Excess Injury of Calcium and Magnesium in the Crops

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Intensive cultivation in glass and plastic houses has been gradually extended to the production of many horticultural crops during last twenty years in Japan. Accumulation of salt in the soil has scarcely occurred in the open fields owing to rainy climate in this country.

On the contrary, acidification accompanied by leaching some bases out of the soil has been observed in many districts in Japan. Under the covered conditions, however, the nutrient components maintained the soil tend to accumulate gradually because of the particular water movement condition; consequently, salt injury has been observed in many commercial-growing crops. Mineral components in the irrigated water also cause salt injury. Generally, accumulation of many cations in the soil is found extensively in dry climate conditions.

It has been elucidated that large quantities of calcium and magnesium were accumulated in the soil under glass and plastic houses conditions. Some investigators have reported excessive injury to the crops caused by calcium or magnesium[4),6),7)]. Studies on calcium have been mainly carried out on soil pH or with osmotic pressure. Magnesium deficiency has been reported in many crops by previous investigators, but scant attention was focused on excessive injury attributed to magnesium[2),3),5)]. From these points of view, this article takes up the effect of the crops hit by excessive amount of calcium or magnesium.

Growth and mineral constituent of cucumber plants grown in the medium added excess calcium or magnesium

The differences in the salt tolerance for calcium, magnesium, potassium and sodium were examined in cucumber plants with sand culture. Nutrient solutions used in the experiment were prepared by individual addition of chloride of these cations into the 0.5 strength Hoagland nutrient solution, and its osmotic pressure was adjusted altogether to 1.49 atmospheres.

Plant height of the cucumber was depressed remarkably as a result of 4 weeks treatment by excessive magnesium (Table 1). Calcium showed less inhibitive effect for the plant height in comparison with magnesium. The effect by sodium was observed slightly. In the case of potassium, plant height was similar to that of controlled plant.

Depressions in the top weight by excessive cations were caused in the order of magnesium, calcium, sodium and potassium. Magnesium showed the most depressive effect for the root weight, and it was evaluated by 40 per cent of control. In the other cations, root development was depressed in the order of sodium, calcium and potassium. Generally, inhibitions for plant growth by excessive cations tended to appear more conspicuously in the roots than in the tops.

Table 1. Cucumber plants grown in high levels of Ca, Mg, K and Na

Salt added	Plant height	5th leaf		6th leaf		Top		Root	
		Width	Length	Width	Length	F. W.	D. W.	F. W.	D. W.
	cm	cm	cm	cm	cm	gr	gr	gr	gr
Control	96.7	18.7	15.5	18.7	15.7	82.2	3.58	27.2	2.05
Ca	82.3	18.0	15.0	18.0	15.5	67.5	6.47	23.1	1.30
Mg	76.3	16.6	13.6	17.3	14.3	59.3	5.98	15.5	0.83
K	96.3	18.0	15.3	19.3	16.0	82.1	8.55	24.0	1.55
Na	90.8	16.5	14.0	17.3	14.3	70.0	7.04	20.5	0.93

Table 2. Mineral content in cucumber plants as affected by salts

Part	Salts added	Mineral content (% of dry matter)							
		N	P	K	Ca	Mg	Na	Fe	Mn
Top	Control	2.81	0.30	4.04	2.38	1.04	1.65	0.10	0.008
	Ca	2.72	0.24	4.17	2.72	0.78	1.86	0.09	0.010
	Mg	2.83	0.32	4.04	1.60	2.24	1.40	0.09	0.013
	K	3.21	0.32	5.22	1.95	0.86	1.97	0.09	0.009
	Na	3.23	0.34	4.41	2.06	0.96	2.55	0.10	0.006
Root	Control	2.20	0.23	4.39	0.89	1.08	1.94	0.74	0.039
	Ca	2.03	0.20	4.32	1.20	0.76	1.53	0.89	0.042
	Mg	2.47	0.27	5.25	0.53	1.12	1.81	0.55	0.033
	K	1.85	0.23	5.41	0.76	0.46	1.92	0.51	0.030
	Na	2.39	0.27	3.06	0.91	0.58	3.21	0.67	0.034

Mineral components in cucumber plants are shown in Table 2. Calcium content in the plant top increased, while phosphorus and magnesium contents decreased by excessive calcium. Excessive magnesium in the nutrient solution remarkably increased in magnesium and decreased in calcium in the plant top. A counteraction between magnesium and calcium was evident in this experiment.

