

## The effects of calcium to magnesium ratio on the increments of dry weight and protein content in tomatoes

J. BUCZEK and K. LEONOWICZ-BABIAK

Institute of Botany and Biochemistry, University of Wrocław, Poland

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### Abstract:

A change in the ratio of Ca: Mg ions in the medium or soil from optimum (3:1) to disfavoured values (3:30) causes dwarfing of tomatoes, a decrease of dry weight increment and of protein content. Total-N and protein-N also decrease, whereas the amount of soluble-N increases. It is suggested that the low ratio of Ca:Mg in the medium or soil affects nitrogen metabolism causing a restriction of protein synthesis.

### INTRODUCTION

It is known that a high magnesium content in soils formed from degraded serpentine rock and calcium deficit are the cause of dwarfing of various plant species (Walker 1954; Krause 1958). There is no doubt that the occurrence of dwarf forms is due to the unfavorable cation balance in the soil solution, and particularly the calcium-magnesium ratio (Loew 1932; Martin 1939; Libbert 1953; Sulej et al. 1970). Moreover, a high content of a number of microelements in serpentine soils (Nemec 1951 a and b; Walker 1954; Sulej et al. 1970) may have a noxious influence on plant growth. Of decisive importance however, would seem here the Ca:Mg ratio. In this connection comparative experiments were made with plants growing on serpentine soils and in aqueous media in which the Ca:Mg ratio and pH corresponded to that in the soil tested. These media did not, however, contain the additional amount of microelements characteristic for serpentine soils.

In the previous paper (Sulej, Slesak, Leonowicz-Babiak, Buczek, 1970) the suggestion was advanced that the unfavourable Ca:Mg ratio affects the nitrogen metabolism of the plants, and this in turn may be the cause of their poor development. The present paper aimed at the investigating of the effect of extremely Ca:Mg ratio on the increments of plant weight, total protein and individual fractions of nitrogen.

## MATERIAL AND METHODS

Tomato (*Lycopersicum esculentum*, variety 'Best of all') seedlings after germination on filter paper were transferred to pots filled with soil for further culture or placed in 1-litre jars filled with culture medium (water cultures). The jars and pots were placed in a greenhouse for three weeks. Thereafter the particular parts of the plants (roots, leafless shoots and leaves) were separately analysed for potassium, calcium and magnesium, particular nitrogen fractions, protein content, and dry weight increment, and elongation growth of the shoot and root was measured. The experiments were made in July and August 1969.

Water cultures. The basic and at the same time control solution was Knop's medium prepared with redistilled water. In this medium the Ca:Mg ion ratio was 3:1. The tested medium differed from the control one only by higher magnesium dose, the other components remaining the same. The Ca:Mg ratio in the medium tested was 3:30 (1:10), this corresponding approximately to the relation in the soil examined. Since an excess of magnesium was introduced into the medium in the form of sulphate, the control medium was supplemented with sodium sulphate in an amount corresponding to the quantity of sulphate ions in the test medium. The composition of the media is given in table 1.

Table 1  
Composition of culture medium (g per liter)

Ca:Mg ratio	Ca(NO <sub>3</sub> ) <sub>2</sub>	KNO <sub>3</sub>	KH <sub>2</sub> PO <sub>4</sub>	MgSO <sub>4</sub>	Na <sub>2</sub> SO <sub>4</sub>	Concentration
3:1	0.354	0.368	0.143	0.143	4.874	5.882
3:30	0.354	0.368	0.143	4.282	—	5.147

All the media contained the same amount of microelements counted in milligrams per litre of solution: Fe-citrate — 50.0; H<sub>3</sub>BO<sub>3</sub> — 0.5; MnSO<sub>4</sub>·5H<sub>2</sub>O — 0.05; CuSO<sub>4</sub>·5H<sub>2</sub>O — 0.05; H<sub>2</sub>MoO<sub>4</sub>·4H<sub>2</sub>O — 0.05. The experiments with the water cultures were performed with two pH variants 7.0 and 6.0. In the course of the experiment pH was measured daily, and, if necessary, adjusted by means of 0.1 N NaOH. All the cultures were aerated. Each combination included six 1-litre jars, each with three plants. Every plant was separately analysed. The results are means from 18 replications.

