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MEASUREMENT OF UPTAKE OF CHELATED AND UNCHELATED Ca AND Sr FROM SOLUTION CULTURE*

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SUMMARY

The uptake of Ca and Sr by three-week old tomato (*Lycopersicon esculentum*) plants from solutions containing Ca^{++} and Sr^{++}, and chelated Ca and Sr (CaL and SrL) was measured over a two-day period. The solution was double-labelled with Ca^{45} and Sr^{85}. Two chelates, EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid) were used at five chelate-cation ratios. When the Ca and Sr content of the solution was held constant, addition of chelate reduced uptake. The reduction was greater with EDTA than with DTPA.

The Ca/Sr ratio of uptake was used to measure the proportion of uptake as the chelated and unchelated species. The Ca^{++}/Sr^{++} ratio was different from the CaL/SrL ratio in solution because of the different equilibrium reactions of Ca and Sr with L. Direct uptake of the CaL and SrL was indicated. In solutions where Ca^{++} = CaL, uptake of CaEDTA was 0.47 of uptake of Ca^{++} and uptake of CaDTPA was 0.95 of uptake of Ca^{++}.

INTRODUCTION

Soluble metal chelates and chelating agents are widely used in soils and solution culture to increase ion uptake and reduce nutrient deficiency [1]. The way chelates increase ion absorption by plants is not well understood [7]. Evidence from experiments using C^{14}-labelled chelate complexes has indicated some direct absorption of the chelate, although less chelate than chelate ion was absorbed [4 8]. Absorption of the chelated ions may occur through broken roots [9].

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The chelate may also serve to buffer the level of the unchelated cation (M^{x+}) in solution.

Chaney, Brown, and Tiffin [3] have evidence that the plant root releases a reducing agent that reduces ferric Fe in the chelate to ferrous Fe which releases it from the chelate before root absorption. This would explain a mode of action for reducible cations.

When a soluble chelating agent, L^{x-}, is added to a solution containing cations M^{x+}, an equilibrium will be formed between L^{x-}, M^{x+} and the chelated metal, ML.

$$M^{x+} + L^{x-} \leftrightarrows ML \qquad [1]$$

The plant root may absorb M as M^{x+} or ML or both. When more M^{x+} than ML ions are absorbed, the initial equilibrium in solution will shift to the left and ML may dissociate giving M^{x+} in solution that may then be absorbed. Hence, there are three sources of M that may be absorbed ML, M^{x+} initially present at equilibrium, and M^{x+} that has dissociated from ML.

The objective of this research was to develop a procedure and measure the rate of uptake of Ca and Sr from the unchelated and chelated forms. The approach used was to determine the Ca/Sr ratio of uptake of Ca and Sr from solutions containing chelated and unchelated Ca and Sr. Because Ca and Sr are chelated with different strengths the Ca^{++}/Sr^{++} ratio will differ from the CaL/SrL ratio in an equilibrium solution of Ca, Sr, and L. Also, we have observed [2] that some plant species such as tomato (*Lycopersicon esculentum*) absorbed ionic Ca and Sr indiscriminantly, particulary where $Ca \gg Sr$. Hence, the Ca/Sr ratio of absorption by tomato roots can be used to calculate the amount of Ca and Sr coming from each of the unchelated and chelated forms in solution.

MATERIALS AND METHODS

Tomatoes were grown in solution cultures in the greenhouse for three weeks prior to their use in a two-day study of Ca and Sr absorption from solutions containing soluble chelates. The chelating agents used were EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid). Five tomato plants were grown in each of 40 three-liter pots. The solution for the first two weeks was a modified one-fifth strength Hoagland solution containing enough Sr to give a Ca/Sr ratio of 100 with a Ca concentration of 1.0 m*M*. For the third week Ca and Sr concentrations were reduced to one-fourth these concentrations. After three weeks the plants were transferred to Ca^{45}- and Sr^{85}-labelled solutions of $CaCl_2$, $SrCl_2$ and the

chelating agent. High specific activity Ca^{45} and Sr^{85} were added at the rate of 50 μc each per pot containing 2750 ml of solution. Four pots were harvested at the start of the two-day experiment for initial measurements. The treatment combinations used are given in Table 1. They consisted of five levels of chelate addition for each chelating agent where total Ca and total Sr remained constant. In addition, chelate-free solutions were included with Ca and Sr concentrations equal to the Ca^{++} and Sr^{++} concentrations in solutions for the three lowest levels of chelate addition. Two replicates were used. The solutions were maintained at a pH of 7.0 to 7.5 during the two-day absorption period.

