumulation with almost unchanged Ca^{2+} level in the tissues. Only a 3-fold increased calcium dose in the solution (Ca:Mg = 9:30) raised the Ca^{2+} accumulation value to a level corresponding to that in control plants and

Table 5
Nitrogen fractions level in leaves of 3-week-old tomato seedlings
(mg/100 g dry weight)
Means from 4 analyses

Nutrient solution Ca:Mg	N-s mg	N-i mg	N-t mg
3:1	0.77	5.79	6.56
3:30	1.12	3.48	4.60
6:30	0.80	5.10	5.90
9:30	0.82	5.28	6.10

Table 6

K^+ , Ca^{2+} and Mg^{2+} accumulation (in mg/100 mg dry weight) and endogenous Ca:Mg ratio after 3 weeks of growth of tomato seedlings

Means from analyses

Organs	Nutrient solution Ca:Mg	K	Ca	Mg	Endogenous Ca:Mg ratio
		mg	mg	mg	
Leaves	3:1	2.20	0.93	0.59	1:0.6
	3:30	2.80	0.54	3.43	1:6.0
	6:3	3.60	0.93	1.92	1:2.0
	9:3	3.00	0.93	1.92	1:2.0
Shoots	3:1	5.10	0.67	0.47	1:0.7
	3:30	6.80	0.26	1.83	1:7.0
	6:30	8.40	0.30	1.47	1:5.0
	9:30	8.26	0.32	1.33	1:4.0
Roots	3:1	2.00	0.80	0.43	1:0.5
	3:30	3.27	0.39	2.20	1:6.0
	6:30	3.53	0.45	2.00	1:5.0
	9:30	4.10	0.60	1.63	1:3.0

simultaneously reduced Mg^{2+} accumulation. This resulted in a change in the internal Ca : Mg ratio to about 1 : 3.

K^+ accumulation increased under the influence of increasing doses of bivalent cations. With a Ca : Mg ratio 3 : 30 in the solution, K^+ accumulation was higher by about 40 per cent than in the control. The K^+ content in the tissues increased still more in the combinations with 2- and 3-fold Ca^{2+} dose in the solution.

DISCUSSION

The above reported results show that a doubled Ca^{2+} dose in a nutrient medium with a high Mg^{2+} content efficiently protected the tomato seedlings from the effect of Mg^{2+} excess by changing the external Ca : Mg ratio from 3 : 30 to 6 : 30. The external symptoms of Ca^{2+} deficit disappeared, although accumulation of this component in the seedling tissue did not change in the 6 : 30 combination. The abolition of Ca^{2+} deficit symptoms was the result of a lower Mg^{2+} accumulation, this leading to a change in the internal Ca : Mg ratio. It would seem, therefore, that the disturbances in the studied metabolic processes caused by the unfavourable Ca : Mg ratio in the medium (3 : 30) were the result of calcium deficit in the tissues due to an excess of Mg^{2+} in the nutrient solution.

The unfavourable Ca : Mg ratio in the solution depressed in the first place NR activity after 3 weeks of seedling growth. It might be supposed

that this inhibition was the result of restricted NO_3^- uptake observed also in the previous study (Suder-Moraw and Buczek, 1977), therefore, of lack of substrate for RN induction. This, however, does not seem probable since accumulation of NO_3^- ions, although lower in the combination with ratio 3:30 as compared with that in the control, occurred at a rather high level. It would seem probable, on the other hand, that the disappearance of RN activity in tomato seedlings after 3 weeks of growth on nutrient solution with Ca:Mg ratio 3:30 was caused by a calcium deficit in the tissues. This may have been caused both by a lack of calcium in the solution and by the change in the endogenous Ca:Mg relation owing to excessive Mg^{2+} accumulation as was the case in previous experiments.

This supposition seems supported by the fact that the change in the Ca:Mg ratio from 3:30 to 6:30 was quite sufficient to maintain NR activity at the same level as in the control plants both in the leaves and roots. The present experiments demonstrated, namely, that a doubled Ca^{2+} dose did not enhance calcium accumulation in the tissues, but it reduced magnesium accumulation, therefore, the endogenous Ca:Mg ratio 1:6 changed to 1:4 in the leaves and 1:5 in the roots. This conclusion finds confirmation in the investigations of Morgan et al. (1972). The latter authors observed a diminished $^{15}\text{NO}_3^-$ assimilation in the roots of *Lolium perenne* in the presence of Mg^{2+} as compared to that in the presence of Ca^{2+} . They suggest that the cause of this lies in calcium deficit. Similarly Paulsen and Harper (1968) and Harper and Paulsen (1969) observed NR activity inhibition in several-weeks-old wheat seedlings growing on a calcium-deficient nutrient medium. The above mentioned authors report an enhancement of NR activity after increasing the Ca^{2+} concentration in the solution.

