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Accumulation and subcellular distribution of cations in relation to the growth of potassium-deficient barley

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Abstract. Growth of barley (*Hordeum vulgare* L., cv. Georgie) was insensitive to soil K content above about 150 mg kg^{-1}, but at lower levels it declined. The reduction in yield was greater in soils containing approximately 10 mg Na kg^{-1} than in soils with about 90 mg kg^{-1} of Na. Growth was unaffected by changes in shoot K concentration above 75 mol m^{-3}, but declined at lower concentrations, and the decrease was less in plants grown in soils with high Na. Growth responses were not simply related to tissue K concentrations because plants grown in soils with extra Na had higher yields but lower K concentrations.

When soil Na was low, plants accumulated Ca as tissue K declined, but when Na was provided this ion was accumulated. Plant Mg concentrations were generally low but increased as K decreased. The Ca and Mg were osmotically active. There were highly significant inverse linear relationships between yield and either the Ca or Mg concentrations in the shoots.

X-ray microanalysis was used to examine the compartmentation of cations in leaves from barley plants (cv. Clipper) grown in nutrient solutions with high and low K concentrations. In plants grown with 2.5 mol m^{-3} K, this was the major cation in both the cytoplasm and vacuole of mesophyll cells. However, in plants grown with 0.02 mol m^{-3} K it declined to undetectable levels in the vacuole, although it was still detectable in the cytoplasm. In all plants, Ca was mainly located in epidermal cells. The implication of the results for explaining responses to K in terms of compartmentation of solutes is discussed.

Key-words: Hordeum vulgare L.; Gramineae; barley; growth responses; solute compartmentation; potassium; X-ray microanalysis; vacuole; cytoplasm.

Introduction

Potassium salts are accumulated to high concentrations in the vacuoles of many plants and are important for the generation of turgor (e.g. Läuchli & Pflüger, 1979; Wyn Jones, Brady & Speirs, 1979; Leigh & Wyn Jones, 1984). When the supply of K is limited, however, its concentration in the vacuole declines and other solutes must be accumulated to maintain turgor (Leigh & Wyn Jones, 1984). The nature of these solutes varies considerably. In barley, for instance, K may be replaced to varying extents by Na, Ca, Mg or reducing sugars (Flowers & Läuchli, 1983; Forster & Mengel, 1969; Leigh & Johnston, 1983; Pitman, Courtice & Lee, 1968; Pitman, Mowat & Nair, 1971). Carrot explants show a similarly wide variation in their ability to replace K (Mott & Steward, 1972).

This variable behaviour is consistent with the suggestion that the lack of biochemical processes within the vacuole allows a wide range of solutes to be accumulated without direct, deleterious effects on metabolism (Wyn Jones *et al.*, 1979). Using this as a basis, Leigh & Wyn Jones (1984) suggested that when inorganic cations were accumulated to replace vacuolar K, growth would be decreased only when there was insufficient K to maintain cytoplasmic concentrations of this ion. Thus, growth was not expected to be sensitive to the nature of the cation in the vacuole. However, when barley was grown in K-deficient soils, in the field at Rothamsted, growth was decreased even though K was mainly replaced by Na and Ca (Leigh & Johnston, 1983). These plants contained 50–70 mol m^{-3} K, which is above the level at which direct effects on cytoplasmic K concentration might be expected (see Leigh & Wyn Jones, 1984). This raises the possibility that there may be effects associated with the replacement of vacuolar K by Na or Ca.

In this paper, relationships between growth and the cation composition of barley are described. To duplicate compositional responses observed in the field (Leigh & Johnston, 1983), plants were grown in soil mixtures containing a range of K concentrations. The soils were from a long-term experiment in which fertilizer treatment had specifically decreased K content. X-ray microanalysis was used to make qualitative investigations of the compartmentation of cations in K-deficient leaves. For practical reasons, the plants used for X-ray microanalysis had to be grown in modified Hoagland's nutrient solution.