After a two-day absorption period, the plants were removed from the solutions and the free space and exchangeable Ca^{45} and Sr^{85} were displaced from the roots by washing in an unlabelled Ca and Sr solution. Plant measurements made were top weight, root weight, root length, and uptake of Ca^{45} and Sr^{85}. Root length was measured by the line-intercept method of Newman [5]. The plant tops and roots were dry-ashed and the ash brought into solution with HCl. Additional Ca and Sr were added, the Ca and Sr precipitated as oxalates, the precipitate washed and dried, and 0.5 gram made into a pellet in an Al planchet. Ca^{45} and Sr^{85} were counted separately using a thin window gas flow detector and a NaI crystal scintillation detector, respectively. The beta count for Ca was corrected for the effect of the gamma radiation from Sr. Samples from the solution cultures were also measured in the same manner.

CALCULATIONS

The concentrations of all species in the equilibrium of the chelating agents with the Ca and Sr solutions used were calculated. The calculations were based on formation constants of the cation-chelate complex reported by Sillen and Martell [6] and the following parameters of the system: (i) $Ca^{++} + CaL$ = total Ca in the system = Ca_T. (ii) $Sr^{++} + SrL$ = total Sr in the system = Sr_T. (iii) $Sr_T + Ca_T \geq L_T$. (iv) (CaL + SrL) at pH above 7 = total L in the system = L_T. In treatments where L/Ca_T was three (Table 1) all the Ca and Sr was assumed to be complexed by the chelating agent. Calcium and Sr were the only competing ions for the chelate in the system. By maintaining the solution at pH 7.0–7.5 the competition by H^+ was negligible. The formation constants used in the calculations for the various metal ion-chelate reactions were:

$$Ca^{++} + EDTA \leftrightarrows CaEDTA \qquad \log K_{CaEDTA} = 10.85$$
$$Sr^{++} + EDTA \leftrightarrows SrEDTA \qquad \log K_{SrEDTA} = 8.64$$
$$Ca^{++} + DTPA \leftrightarrows CaDTPA \qquad \log K_{CaDTPA} = 10.89$$
$$Sr^{++} + DTPA \leftrightarrows SrDTPA \qquad \log K_{SrDTPA} = 9.68$$

By combining the equilibrium relations for the reactions of Ca^{++} and Sr^{++} with L^{x-} we can write:

$$\frac{K_{CaL}}{K_{SrL}} = \frac{(CaL)(Sr^{++})}{(SrL)(Ca^{++})} \qquad [2]$$

By combining equation [2] with relationships i, ii, iii, and iv, we can solve for the amounts of each species present at equilibrium. This solution involves development of equation [3] a quadratic equation of the second order.

$$A(CaL)^2 + BCaL - D = 0 \qquad [3]$$

from which

$$CaL = (-B \pm \sqrt{B^2 + 4AD})/2A \qquad [4]$$

where

$$B = K_{SrL}Sr_T - (K_{SrL} - K_{CaL})(L_T) + K_{CaL}Ca_T$$
$$A = K_{SrL} - K_{CaL}$$
$$D = K_{CaL}Ca_TL_T$$

Uptake rate calculation

The mean uptake rate for the two-day absorption period was determined using equation [5] [10].

$$\text{Mean uptake rate} = \frac{\text{Total uptake}}{\text{time}} \times \frac{\ln(L_2/L_1)}{(L_2 - L_1)} \qquad [5]$$

where L_1 and L_2 are the root lengths at the beginning and end of the uptake period.

Calculation of uptake of chelated and unchelated Ca and Sr

The source of Ca and Sr absorbed by the plant was determined using the Ca/Sr ratio of uptake, U_{Ca}/U_{Sr}, and the Ca^{++}/Sr^{++} and CaL/SrL ratios in the solution bathing the roots. We have shown experimentally [2] that the uptake of Ca^{++}, $U_{Ca^{++}}$, and Sr^{++}, $U_{Sr^{++}}$, was in the ratio of Ca^{++}/Sr^{++} in solution. We assume that the uptake of the chelated species of Ca and Sr will be in the CaL/SrL ratio in solution since CaL and SrL should be very similar.

