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THE INFLUENCE OF HUMIDITY ON THE MINERAL COMPOSITION OF TOMATO PLANTS WITH SPECIAL REFERENCE TO CALCIUM DISTRIBUTION

by M. J. ARMSTRONG and E. A. KIRKBY

Department of Plant Sciences, The University of Leeds, Leeds LS2 9JT, England

KEY WORDS

Ash alkalinity Calcium Cation distribution Humidity Magnesium Mineral composition Oxalate Pectate Tomato

SUMMARY

Tomato plants grown in water culture under two different humidity regimes (50% R.H. and 95% R.H.) were harvested at intervals over the growth period and cation uptake and calcium distribution investigated. The following results were obtained:

1. Plants in the high humidity regime initially grew faster but at the final harvest after 22 days, dry matter yields were the same.

2. In the high humidity treatment particularly towards the end of the experiment, the growth of the young leaves was disturbed and the plants showed symptoms resembling Ca or B deficiency. Analysis revealed that these tissues were lower in Ca than comparative tissues of the low humidity plants. The converse was true for B.

3. Cation uptake was little affected by the humidity treatment. However, the distribution of calcium within the plant was markedly influenced by humidity. In the high humidity treatment the level of Ca in the young leaves was very low and Ca accumulated in the stems. A high proportion of both Ca and Mg in these younger Ca deficient leaves was found to be associated with pectate. The same trend was observed in the stems, more of the Ca and Mg in this case being bound to oxalate as well as pectate. These results further indicate the possible significance of exchange movement of Ca particularly under low transpiration conditions when Ca transport by mass flow is restricted.

4. Xylem sap analysis showed a close cation-anion balance in all samples. NO_3-ion was the predominant anion and the two major cations were K and Ca. This provides further evidence that the upper plant parts provide the major site of NO_3-reduction in the tomato plant.

INTRODUCTION

Calcium related disorders are becoming of increasing importance in a number of agricultural and horticultural crops[7,16,22,23]. Soil/plant studies have shown that although the soil may be adequately supplied with calcium, deficiency disorders can still occur[7,16]. The uptake and distribution of Ca within the plant is thus a very important aspect of this soil/plant problem.

Calcium transport in plants is restricted almost exclusively to the xylem in which it moves upwards from the roots[4,14,16,18,28]. In a study of xylem exudate collected at different times after decapitation of tomato plants, we observed a very close relationship between the movement of Ca and water through the roots[7]. Regardless of the weight of sap exuded, the Ca concentration in the exudate always remained approximately the same as that of the outer solution. This relationship between Ca and water led us to consider the influence of water movement on the uptake and distribution of Ca in the intact plant. Here transpiration as well as root pressure plays a decisive role on Ca movement and both can have a direct bearing on the occurrence of Ca deficiency disorders.

Earlier findings of the influence of transpiration on Ca uptake are at variance. Hylmö[5], working with pea plants, reported that more than 75% of the total uptake of Ca could occur directly by mass flow with water. On the other hand, Michael and Marschner[20], working with oats and mustard plants, observed only a slight depression in Ca uptake in plants grown when the transpiration was depressed by 7–10 times that of the control plants. A poor correlation between Ca uptake and transpiration has also been reported by other authors using different plant species[13,26].

In the present work cation uptake and calcium distribution were investigated in tomato plants grown under two very different humidity regimes. Boron was also determined because of its similarity in behaviour to Ca in uptake and distribution[21]. In order to follow Ca transport, xylem exudates from decapitated plants were also collected. The aim of this study was thus to assess the importance of transpiration in cation uptake and the distribution of Ca by intact tomato plants. In view of the increasing interest in the importance of exchange reactions in Ca transport in plants[1,6,11,27,28], Ca and Mg were fractionated in the different plant tissues to estimate pectate and oxalate binding.

METHODS

Tomato plants (*Lycopersicon esculentum* var. Ailsa Craig) were germinated and grown in the glasshouse in a soil compost mixture. At the 4 leaf stage of growth the plants were removed and their roots thoroughly rinsed with distilled water to remove any adhering soil particles. Plants of similar size and weight were transferred to 52 litre tanks holding aerated nutrient solution. Each tank supported 12 plants.