Pot cultures. The pots were filled with compost soil (control) or 4 kg of the tested soil. The soil was taken from areas where the bedrock contained about 80 per cent of serpentine minerals. A characteristic of these soils and its composition are given in the preceding paper (Sulej et al. 1970) therefore below only the most important characteristics are mentioned: the pH of the soil was 7.0—7.2 and the Ca:Mg ratio was around 1:10. One tomato seedling was planted in each pot. After the experiments

each plant was analysed separately. Each combination included 18 pots. The results are given as means from 18 replications.

**Analyses.** After the end of the vegetation experiments, the length of the shoot and of the entire root starting from the root-neck region were measured. The plants were separated into the particular parts (root, leaves, leafless shoot). Dry weight was determined at 105°. The particular nitrogen fractions: total-N and water-soluble-N were determined by the Kjeldahl micromethod (Mejbaum and Mochnacka 1968) after dissolution of the material in concentrated sulphuric acid. Soluble-N was extracted with hot water, soluble protein was precipitated with cadmium hydroxide and soluble-N was determined in the filtrate. Protein nitrogen was calculated from the difference between total and soluble-N. Protein content was calculated by multiplying protein N by 6.25. Potassium, calcium and magnesium content was determined after dissolution of the particular plant parts dried to constant weight in concentrated sulphuric acid with hydrogen peroxide added, and whereafter making up with water to constant volume. Potassium and calcium were determined in a Zeiss flame photometer (after Nowosielski, 1968) and magnesium in a colorimeter by means of p-nitrobenzeneazoresorcinol (Nowosielski).

Statistical elaboration consisted in the calculation of the means of the arithmetic mean.

## RESULTS

Tomato seedlings growing on the tested soil developed distinctly shorter shoots as compared with the control plants (Table 2). The seedlings in water culture showed a similar reaction. A change in the Ca:Mg ions ratio from 3:1 to 3:30 caused a shortening of the shoot length independently of the pH of the medium. Quantitative differences were, however, larger at neutral pH than at weakly acid. It should be noted that pH of the soil examined was about 7.2, and the Ca:Mg ions ratio was 1:10. The medium with pH 7.0 corresponded, thus, to the pH conditions and Ca:Mg ratio in the soil.

Table 2  
Average length of plant and average amount of leaves per plant

Conditions of cultivation		Ca:Mg ratio	Root mm    %		Shoot mm    %		Leaves amounts    %	
Water cultures	pH 6.0	3:1	205		87		4	
		3:30	83	41	59	68	3	75
	pH 7.0	3:1	200		104		4	
		3:30	48	24	55	53	3	75
Compost soil	pH 6.2	3:1	—		89		5	
Serpentine soil	pH 7.2	1:10	—		39	44	3	60

Table 3

Average yield (dry weight) and average content of protein per plant

Condition of cultivation	Ca:Mg ratio	Root			Shoot without leaves			Leaves			Whole seedling		
		Dry weight mg	%	Protein mg	%	Dry weight mg	%	Protein mg	%	Dry weight mg	%	Protein mg	%
Water cultures	pH 6.0	19	26	4.9	20	34	38	6.9	32	100	34	34.9	28
	3:30	5	13	1.0	3.1	13	33	2.1	6.1	34	87	9.9	28
	pH 7.0	13	23	3.1	19	33	24	6.1	20	87	20	25.3	23
Compost soil	3:30	3	3	0.6	19	8	8	1.2	20	20	23	5.8	23
	pH 6.2					65		9.0		162		56.2	
	3:1												
Serpentine soil	pH 7.2					9	14	0.9	10	34	21	8.2	15
	1:10									43	19	8.9	13

As seen from table 2, roots of plants are more sensitive to changes in the ion ratio in the medium than are the shoots. The root length was reduced by about 76 per cent as compared to that in the controls. The shoot length in the same combination (water medium pH 7.0) decreased only by 47 per cent. It is noteworthy that the change in the ion ratio in the medium reduced the number of leaves.

The changes in the Ca:Mg ions ratio towards a higher magnesium value, calcium remaining unchanged, caused a distinct decrease of the dry weight increment (Table 3). This decrease concerned the whole plant, root, shoot and leaves. Protein content (Table 3) converted to one plant was also considerably lower. It is interesting that the relative diminution of the protein weight corresponds fairly well to the reduced relative dry weight. This fact may indicate that the diminution of plant weight due to the Ca:Mg ions in the medium mainly involves protein weight decrease. This relation is most pronounced in the water cultures at pH 7.0. Changes in pH of the medium (6.0 or 7.0) did not have any major influence on the dry weight and protein content.