The method of calculating the amount of Ca and Sr absorbed as the chelated and unchelated species was as follows:

Let $U_{Ca}/U_{Sr} = R$ = Ca/Sr in plant uptake
$U_{CaL}/U_{SrL} = R_1$ = CaL/SrL in solution
$U_{Ca^{++}}/U_{Sr^{++}} = R_2$ = Ca^{++}/Sr^{++} in solution

Then

$$R = U_{Ca}/U_{Sr} = (U_{CaL} + U_{Ca^{++}})/U_{Sr} \qquad [6]$$
$$= U_{CaL}/U_{Sr} + U_{Ca^{++}}/U_{Sr} \qquad [7]$$
$$= R_1\, U_{SrL}/U_{Sr} + R_2\, U_{Sr^{++}}/U_{Sr} \qquad [8]$$
$$= R_1\, (U_{Sr} - U_{Sr^{++}})/U_{Sr} + R_2\, U_{Sr^{++}}/U_{Sr} \qquad [9]$$
$$= R_1 - R_1\, U_{Sr^{++}}/U_{Sr} + R_2\, U_{Sr^{++}}/U_{Sr} \qquad [10]$$
$$= R_1 + U_{Sr^{++}}((R_2 - R_1)/U_{Sr}) \qquad [11]$$

$$U_{Sr^{++}} = ((R - R_1)/(R_2 - R_1))\, U_{Sr} \quad [12]$$

$$U_{Ca^{++}} = R_2\, U_{Sr^{++}} \quad [13]$$

Uptake of CaL and SrL were determined by subtracting the calculated values for $U_{Ca^{++}}$ and $U_{Sr^{++}}$ from U_{Ca} and U_{Sr}.

RESULTS AND DISCUSSION

The calculated concentrations of Ca^{++}, CaL, Sr^{++}, and SrL in solution for the treatments used in this research are shown in Table 1.

TABLE 1

Equilibrium concentrations (μM) of Ca^{++}, CaL, Sr^{++}, and SrL in the solutions used

L/Ca_T	Ca_T	Sr_T	L_T	CaL	SrL	CaL/SrL	Ca^{++}	Sr^{++}	Ca^{++}/Sr^{++}
EDTA									
3.0	250.0	2.50	750.0	250.0	2.50	100			
1.0	250.0	2.50	250.0	248.6	1.34	186	1.4	1.16	1.25
0.9	250.0	2.50	225.0	224.9	0.13	1720	25.1	2.37	10.61
0.7	250.0	2.50	175.0	175.0	0.035	4937	75.0	2.46	30.44
0.5	250.0	2.50	125.0	125.0	0.015	8160	125.0	2.48	50.31
—	25.1	2.37	0.0	—	—	—	25.1	2.37	10.61
—	75.1	2.46	0.0	—	—	—	75.1	2.46	30.44
—	125.0	2.48	0.0	—	—	—	125.0	2.48	50.31
DTPA									
3.0	250.0	2.50	750.0	250.0	2.50	100			
1.0	250.0	2.50	250.0	247.8	2.19	113	2.2	0.31	6.99
0.9	250.0	2.50	225.0	224.1	0.87	257	25.9	1.63	15.88
0.7	250.0	2.50	175.0	174.7	0.31	558	75.3	2.19	34.55
0.5	250.0	2.50	125.0	124.9	0.14	862	125.1	2.36	53.14
—	25.9	1.63	0.0	—	—	—	25.9	1.63	15.88
—	75.3	2.19	0.0	—	—	—	75.3	2.19	34.55
—	125.1	2.36	0.0	—	—	—	125.1	2.36	53.14
—	250.0	2.50	0.0	—	—	—	250.0	2.50	100.00

The total Ca and total Sr absorbed, their ratio, and the mean rates of uptake of Ca and Sr by the tomato plants over the two-day uptake period are given in Table 2. Since there was variation in mean plant size, uptake rate per cm of root rather than total uptake was used to compare treatments. Calcium uptake rate decreased as L increased where Ca_T remained constant. This decrease was greater with EDTA than with DTPA. These results indicate that CaL was less available than Ca^{++}.