The plants were grown in growth chambers at two different relative humidities, one growth chamber at 50% R.H. and the other at 95% R.H. Other environmental factors were the same in both treatments, *i.e.* a light intensity of 80 W m^{-2} provided by warm white fluorescent tubes and a 16-h light and 8-h dark cycle at temperatures of 23°C and 18°C respectively. Each growth chamber contained 3 tanks.

The nutrient solution used was as follows: $Ca(NO_3)_2$ (10 meq/l); K_2SO_4 (1 meq/l); NaH_2PO_4 (1 meq/l) and $MgSO_4$ (2 meq/l). The micronutrients and iron were added as described previously[9]. In

order to maintain the concentration of the nutrient medium as constant as possible the nutrient solutions were changed daily.

Plants were harvested after 5, 10, 14, 18, 20 and 22 days of growth in the nutrient medium. For the first 4 harvests, 6 plants were taken from both treatments and divided into tops and roots. The weights of fresh and oven dried (85°C) material from each plant were recorded.

At harvest 5 and 6 (after 20 and 22 days) 6 plants were harvested in both of the humidity treatments. Initially the plants were decapitated 1 cm above the root system and divided into young leaves, old leaves and stems. For each plant the fresh and oven dried (85°C) weights were recorded. The roots with 1 cm of the stem remaining were used for sap collection. The stump was fitted with a short length of PVC tubing to allow the exudate to collect. This was removed by means of a syringe. Exudates obtained during the first 10 minutes after decapitation were discarded. Sap was then collected and removed and weighed at 5 minute intervals for the next 20 minutes. On completion of sap collection, fresh weights were taken of the remaining portions of stem and of the total roots. Both tissues were then separately oven dried and weighed.

Chemical analyses for Ca, Mg, K and Na were carried out on the bulked material of the individual dried plant parts for each harvest. In addition, at harvests 5 and 6 dried plant material from the different plant tissues was extracted four times with boiling water and Ca, Mg, oxalate and total organic anions (alkalinity of ash) estimated in the insoluble residues. Pectate was calculated as the difference between the total negative organic charge (ash alaklinity) and oxalate[10]. Boron was also estimated on the dried plant material in samples from the last two harvests. The sap was analysed for inorganic cations and anions. The methods used are described elsewhere[3,9].

RESULTS

The influence of humidity on the growth and cation uptake and content of tomato plants is shown in Table 1. The different humidity treatments exerted a pronounced influence on plant development. Plants of the high humidity treatment (low transpiration) grew more rapidly up to the fourth harvest (18 days) but as the experiment progressed yield differences became smaller, until at the final harvest the yields of both treatments were identical.

Not only did the growth of the plants differ between the two humidity treatments but so also did their appearance. These differences became particularly marked after the fourth harvest. In contrast to the plants grown under normal humidity conditions (low humidity), plants from the high humidity treatment showed abnormal leaf growth. The leaves in this treatment were darker green and the younger leaves in particular were poorly developed and curled. The whole appearance of the plants resembled Ca or B deficiency.

Cation uptake reflected the trend in growth rate. Until the final harvest the total uptake of cations was somewhat higher in the high humidity treatment. The uptake of the individual cations followed the same pattern. Expressed as a percentage of the dry matter there was thus little difference between the two humidity treatments in cation content on a whole plant basis.