The difference in protein content in reference to 1 g of dry weight (Table 4) is not so wide as when converted to one plant. Statistical analysis demonstrated, however, that the differences in protein content between the experimental plants and the controls are significant for all combinations. From among the organs analysed, the greatest differences in protein content were found in the roots amounting to about 25–31 per cent, and the smallest in the leaves.

The changes in ion ratio in the medium, from normal values to extreme with a ten times higher magnesium dose, reduced the amount of total nitrogen, although the differences were not significant in all combinations. They were nonsignificant in the combination with pH 6.0 except for the roots (Table 4). Protein N also decreased in all combinations. In all the organs analysed, however, the soluble N content increased. This was most pronounced in shoots and roots and least in leaves.

Accumulation of potassium, calcium and magnesium ions in relation to dry weight (Table 5) is directly dependent on the Ca:Mg ions ratio in the medium. pH exerts only a slight influence on the uptake of these elements. Changes in this ratio in the medium from 3:1 to 3:30 depressed potassium accumulation in all the analysed parts. Calcium ions content in reference to dry weight was also markedly decreased with the exception of that in root at both pH values although the differences were not significant. The unfavourable Ca:Mg ions ratio caused in all the analysed parts of the plants and in all combinations an increase of magnesium accumulation.

Table 6 gives the results concerning potassium, calcium and magnesium ion accumulation by the roots and leaves, converted to protein weight units. As seen from these data, excessive magnesium quantities in the medium induced an increased accumulation of this element per unit of protein produced. Differences were, however, noted in Ca and K ions accumulation in reference to protein weight in roots and leaves. In roots in which the greatest decrease of total protein is noted (Table 4), there were no differences in calcium content between the experimental and the control

Table 4

Average content of protein and nitrogen fractions per gram of dry weight

Condition of cultivation	Ca:Mg ratio	Total-N		Soluble-N		Protein-N		Protein	
		mg	%	mg	%	mg	%	mg	%
Water cultures	pH 6.0	46.4	86	4.3	126	42.5	81	303.9	82
	3:1	40.1		5.4		36.4		249.9	
	3:30	46.3		5.0		41.4		258.8	
	Seedling	39.0	84	6.5	129	32.5	79	203.0	79
	3:1	46.3		5.1		41.2		260.2	
	3:30	39.2	85	6.8	133	32.4	79	196.2	75
	Root	42.8		4.8		38.1		237.5	
	3:1	32.4	76	6.3	131	26.1	70	163.1	69
	3:30	35.3		4.1		31.2		194.9	
	Shoot	31.3*	89	6.3	154	25.0	80	156.5	80
	3:1	31.8		2.5		29.3		183.1	
	3:30	28.6*	90	4.5	180	24.1	82	150.5	82
Compost soil Serpentine soil Compost soil Serpentine soil Compost soil Serpentine soil	pH 6.0	59.8	87	4.3	116	55.5	84	346.5	84
	3:1	51.8*		5.0		46.8		291.9	
	3:30	52.8	84	4.9	118	47.6	81	299.0	81
	Leaves	44.5		5.8		38.7		241.0	
	3:1	49.6	66	3.5	124	46.1	62	289.0	62
	3:30	33.0		4.3		28.7		179.5	
	Seedling	24.4	80	2.1	190	22.3	70	139.2	70
	1:10	19.6		4.0		15.6		97.7	
	Shoot	58.8	83	3.4	120	55.4	80	347.5	80
	3:1	48.5		4.1		44.4		277.5	
	1:10								
	Leaves								

\* różnice statystycznie nieudowodnione  
differences statistically nonsignificant