Materials and methods

Plant material

Seeds of barley (*Hordeum vulgare* L. cv. Georgie or Clipper) were germinated in the light in trays covered

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Table 1. Chemical analyses of some of the soils, as collected from the field

Soil	pH	$NaHCO_3$-soluble P (mg kg^{-1} dry soil)	Exchangeable cation concentration (mg kg^{-1} dry soil)			
			K	Ca	Mg	Na
1	7.3	44	33	1847	44	4.1
2	7.4	33	105	1718	40	3.2
3	7.1	45	177	1647	39	7.9

with polyethylene and lined with damp filter paper. Seedlings were transferred to the experimental growth conditions at about 1-week of age when the coleoptiles were about 2 cm long and roots about 5 cm. Freshly harvested leaves of spinach (*Spinacia oleracea*) and sugar beet (*Beta vulgaris* L.) were kindly provided by Dr S. G. Gutteridge and Mr T. Pocock.

Preparation of soils and growth of plants

A sandy loam soil of the Stackyard Series (Catt *et al.*, 1980) was collected from various plots of the Woburn Reference Plot Experiment (Widdowson, Penny & Hewitt, 1982) at Woburn Experimental Farm, Bedfordshire. After drying at room temperature the soils were sieved through a 3 mm mesh and samples were taken for analysis. Based on the results of these analyses (Table 1), the soils were combined in various proportions to give mixtures with a range of exchangeable K concentrations (Table 2). Four hundred and fifty grams of each mixture were placed in 12 cm plastic plant pots and solutions of NH_4NO_3, $NH_4H_2PO_4$ and $MgSO_4$ were added to give final cocentrations of N, P and Mg of 200, 70 and 70 mg kg^{-1} dry soil, respectively. If required, additional K and Na were also added as solutions of K_2SO_4 or Na_2SO_4. The next day the soil from each pot was thoroughly re-mixed, 400 g were replaced in the pot and the remainder retained for a final analysis, the results of which are used here.

The day before the seedlings were planted, the soil was thoroughly dampened with deionized water and five seedlings (cv. Georgie) were planted in each pot. More water was then added to bring the soil to approximately 50% of its saturated water-holding capacity of about 200 cm^3 kg^{-1} dry soil. This was maintained throughout the experiment by watering the pots to weight once or twice daily. The pots were arranged randomly in a Saxcil controlled environment cabinet. The growing conditions were: 14 h d, 20/16 °C day/night temperature, and 70/90% day/night relative humidity. Light was provided by standard fluorescent tubes colour no. 35 with supplementary tungsten, giving approximately 425 μE m^{-2} s^{-1} at the soil surface. Plants were grown for up to 30 d and then all aerial plant parts from a pot were harvested. Total fresh weight was determined and representative samples were taken for sap, and sometimes, sugar extraction. The remainder was dried at 80 °C overnight then ground before extraction of cations.

Growth of plants for X-ray microanalysis

X-ray microanalysis studies were performed at the CSIRO Division of Horticultural Research, Merbein. Soils of similar composition to those at Rothamsted were not available, and so barley (cv. Clipper) was grown in a modified Hoagland's nutrient solution containing 2.5 or 0.02 mol m^{-3} K (as K_2SO_4), 1 mol m^{-3} $MgSO_4$, 1 mol m^{-3} $NH_4H_2PO_4$, 5 mol m^{-3}

Table 2. Expected and measured concentrations of exchangeable cations in some soil mixtures

Exchangeable cation concentration (mg kg^{-1} dry soil)											
Expected*				Measured							
				Before amendment				After amendment‡			
K	Ca	Mg	Na	K	Ca	Mg	Na	K	Ca	Mg	Na
50	1456	47	8.5	47	1398	46	6.2	51	1574	73	88
100	1680	39	5.5	96	1654	39	6.0	98	1697	63	87
150	1684	40	8.0	139	1614	38	5.3	147	1745	66	84
300†	1647	39	7.9	ND	ND	ND	ND	291	1630	62	80

*Based on analyses of original soils and the proportions in which they were mixed.
†Extra K added as K_2SO_4.
‡Extra Mg and Na added to give expected final concentrations of approximately 70 and 90 mg kg^{-1} dry soil, respectively.
ND = not determined.