The distribution of dry weights between different plant tissues for the two humidity treatments at harvest are shown in Table 2. The results reinforce the

Table 1. The influence of humidity on the dry weight yields (g/plant) cation uptake (meq/plant) and cation content (% dm) during the growth of tomato plants

Humidity	Plant dry weight (g/plant)		K		Ca		Mg		Na		Total
			meq	%	meq	%	meq	%	meq	%	
Harvest No 1											
Low	0.38	***	0.5	(5.1)	0.5	(2.6)	0.2	(0.6)	0.1	(0.6)	*1.3*
High	0.52		0.8	(6.0)	0.6	(2.3)	0.2	(0.5)	0.2	(0.9)	*1.8*
Harvest No 2											
Low	1.49	***	2.4	(6.3)	2.3	(3.1)	0.7	(0.6)	0.4	(0.6)	*5.8*
High	2.12		3.9	(7.2)	3.0	(2.8)	1.2	(0.7)	0.5	(0.5)	*8.6*
Harvest No 3											
Low	3.44	***	5.6	(6.4)	5.8	(3.4)	2.0	(0.7)	0.8	(0.5)	*14.2*
High	4.89		9.2	(7.3)	8.2	(3.4)	2.8	(0.7)	1.1	(0.5)	*21.3*
Harvest No 4											
Low	8.99	**	14.8	(6.4)	15.2	(3.4)	4.7	(0.6)	1.5	(0.4)	*36.2*
High	10.25		18.7	(7.1)	16.5	(3.2)	5.2	(0.6)	1.8	(0.4)	*42.2*
Harvest No 5											
Low	13.12	N.S.	21.3	(6.3)	17.3	(2.6)	6.4	(0.6)	3.2	(0.6)	*48.2*
High	14.04		26.0	(7.2)	17.5	(2.5)	6.9	(0.6)	3.3	(0.5)	*53.7*
Harvest No 6											
Low	18.41	N.S.	32.1	(6.8)	26.0	(2.8)	9.1	(0.6)	3.8	(0.5)	*71.0*
High	18.41		33.1	(7.0)	24.6	(2.7)	8.9	(0.6)	3.8	(0.5)	*70.4*

*** Significant at 0.1% level; ** Significant at 1% level; N.S. Non significant.

Table 2. The influence of humidity on the distribution of dry matter yields (g/plant) between plant tissues of tomato plants at harvest 6

Humidity	Dry weight (g/plant)								
	Young leaves	(%)	Old leaves	(%)	Stems	(%)	Roots	(%)	Total
Low	3.15	(17.1)	6.86	(37.3)	5.93	(32.2)	2.47	(13.4)	*18.41*
	***		***		***		N.S.		
High	2.13	(11.6)	5.46	(29.7)	8.63	(46.9)	2.19	(11.9)	*18.41*

*** Significant at 0.1% level; N.S. Non significant.
Figures in parentheses indicate % distribution.

Table 3. The influence of humidity on the total uptake and concentrations of Ca (meq/100 g dry weight and B (mg/100 g dry weight) in different tissues of the tomato plant

Humidity	Young leaves	(%)	Old leaves	(%)	Stems	(%)	Roots	(%)	Total
					Calcium				
Low	103	(12.7)	216	(56.9)	114	(25.8)	(48)	(4.6)	*26.0*
High	78	(6.3)	226	(50.0)	113	(39.4)	32	(3.6)	*24.6*
					Boron				
Low	2.7	(18.0)	3.8	(52.0)	1.8	(20.0)	2.0	(10.0)	*0.50*
High	3.7	(13.6)	3.9	(35.6)	2.6	(37.3)	3.5	(13.6)	*0.59*

Figures in parentheses indicate the % Ca and B distribution between tissues.

recorded visual symptoms. In the high humidity treatment a considerably greater proportion of the total weight was accounted for in stem material (approx. 46% against 32%). The proportion in the old leaves and particularly in the young leaves was correspondingly lower.

Table 3 shows the distribution of Ca and B between tissues at harvest 6. The most striking observation in these results is the lower Ca concentration observed in the young leaves of the high humidity treatment. This lower value is associated with a depression in dry matter production (Table 2). The concentrations of Ca in other tissues are similar in both treatments. However, there is a Ca accumulation in the stems of the high humidity treatment because of the higher yield of these tissues. The uptake of B is slightly higher in the high humidity plants. Distribution follows a similar pattern to that of Ca with a large fraction occurring in the old leaves and stem of the high humidity treatment. In contrast to the Ca distribution, however, the younger leaves of the high humidity treatment are higher in B.