Table 5

Effect of Ca:Mg ratio on ion accumulation mg/g dry weight

Conditions of cultivation		Ca:Mg ratio	K mg	Ca mg	Mg mg
Water culture	pH 6.0 pH 7.0	3:1	69.8	23.9	4.4
		3:30	42.7	8.2	16.4
		3:1	60.3	20.2	5.8
		3:30	39.0	7.6	21.5
	pH 6.0 pH 7.0	3:1	66.6	8.2	6.3
		3:30	26.4	5.7*	15.1
		3:1	56.6	10.9	6.9
		3:30	39.0	8.2*	18.4
	pH 6.0 pH 7.0	3:1	116.2	12.0	3.8
		3:30	88.1	6.3*	11.5
		3:1	110.5	8.9	5.0
		3:30	85.6	6.3	15.2
	pH 6.0 pH 7.0	3:1	48.5	32.7	4.4
		3:30	27.7	8.8	17.6
		3:1	41.5	27.7	5.7
		3:30	21.4	8.2	20.3
Compost soil	pH 6.2	3:1	58.6	28.3	3.1
Serpentine soil	pH 7.0	1:10	38.3	8.8	18.3
Compost soil	pH 6.2	3:1	90.5	21.4	4.8
Serpentine soil	pH 7.2	1:10	61.6	13.8	14.0
Compost soil	pH 6.2	3:1	45.9	30.8	2.5
Serpentine soil	pH 7.2	1:10	32.0	7.6	14.5

\* różnice statystycznie nieudowodnione  
differences statistically nonsignificant

Table 6

Effect of Ca:Mg ratio on ion accumulation µg/mg of protein

Condition of cultivation		Ca:Mg ratio	K µg	Ca µg	Mg µg
Water cultures	pH 6.0 pH 7.0	3:1	256	32	24
		3:30	135	29	77
		3:1	238	46	28
		3:30	239	50	113
	pH 6.0 pH 7.0	3:1	140	95	13
		3:30	95	30	59
		3:1	139	93	19
		3:30	89	34	83
	pH 6.2 pH 7.2	3:1	132	89	7
		1:10	115	27	52

plants. In the combination with pH 7.0 the potassium content was also equal, but it was lower at pH 6.0. In the leaves of plants in water culture, excess of magnesium significantly depressed calcium and potassium accumulation in reference to one protein unit produced, whereas in the leaves of plants growing on serpentine soil only the calcium level decreased.

## DISCUSSION

A markedly lowered protein content (by ca. 38%) in plants growing on the tested soil with an average Ca:Mg ions ratio 1:10 (Sulej, Slezak, Babiak-Leonowicz and Buczek 1970) seems to confirm the supposition that a disturbed nitrogen metabolism is one of the symptoms of intolerance in plants of a magnesium ions excess in the soil. The experiments with the water cultures in two different combinations as regards Ca:Mg ratio (without reducing the calcium dose), one of which corresponded to the relations in the soil, confirmed the above mentioned supposition. Analyses demonstrated that a change in the Ca:Mg ratio in the medium from optimal (3:1) to extreme unfavourable values (3:30) reduced the protein content with simultaneous increase in the soluble N fraction. A slight decrease of total N was also noted. These data seem to suggest that an unsuitable Ca:Mg ratio in the soil or the culture medium causes certain, although not clearly understood, disturbances in protein synthesis.

Analyses of the particular plant organs give reason to believe that protein synthesis is restricted above all in the roots. In these organs the greatest depression of the protein increment is observed with a simultaneous increase in the soluble N fraction. In leaves, on the other hand, protein synthesis processes seem less disturbed. Since the plants were cultured on media differing solely by the Ca:Mg ions ratio, the amount of calcium remaining the same, it is reasonable to believe that the unfavourable ratio of these ions in the medium was the main factor limiting both the growth of the plants and the dry weight and protein content increments.

pH of the medium plays here also a certain role. In general, plants in a solution with pH 6.0 were more resistant to the disfavoured proportion of the cations in the medium as manifested by a less reduced dry weight or protein weight increment and by a somewhat better growth. These differences are, however, quantitative not qualitative, so that the general type of response of the plants to the changes remained similar at weakly acid pH.

The dwarfed size of the tomato seedlings on soils derived from degraded serpentine rock might be ascribed as in the previous investigations (Sulej et al., 1970) to the low calcium content in these soils. This supposition seems correct in the light of the studies of Walker, Walker and Ashworth (1955). These authors found that addition of calcium to serpentine soils distinctly improved the growth of tomato seedlings. The experiments of Madhok and Walker (1969), however,



with water culture of two sunflower varieties, one of which was a form endemic for serpentine soils, demonstrated that the cause of intolerance of some plant species to serpentine soils is above all the excessive accumulation of Mg ions in the plants.