It is a known fact that the presence of Ca^{2+} in the nutrient solution affects cation (Epstein, 1977) and anion absorption (Hyde, 1966; Viets, 1944). Moreover, calcium is essential for processes of absorption and translocation of ions in plant tissues (Morgan et al., 1972). Since, according to Ritenour et al. (1967), reduction of NO_3^- to ammonia occurs in various compartments of the cell, it would seem that the role of calcium in ion absorption and translocation processes within the cells explains to some extent the NR activity inhibition in the case of Ca^{2+} deficit in the tissues, observed in the present experiments. The direct action of Ca^{2+} and Mg^{2+} ions on NR activity is but little probable, the more so since the present experiments (Table 5) do not seem to point to a direct dependence of the enzyme activity on the presence of calcium or magnesium.

The presence of K^+ ions in the solution and tissues is not without significance for NR activity. Nitsos and Evans (1966) and Oji and Izawa (1969) demonstrated that K^+ ions stimulate NO_3^- assimilation.

These authors believe that the function of K^+ may involve enhanced NR and nitrite reductase synthesis. In the present experiments a diminished K^+ accumulation was not observed under the influence of increased Mg^{2+} doses in the solution. It was found, on the other hand, that an excess of Mg^{2+} stimulated K^+ accumulation in all the examined organs of tomato seedlings. Similar results were reported by Korczewski and Majewski (1932), Walker (1954) and Scharrer and Mengel (1960). It seems, therefore, that the amount of K^+ accumulated in the case of a Ca: Mg ratio = 3:30 has no significant effect on NR activity. Higher calcium doses in the nutrient medium, however, changing the ratio from 3:30 to 6:30 or 9:30 markedly increased the potassium balance of the seedlings and this may affect NO_3 ions transport and nitrate reduction (Oji and Izawa, 1969, Ben-Zoni et al. 1971; Mengel and Simic, 1973).

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Authors' address:

*Dr Anna Suder-Moraw & Doc. dr Józef Buczek
Institute of Botany University of Wrocław,
Kanonia Str. 6/8; 50-328 Wrocław; Poland*

Asymilacja azotu i aktywność reduktazy azotanowej w siewkach pomidorów w zależności od stosunku Ca:Mg w pożywce

II. Wpływ wzrastających dawek wapnia w obecności wysokich stężeń magnezu na redukcję azotanów i poziom białka

Streszczenie

Badano wpływ wzrastających koncentracji Ca^{2+} w pożywce przy wysokiej ilości Mg^{2+} na zmiany w aktywności NR, poziomie niektórych frakcji azotu, akumulację K^+ , Ca^{2+} i Mg^{2+} oraz wzrost i plon siewek pomidorów.

Stosunek Ca:Mg w pożywce 3:30 wywołuje w siewkach pomidorów objawy niedostatu Ca^{2+} na skutek nadmiernej akumulacji jonów Mg^{2+} i obniżonej akumulacji Ca^{2+} . W rezultacie obserwuje się obniżenie przyrostu suchej masy białka i zahamowanie aktywności NR. Podwojenie dawki Ca^{2+} w pożywce, czyli zmiana stosunku Ca:Mg do wartości 6:30 powoduje ustąpienie zewnętrznych objawów niedostatku wapnia oraz wpływa na zmniejszenie akumulacji Mg^{2+} przy niezmienniej akumulacji jonów Ca^{2+} . Równocześnie obserwuje się zwiększenie aktywności NR w siewkach do wartości zbliżonej dla roślin kontrolnych. Siewki pomidorów rosnące na potrójnej dawce Ca^{2+} (Ca:Mg = 9:30) nie różniły się zupełnie od siewek kontrolnych. Zwiększenie stężenia wapnia w pożywce wobec stałej wysokiej dawki magnezu powoduje wzrost akumulacji K^+ co może mieć wpływ na absorpcję i redukcję azotanów. Przypuszcza się, że niedobór Ca^{2+} w tkankach roślin wywołany nadmierną akumulacją Mg^{2+} przy niewłaściwym stosunku Ca:Mg w pożywce jest główną przyczyną zaburzeń w asymilacji N-NO_3 .