The ionic balances of the water insoluble fractions from plant tissues at harvest 6 are shown in Table 4. Calcium and Mg are also expressed as a percentage of their total concentrations in the individual plant tissues. In the balance the cations are made up by insoluble Ca and Mg whereas the anions are the negative organic charges of oxalate and pectate. The balance between total cations and anions is reasonably good suggesting that the most important contributing charges have been taken into account.

The comparison of results from similar tissues in this table provides further information concerning Ca distribution. In the young leaves of the high humidity treatment where Ca concentration was depressed (Table 3) a much higher proportion of the total Ca was present in insoluble form (75.6% against 47.6%).

Table 4. The influence of humidity on the cation-anion balance (meq/100 g dry weight) in the water insoluble residues of different tissues of the tomato plant at harvest 6

	Humidity	Ca	(%)	Mg	(%)	Total cations	Oxalate	Pectate	Total anions
Young leaves	Low	49	(47.6)	3	(7.7)	*52*	26	24	*50*
	High	59	(75.6)	8	(23.5)	*67*	24	38	*62*
Old leaves	Low	93	(43.1)	2	(4.0)	*95*	47	43	*90*
	High	88	(38.9)	4	(7.7)	*92*	39	46	*85*
Stems	Low	72	(63.2)	4	(8.0)	*76*	50	30	*80*
	High	90	(79.7)	11	(22.9)	*101*	64	40	*104*
Roots	Low	28	(58.3)	11	(18.0)	*39*	7	28	*35*
	High	28	(71.8)	7	(13.0)	*35*	8	29	*37*

Figures in parentheses indicate % of the total concentrations of Ca and Mg respectively.

A higher proportion of Mg was also found in the insoluble fraction. Oxalate values were similar in both treatments, and the increase in cation charge in the high humidity treatment was balanced by an increase in pectate. A similar but less pronounced effect on the Ca and Mg insoluble fractions was observed in the stem. In this case oxalate as well as pectate contributed to balancing the increases in Ca and Mg charge. The increase in percentage of insoluble Ca in the roots of the high humidity plants is of less quantitative importance because of the lower total root Ca amounts.

The influence of humidity on the weight and cation-anion balance of exuded sap at the 20 minute sampling time is shown in Table 5. As all sampling times investigated the amount of exudate obtained from the low humidity treated plants was approximately twofold that given by the high humidity plants. There was also a very close agreement between total cations and total anions in all

Table 5. The influence of humidity on the weight (g) and cation-anion balance of sap exuded at the 20 minutes sampling time. (The experiment began 10 minutes after decapitation. Sap exuded prior to this was discarded because of possible phloem contamination)

Humidity	Sampling time	Exudate weight	Cations (meq/l)					Anions (meq/l)				
			K	Ca	Mg	Na	Total	NO_3	SO_4	H_2PO_4	Cl	Total
Low	20	1.1	16.9	12.4	3.3	1.3	*33.9*	28.9	3.0	1.7	1.0	*34.3*
High	20	0.6	19.2	12.9	3.3	1.8	*37.2*	29.0	3.4	1.7	1.6	*35.7*

samples. This indicates that the main ionic components have been accounted for and are inorganic. The quantitatively most important charges making up the balance are K-ions, Ca-ions and NO_3-ions.

DISCUSSION

The stimulation in early growth of plants of the high humidity treatment (Table 1) is consistent with the findings of Michael and Marschner[20]. This effect probably results from the more fully open stomates in these plants which enabled better CO_2 assimilation and thus faster leaf expansion. This is in agreement with much recent evidence of a direct stomatal response to humidity[12,24].

The loss of this advantage in growth of the high humidity treatment was accompanied by an onset of symptoms in the young leaves of these plants resembling Ca or B deficiency. As Ca and B behave similarly in uptake and distribution[21] it was essential to distinguish which of these nutrients was causing the disorder. At both humidities the distribution patterns of Ca and B were found to be similar (Table 3). However, the Ca levels of the young leaves of the high humidity plants were somewhat lower than in the plants of the low humidity treatment whereas the reverse was true for B. This clearly indicates that the deficiency in the young leaves was due to Ca and not B.