In the roots, there was no remarkable alteration except an increase in calcium content and a decrease in magnesium content by excessive calcium in the medium.

Excessive addition of magnesium into the nutrient solution tended to increase in phosphorus, potassium and magnesium contents and to decrease in calcium, ferric and manganese contents in the roots. It is noted that magnesium contents greatly increased in the plant top, showing little increase in the roots by excessive magnesium in the medium. Thus, when compared with the isosmotic solutions between excessive calcium and magnesium solutions, considerable difference was observed in the growth of the cucumber plant, especially inhibition in the root by excessive magnesium. As for the alternate cation content in the plants, calcium acts antagonistically to magnesium, therefore the mineral contents are changed markedly.

Effects of calcium and magnesium on TTC (2, 3, 5-triphenyl tetrazorium chloride) reduction activity of plant roots[8]

Since the toxic effects of excessive cations appeared especially in the roots, it was examined whether the TTC reduction method is suitable for the histochemical estimation of root activity to ascertain easily the injury of excessive salt. TTC reduction activity of the intact roots of cucumber seedlings after dipping the roots into single salt solutions varied with levels of concentration or kinds of the solution, and these responses occurred before the plants showed any visible symptom of excessive salt injury.

From this result, it was confirmed that TTC reduction activity in the roots was one of the suitable indexes for the decision of root injury by these salts.

Under the TTC reduction method, critical concentrations inducing excessive injury were examined in cucumber roots for individual salts respectively (Table 3). Cucumber roots

Table 3. Inhibitive salt concentration evaluated by the TTC method in cucumber plants

Salts	Salt conc.		Osmotic pressure atm.	Ionic strength
	mM	m.e.		
$CaCl_2$	35	70	2.35	0.105
KCl	30	30	1.59	0.030
NH_4Cl	4	4	0.18	0.004
$NgCl_2$	1.5	3	0.11	0.005
$Ca(NO_3)_2$	30	60	2.71	0.090
KNO_3	25	25	1.75	0.025
NH_4NO_3	8	8	0.26	0.008
$Mg(NO_3)_2$	1	2	0.07	0.003
K_2SO_4	7.5	15	0.47	0.023
$(NH_4)_2SO$	3	6	0·16	0.009
$MgSO_4$	0.75	1.5	0.03	0.003
KH_2PO_4	10	10	0.44	0.010
$NH_4H_2PO_4$	3	3	0.19	0.003
K_2HPO_4	5	10	0.23	0.015
$(NH_4)_2HPO_4$	4	8	0.19	0.012

showed very low tolerance for magnesium and ammonium ions, but high tolerance for calcium and potassium ions.

No correlation was observed between these critical concentrations and characteristics of solution, such as osmotic pressure, ionic strength and pH value. Therefore, these critical concentrations are dependent upon the results of physiological behavior of cations.

In order to compare the responses to cations among the species, TTC reduction activity was determined in the roots of rice and rush plants with the same method as determined in the cucumber. The critical concentrations for the inhibition of the root activity were higher in these plants than that in the cucumber, but the critical concentrations for magnesium were lower in both plants than those for calcium as shown in the cucumber[9].

Mineral composition of mitochondria prepared from cucumber roots as affected by magnesium or calcium[10]

The surrounding tissues of growing point and cambium in cucumber roots are colored with TTC or Janus green B. Since mitochondria is markedly colored with the same dyes in the cells, it means that decreased TTC reduction activity of plant roots through excessive salts will be caused by altering the mitochondrial components. Table 4 shows the

Table 4. Mineral content in mitochondria of cucumber roots as affected by Ca- and Mg-salts

Salts treated	Mineral content (γ/mg protein)					
	Ca	Mg	P	K	Fe	Mn
Control	5	2	11	13	4	0.8
Ca 4 mM	12	2	9	13	3	0.8
Ca 40 mM	11	2	9	10	4	0.6
Mg 0.5 mM	6	2	11	13	4	0.8
Mg 4 mM	6	5	14	5	2	0.5

mineral contents of mitochondria prepared from cucumber roots treated by two concentrations of calcium or magnesium solutions. In cucumber roots, magnesium content was scarcely increased by magnesium treatment (Table 2). On the contrary, in the mitochondria a large amount of magnesium accumulated, and phosphorus was also slightly increased (Table 4), while, the contents of iron, potassium and manganese decreased in the mitochondria.