Comparisons between chelate-free solutions and chelate-contain-

TABLE 2

Total and rate of Ca and Sr uptake by tomato plants as affected by chelate treatments

L/CaT	Ca uptake		Sr uptake		Ca/Sr
	Total μ moles/pot	Rate moles cm^{-1} sec^{-1} $\times 10^{14}$	Total μ moles/pot	Rate moles cm^{-1} sec^{-1} $\times 10^{16}$	
EDTA					
3.0	2.24	0.36	.04	0.69	52.3
1.0	63.41	6.22	1.67	16.45	37.9
0.9	82.93	6.85	1.68	14.14	48.9
0.7	98.02	8.07	1.59	13.15	61.4
0.5	102.00	9.51	1.38	12.90	73.5
NoL (0.9)*	35.73	2.90	3.22	26.22	11.1
NoL (0.7)	54.89	6.56	1.81	21.77	30.1
NoL (0.5)	133.60	9.40	2.65	18.66	50.4
$S_{\bar{x}}$	9.20	0.60	.16	1.82	—
DTPA					
3.0	51.44	4.15	1.00	8.10	51.0
1.0	63.72	6.95	0.70	7.64	90.7
0.9	60.06	7.73	0.59	7.66	93.0
0.7	82.01	7.83	0.84	8.00	97.6
0.5	81.38	8.60	0.82	8.79	98.1
NoL (0.9)*	23.20	2.01	1.42	12.40	16.3
NoL (0.7)	42.57	6.02	1.19	16.80	35.7
NoL (0.5)	100.15	8.07	1.86	15.05	53.6
NoL	114.32	10.00	1.10	9.72	102.8
$S_{\bar{x}}$	7.67	0.39	.16	1.1	—

* No chelate, Ca^{++} and Sr^{++} concentrations the same as Ca^{++} and Sr^{++} concentrations in indicated chelate treatments.

ing solutions where Ca^{++} concentrations were the same indicate that, adding CaL increased Ca uptake rate for both chelates. This increased uptake rate from the chelate indicates a source of Ca available to the plant other than the Ca^{++} present in solution initially.

The Ca/Sr ratio in plants grown in chelate-free solutions was approximately the same as the Ca/Sr ratios in the culture solution (Tables 1 and 2) confirming the nondiscriminatory absorption of Ca and Sr by tomato plants. However, the Ca/Sr ratio in plants grown in the EDTA and DTPA containing solutions fell between the Ca^{++}/Sr^{++} and CaL/SrL ratios initially in the solution. These ratios can be explained by assuming absorption of Ca from CaL as well as

from Ca^{++} and Sr from SrL as well as Sr^{++}. This agrees with the observation that adding CaL to solutions of Ca^{++} increased Ca uptake. It may also be noted that adding CaL increased uptake less than from adding an equivalent amount of Ca^{++}.

Calcium may be supplied to the root from CaL either by absorption into the root directly as CaL or as absorption of Ca^{++} after it is released into solution from CaL. When the root absorbs Ca^{++}, the initial equilibrium will be upset, some CaL will dissociate giving Ca^{++} and L^{x-}, and the system readjusts to a new equilibrium. However, if only Ca^{++} absorption occurs the proportion of L to Ca in solution will increase, and a greater proportion of the remaining Ca will be in the CaL form. This shift in equilibrium may be viewed as competition between the ligand and the plant root for the Ca^{++} in solution. With a greater proportion of L to Ca less Ca^{++} will be present in solution and its uptake will be reduced. When the root also absorbs CaL directly it does so at a slower rate than Ca^{++} so that the total rate of Ca uptake (Ca^{++} plus CaL) is reduced.

When EDTA and DTPA treatments are compared, the rate of Ca uptake with DTPA was much greater than with EDTA, particularly where L/Ca_T was three and essentially all Ca was as CaL. This large difference cannot be explained solely on the basis of ligand-plant root competition for Ca^{++} because CaDTPA has a higher formation constant (7.76×10^{10}) than CaEDTA (7.08×10^{10}), thus higher not lower rates of Ca uptake would be expected with EDTA. A higher formation constant provides more competition with the plant root for Ca. The greater uptake of Ca from DTPA than EDTA treatments can most logically be explained by assuming direct uptake of CaL and a faster absorption rate for CaDTPA than CaEDTA. This does not rule out some absorption of Ca^{++} after it is released from CaL.

Comparison between Ca^{++}/Sr^{++} and CaL/SrL ratios at the beginning of the experiment (Table 1) and those at the end of the experiment (Table 4) shows that in most cases the ratios decreased. If the plants absorbed only Ca^{++} and Sr^{++}, both that initially in solution and that which dissociated from the chelate, the Ca/Sr ratio of uptake should have been between the initial and final Ca^{++}/Sr^{++} ratios in solution. Actually the Ca/Sr ratios of uptake were much greater indicating that CaL and SrL must have been absorbed.