The uptake of Ca and other cations was little affected by humidity. The similarity in behaviour of Ca, an ion which is largely taken up passively[15] with that of K where metabolic regulation is important[19,25] probably relates to the role of both these two ions as counterions to NO_3 ion uptake and transport (Table 5). The relationship probably also accounts for the absence of an effect of humidity on Ca uptake, for an increase in transpiration can only stimulate Ca uptake if NO_3 uptake is also increased.

The marked effect of humidity in restricting Ca distribution can be seen in Table 3. Calcium accumulated in the stems and only low amounts reached the young leaves. With the slower rate of water flow through the high humidity plants it may be supposed that more NO_3–N associated with Ca ions was assimilated in the stems. Oxalate formation following NO_3-reduction was thus stimulated[8] and Ca depleted from the xylem stream by fixation as Ca oxalate. Moreover, Ca is immobile in the phloem[4,16] so that its movement is entirely dependent on the xylem pathway. This contrasts markedly with K and N the other two major constituents of the xylem stream since both these elements are also highly mobile in the phloem and can thus be transported to the younger tissues despite high humidity conditions.

There is increasing evidence that Ca movement in plants takes place not only

by mass flow but also by a series of exchange reactions along negatively charged sites of the xylem vessels[1,6,17,27]. The results of Table 4 indicates the possible importance of exchange reactions in Ca transport under low transpiration conditions. In the young leaves of the high humidity plants where the Ca levels were lower than in comparative low humidity plants, a much greater proportion of the Ca was associated with pectate. A similar trend was observable in the stems. Calcium ions are needed to maintain the integrity of membrane structures[16,19]. We therefore also conclude that in these Ca deficient tissues of the high humidity plants the predominant association of Ca with exchange sites precludes the normal physiological roles of Ca.

The behaviour of Mg was somewhat similar to that described above for Ca suggesting that Mg too can be transported by exchange reactions. This is consistent with the report of Ferguson and Clarkson[2] that both Mg as well as Ca follows the apoplastic pathway across the root cortex. Our results indicate, that the proportion of indiffusible Mg of the total Mg is much lower than the corresponding value for Ca. This indicate that a relatively higher proportion of the total Mg was moved by mass flow than by exchange reactions.

The sap analysis (Table 5) further substantiates our view that in tomato plants the upper plant parts provide the principal site of NO_3-reduction[8] and that K and Ca ions are the major accompanying cations in NO_3-transport. The importance of root pressure on Ca transport has recently been stressed by Palzkill and Tibbits[22] who reported that root pressure enabled the inner leaves of cabbage plants to be adequately supplied with Ca. The data presented here also show that root pressure can provide the tops with a source of Ca. It is of interest to note that in our experiment root pressure was depressed in the high humidity treatment. However, the contribution of root pressure to Ca movement in this treatment may well have been considerable because of the relatively more restricting effect of high humidity on the transport of Ca *via* the transpiration stream.

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REFERENCES

1 Bell, C. W. and Biddulph, O. 1963 Translocation of calcium. Exchange versus mass flow. Plant Physiol. **38,** 610–614.

2 Ferguson, I. B. and Clarkson, D. T. 1976 Simultaneous uptake and translocation of magnesium and calcium in barley (*Hordeum vulgare* L.) roots. Planta **128,** 267–269.

3 Hall, D. A. 1971 The influence of varied calcium nutrition on the growth and ionic composition of plants. Ph.D. Thesis, University of Leeds.