It is known (Wall 1940a and b; Vyskrebentseva 1963) that a considerable deficit of potassium in the soil reduces the total and protein nitrogen content with a simultaneous increase of soluble N. Calcium deficit in the medium may also limit protein production. Burstrom (1954) demonstrated that calcium ions are indispensable for nitrate uptake from the medium, and increasing doses of Ca ions enhance nitrate uptake and increase the protein content in wheat seedlings. Paulsen and Harper (1968) and Harper and Paulsen (1969) showed that calcium deficiency in the medium may restrict the activity of nitrate reductase.

In the present experiment on water culture, the potassium and calcium doses were identical in the control and experimental cultures. Therefore it is difficult to explain the fact of lowered protein production by a deficit of these ions in the substrate. A certain decrease in K and Ca ions accumulation is also observed in reference to dry weight in plants growing on media with a disfavoured Ca:Mg ratio. An exception were the roots in which no significant decrease in calcium content was observed as compared with that in the controls. The fact that roots, in which the greatest depression of protein synthesis was noted, did not show significant differences in calcium accumulation, whereas in the leaves protein synthesis was least inhibited, and the differences in Ca and K accumulation as compared with the controls were significant, indicates that this factor did not restrict protein production. This supposition seems reasonable if we compare the results in Table 6. In the roots, namely, the amount of accumulated K and Ca ions in reference to one protein unit produced were the same in the experimental and control plants. Wide differences were, however, found in the leaves, and these facts do suggest that a certain K and Ca ions deficit in plants is not the main factor limiting growth and protein synthesis. On the other hand, it is not excluded that excess of magnesium accumulated caused essential difference in the relations between the particular cations in the plants, and this no doubt could affect the biochemical or physico-chemical processes. Among others the processes of protein synthesis were disturbed, which as it seems are largely responsible for normal weight increment and growth of the plants.

#### SUMMARY AND CONCLUSIONS

The investigations performed allow the following conclusions. The change of the Ca:Mg ratio in the medium or soil from 3:1 to 3:30, all other components remaining unchanged except magnesium, causes development of dwarfed plant forms, reduces the dry weight increment and the protein content. Excess of magnesium in the medium enhances accumulation of this element in the plants. This relation was observed in all the plant organs (root, shoots, leaves). The amount of magnesium accumulated per protein weight unit is highest in the roots in which the increment

of protein decreases most. In leaves in which protein synthesis was less inhibited than in roots accumulation is lower. Excessive magnesium accumulation results in a decreased accumulation of potassium and calcium ions, varying in dependence on the plant organ with the exception of the roots. These facts suggest that it is mainly the excess of accumulated magnesium that is the factor restricting protein production, whereas a limited potassium or calcium uptake affects this process less. It would seem, therefore, that a disfavoured Ca:Mg ratio in the soil or medium influences nitrogen metabolism and in this way reduces protein synthesis.

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*Zależność przyrostu masy roślin i masy białka od stosunku jonów wapnia i magnezu w podłożu*

Streszczenie

Przeprowadzone badania pozwalają wyciągnąć następujące wnioski: Zmiana stosunku jonów Ca:Mg w pożywce czy glebie z wartości 3:1 do wartości 3:30, przy zachowaniu tych samych ilości wszystkich składników z wyjątkiem magnezu, wpływa na wykształcenie karłowatych form roślin, obniżenie przyrostu suchej masy oraz zmniejszenie zawartości białka.

Nadmiar magnezu w pożywce wpływa na zwiększoną akumulację tego pierwiastka przez rośliny. Zależność tę obserwuje się we wszystkich organach rośliny (korzenie, pędy, liście). Ilość akumulowanego magnezu na jednostkę masy białka jest największa w korzeniach, u których stwierdzono najsilniejszy spadek przyrostu masy białka, a stosunkowo niższa w liściach, w których synteza białka była słabiej hamowana niż w korzeniach. Nadmierna akumulacja magnezu pociąga za sobą zmniejszenie akumulacji jonów potasu i wapnia w ilościach różnych w zależności od organu rośliny z wyjątkiem korzeni.

Powyższe fakty sugerują, że czynnikiem ograniczającym produkcję białka jest głównie nadmiar akumulowanego magnezu, natomiast w mniejszym stopniu ograniczenie pobierania potasu czy wapnia. Należy zatem przypuszczać, że nieodpowiedni stosunek jonów Ca:Mg w podłożu wpływa na metabolizm azotowy w kierunku ograniczenia syntezy białka.