These results indicate that localization of mineral nutrients must be considered thoughtfully in discussing mineral components in plant tissues. Calcium content of mitochondria treated by calcium increased by approximately two times as compared with control, but the other mineral contents were not altered.

Effects of calcium and magnesium on the activity of some enzymes in cucumber roots[10]

It has been reported that close correlation was found between the TTC reduction activity and the respiration of the plant tissues. Therefore, it seems that the alteration of TTC reduction activity in the roots includes that of enzyme activities associated with tissue respiration. So the effects of 4 mM calcium or magnesium on the activities of some root enzymes were examined.

The root enzymes determined throughout in this experiment were succinate dehydrogenase (SDH), isocitrate dehydrogenase (IDH), isocitrate lyase, aconitase and catalase. SDH activity of the mitochondrial fractions and IHD activity of the crude extractions of the roots were determined after dipping for 24 hours in calcium or magnesium chloride solutions (4 mM) by using spectrophotometer.

Mitochondrial fractions from cucumber roots were prepared by means of the centrifugation method with sucrose isosmotic solution (pH 7.4). As shown in Table 5, under the treatment of 4 mM magnesium, SDH activity was inhibited by about 40 per cent IDH actiivty was also inhibited by 20 per cent of the control.

The treatment by calcium (4 mM) showed slight inhibition for SDH but not for IHD. Aconitase, isocitrate lyase and catalase activities in cucumber roots treated by calcium or magnesium are shown in Table 6. These enzymes were activated by calcium or magnesium especially, isocitrate lyase and catalase activities were particularly affected.

Aconitase is one of the enzymes in connection with the tricarboxylic acid (TCA) cycle, the other two enzymes are closely related to glyoxylate cycle[1]. These enzymes were activated by the excessive amount of calcium or magnesium, but dehydrogenases involved in the TCA cycle were inhibited only by magnesium.

Table 5. Succinate dehydrogenase and isocitrate dehydrogenase activities of cucumber root as affected by Ca and Mg supplied to roots

Treatment	Succinate dehydrogenase		Isocitrate dehydrogenase	
	Specific activity $\Delta OD \times 10$/mg prot.	Ratio	Specific activity $\Delta OD \times 10$/mg prot.	Ratio
Control	20.5	100	43.8	100
Ca 4 mM	18.8	92	44.4	101
Mg 4 mM	12.5	61	35.1	80

Table 6. Aconitase, isocitrate lyase and catalase activities of cucumber roots as affected by Ca and Mg supplied to roots

Treatment	Aconitase		Isocitrate lyase		Catalase	
	Specific activity $\Delta OD \times 10^3$/mg prot.	Ratio	Specific activity $\Delta OD \times 10^2$/mg prot.	Ratio	Specific activity*	Ratio
Control	83.4	100	13.3	100	0.35	100
Ca 4 mM	106.3	127	20.0	150	0.47	134
Mg 4 mM	132.1	159	50.5	380	1.00	286

Note: * Titres (ml) of 1/200 M $KMnO_4$ per milligram of protein in the enzyme solutions

This fact suggests that excessive magnesium acts in different ways of mechanisms for the TCA and the glyoxylate cycles and that the physiological and the biochemical behaviors of excessive calcium or magnesium are quite different.

The inhibition for dehydrogenases associated with the TCA cycle coincides closely with that for energy production systems. Therefore, it should be considered that crop production will be subject to large limitation by the excessive amount of calcium or magnesium connected to the inhibition of the TTC reduction activity and the nutrient absorption by the roots.

Conclusion

Excessive amount of calcium or magnesium affected the mineral composition of the plants respectively and altered the ionic balance in the plants. Excessive magnesium affected more inhibitively plant growth than the same excessive calcium in the isosmotic solutions. It depresses the TTC reduction activity of the roots because of inhibition for dehydrogenases associated with the respiratory systems. Growth inhibition of plants by excessive calcium differs from magnesium; it seems that the osmotic pressure of calcium solution causes the inhibition for root growth, and excessive calcium makes the alteration of ionic balance in the plants which influences upon the nutrient absorption.

Although currently discussions have been held on calcium or magnesium excessive injury, sufficient consideration should be taken on these problems in the growing of the crops.

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