4 Hanger, B. C. 1979 The Movement of calcium in plants. Commun. Soil Sci. Plant Anal. *In Press.*

5 Hylmö, B. 1953 Transpiration and ion absorption. Physiol. Plant. **6,** 333–405.
6 Isermann, K. 1970 Der Einfluss von Adsorptionsvorgängen im Xylem auf die Calcium-Verteilung in der höheren Pflanze. Z. Pflanzenernaehr. Bodenkd. **126,** 191–203.
7 Kirkby, E. A. 1979 Maximizing calcium uptake. Commun. Soil Sci. Plant Anal. *In Press.*
8 Kirkby, E. A. and Knight, A. H. 1977 Influence of the level of nitrate nutrition on ion uptake an assimilation, organic acid accumulation and cation-anion balance in whole tomato plants. Plant Physiol. **60,** 349–353.
9 Kirkby, E. A. and Mengel, K. 1967 Ionic balance in different tissues of the tomato plant in relation to nitrate, urea or ammonium nutrition. Plant Physiol. **42,** 6–14.
10 Knight, A. H., Unpublished data.
11 Koontz, B. V. and Foote, R. E. 1966 Transpiration and calcium deposition by unifoliate leaves of *Phaseolus vulgaris* differing in maturity. Physiol. Plant. **19,** 313–321.
12 Lange, O. L., Lösch, R., Schulze, E-D and Kappen, L. 1971 Response of stomata to changes in humidity. Planta **100,** 76–86.
13 Lazaroff, N. and Pitman, M. G. 1966 Calcium and magnesium uptake by barley seedlings. Aust. J. Biol. Sci. **19,** 991–1005.
14 Loneragan, J. F. and Snowball, K. 1969 Calcium requirement of plants. Aust. J. Agric. Res. **20,** 465–478.
15 Maas, E. V. 1969 Calcium uptake by excised maize roots and interactions with alkali cations. Plant Physiol. **44,** 985–989.
16 Marschner, H. 1974 Calcium nutrition of higher plants. Neth. J. Agric. Sci. **22,** 275–282.
17 Marschner, H. and Ossenberg-Neuhaus, H. 1977 Wirkung von 2,3,5-Trijodbezoesäure (TIBA) auf den Calcium-transport und Kationenaustauschkapazität in Sonnenblumen. Z. Pflanzenphysiol. Bd. **85,** 29–44.
18 Marschner, H. and Richter, C. H. 1974 Calcium-Transport in Wurzeln von Mais- und Bohnenkeimpflanzen. Plant and Soil **40,** 193–210.
19 Mengel, K. and Kirkby, E. A. 1978 Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland, 593 p.
20 Michael, G. and Marschner, H. 1962 Einfluss unterschiedlicher Luftfeuchtigkeit und Transpiration auf Mineralstoffaufnahme und -Verteilung. Z. Pflanzenernaehr. Bodenkd. **96,** 200–212.
21 Michael, G., Wilberg, E. and Kouhsiahi-Tork, K. 1969 Durch hohe Luftfeuchtigkeit Induzierter Bormangel. Z. Pflanzenerernaehr. Bodenkd. **122,** 1–3.
22 Palzkill, D. A. and Tibbitts, T. W. 1977 Evidence that root pressure flow is required for calcium transport to head leaves of cabbage. Plant Physiol. **70,** 854–856. (1977).
23 Shear, C. B. 1975 Calcium-related disorders of fruits and vegetables. Hortic. Sci. **10,** 361–365. (1975).
24 Sheriff, D. W. 1977 The effect of humidity on water uptake by, and viscous flow resistance of, excised leaves of a number of species: physiological and anatomical observations. J. Expt. Bot. **28,** 1399–1407 (1977).
25 Spanswick, R. M. and Williams, E. J. 1964 Electrical potentials and Na, K and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. J. Exp. Bot. **15,** 193–200.
26 Swallis, A. A. and O'Leary, J. W. 1975 The effect of relative humidity on growth, water consumption and calcium uptake in tomato plants. J. Ariz. Acad. Sci. **10,** 87–89.
27 Van de Geijn, S. C., Petit, C. M. and Roelofsen, H. 1979 Measurement of the cation exchange capacity of the transport system in intact plant stems. Methodology and preliminary results. Commun. Soil Sci. Plant Anal. *In Press.*
28 Wiersum, L. K. 1979 Effects of environmental and cultural practices in calcium nutrition. Commun. Soil Sci. Plant Anal. *In Press.*