TABLE 3

Uptake rates of Ca^{++}, CaL, Sr^{++}, and SrL based on their initial concentration in the culture solution

L/Ca_T	Uptake, moles cm^{-1} sec^{-1}			
	Ca^{++} $\times 10^{14}$	CaL $\times 10^{14}$	Sr^{++} $\times 10^{16}$	SrL $\times 10^{16}$
EDTA				
1.00	0.15	6.07	13.18	3.27
0.90	1.46	5.38	13.82	0.32
0.70	3.98	4.09	13.06	0.08
0.50	6.47	3.04	12.86	0.04
DTPA				
1.00	0.11	6.83	1.61	6.03
0.90	0.83	6.90	5.21	2.45
0.70	2.42	5.41	7.04	0.96
0.50	4.41	4.19	8.30	0.49

TABLE 4

Mean uptake rates of Ca^{++}, CaL, Sr^{++}, and SrL and Ca^{++}/Sr^{++} and CaL/SrL ratios at the end of the experiment as influenced by rate of addition of two chelates

L/Ca_T	Uptake rate* (moles cm^{-1} sec^{-1}) of				Final cultural solution ratio of	
	Ca^{++}	CaL	Sr^{++}	SrL	Ca^{++}/Sr^{++}	CaL/SrL
	$\times 10^{14}$		$\times 10^{16}$			
EDTA						
1.0	0.13	6.10	12.5	4.0	0.78	126
0.9	0.79	6.06	13.4	0.7	0.24	860
0.7	3.92	4.15	13.1	0.1	29.5	4857
0.5	6.53	2.98	12.9	0.04	51.4	8248
DTPA						
1.0	0.06	6.89	1.02	6.62	5.8	95
0.9	0.86	6.87	5.29	2.37	16.3	265
0.7	2.21	5.62	6.91	1.09	32.0	517
0.5	4.03	4.57	8.22	0.57	49.0	795

* Calculated using the means of the Ca^{++}/Sr^{++} and CaL/SrL ratios at the beginning and end of the experiment.

Uptake rates

Uptake of Ca and Sr apportioned into that absorbed as the chelated and unchelated forms is given in Table 3. The values

represent the amount of each that had to be absorbed from the initial solutions to account for the Ca/Sr ratio in the plant. The uptake values of Table 2 were used to calculate the amount of L, Ca_T, and Sr_T remaining in the culture solution at the end of the experiment and from these values, CaL/SrL and Ca^{++}/Sr^{++} ratios in solution at the end of the experiment were calculated. The mean of the ratios at the beginning and end of the experiment were used to calculate the mean uptake rates of CaL, SrL, Ca^{++}, and Sr^{++} given in Table 4. These calculated values show that uptake of CaDTPA was much greater than CaEDTA. Using C^{14}-labelled chelates, Hill-Cottingham and Lloyd-Jones [4], also found differences in uptake between chelates when they compared EDTA and EDHPA (ethylene-bis-(orthohydroxyphenylacetic acid)).

When the rates of uptake are compared at L/Ca_T of 0.5, where initial CaL and Ca^{++} concentrations were approximately equal, the uptake rate of CaEDTA was 0.47 of the uptake rate of Ca^{++}. The uptake rate of CaDTPA was 0.95 the uptake rate of Ca^{++}, reflecting the higher availability of CaDTPA. Uptake rates of CaL will be influenced by the levels of Ca^{++} present and vice versa.

Since the level of Sr in the system was 0.01 the level of Ca and, because Ca was chelated perferentially to Sr, the rates of Sr uptake were influenced by changes in Ca^{++} and CaL levels in solution. However, since we have assumed that the plant does not differentiate between Ca^{++} and Sr^{++} or between CaL and SrL the relative availability of Sr^{++} and SrL should be the same as for Ca^{++} and CaL.

The use of double labelling of Ca and Sr with Ca^{45} and Sr^{85} and the measurement of the Ca/Sr ratio of Ca and Sr absorbed has shown that plant roots absorbed chelated as well as unchelated forms. It also showed that the chelated forms were absorbed more slowly than unchelated forms. This method of measurement also showed a difference between chelate ligands in their relative rates of absorption by the root. This procedure may be used to investigate other ligands and study the relation of ligand type to its rate of absorption. The procedure can also be used to study differences between plant species. This should offer an opportunity to determine the basic nature of the mechanism by which chelates affect nutrient absorption rates by plant roots.

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