$Ca(NO_3)_2$ and with micronutrients at the concentrations given by Sutcliffe & Baker (1974), except that Fe was added as FeNaEDTA. Four seedlings were grown in 2.5 dm^3 of solution in plastic buckets in a controlled environment cabinet. Light (250 $\mu E\ m^{-2}\ s^{-1}$ at plant level) was provided by high-pressure mercury vapour lamps, the day length was 12 h and the day/night temperatures were 23/17 °C. All solutions were aerated and water lost through transpiration was replaced daily. Solutions were changed every 3 or 4 d. After 13 d, three plants from each pot were harvested, weighed, and then analysed for cations as described below. The remaining plant was used for X-ray microanalysis.

X-ray microanalysis

All observations were made on tissue from midway along the first fully expanded leaf. Tissue was mounted in Cu holders and rapidly frozen in melting N_2. The frozen sample was fractured in a modified Balzers SCU 102 preparation chamber with the stage temperature at −100 °C. Cell detail was revealed by subliming ice from the sample by raising the specimen temperature to −86 °C for 2 min. For reproducibility this process was controlled by a Yamatake–Honeywell temperature programmer. After etching, the specimen was re-cooled to −130 °C then evaporatively coated with Cr. The coated specimen was analysed in a Philips 500 scanning electron microscope fitted with an EDAX energy dispersive X-ray analyser. Spectra from 0 to 5 keV were recorded. The accelerating voltage was 12 kV, the emission current 40 μA, the spot diameter was 0.4 μm and data collection time 100 live s. During X-ray analysis the beam was focused in the 'reduced area mode'.

Chemical analyses

Cell sap was extracted using the freeze/thaw technique described by Tomos *et al.* (1984). Sap osmotic pressure was determined using a Wescor 1500 C vapour pressure osmometer. Sugars were extracted and assayed as previously described (Leigh *et al.*, 1979), except that reducing sugars were measured by the Somogyi–Nelson method (Somogyi, 1952). For analysis of cations the sap was diluted with 600 mol m^{-3} HCl. Total cation contents of tissue were determined by extracting ashed (450 °C, 3 h) plant material with 600 mol m^{-3} HCl at 80 °C. The Ca oxalate content of leaf tissue was determined as acetic acid-insoluble Ca (Gallagher, 1975). This was calculated as the difference between total Ca content (HCl extraction of ashed tissue) and that extracted from oven-dried tissue by 1 mol dm^{-3} acetic acid after 2 h at room temperature. Exchangeable cations in soils were extracted with 1 mol dm^{-3} ammonium acetate, as described by Metson (1956). Cation concentrations in all solutions were determined by atomic absorption or using an inductively coupled plasma optical emission spectrometer. Bicarbonate-soluble P was extracted from soils with 500 mol m^{-3} $NaHCO_3$ at pH 8.5 (Olsen *et al.*, 1954) and was measured using a Technicon AutoAnalyser. Soil pH was determined by mixing 10 g soil with 25 cm^3 of water and measuring the pH of the suspension after 1 h.

Replication and variability

All treatments were triplicated and results are reported as the mean for each treatment. Standard errors were always less than 10% of the mean and usually less than 5%. For clarity, standard errors are not indicated in the Figs.

Results

Soil cation concentrations

The experimental plots from which the soils were taken had received different fertilizer treatments between 1960 and 1979. These treatments resulted in different concentrations of exchangeable K in the soils but similar levels of other exchangeable cations, bicarbonate-soluble P, and soil pH (Table 1). By combining the soils in various proportions, new soil mixtures were produced that differed significantly only in their concentrations of exchangeable K (Table 2). To increase the range of K concentrations, K_2SO_4 was added to some mixtures. Extra Mg (as $MgSO_4$) was added to all mixtures, and Na concentrations in some were increased with Na_2SO_4. Analyses of the soils after these additions are given in Table 2.

The effect of Na on the yield of barley grown in soils with different K concentrations

Growth of barley decreased with decreasing K concentration in the soil but the effect was modified by Na (Fig. 1). In soils without extra Na the growth of plants was relatively insensitive to soil K concentration above 150 mg kg^{-1} dry soil. Below this concentration, growth decreased significantly and plants grown in soil with only 38 mg K kg^{-1} gave less than 40% of the fresh weight yield of plants grown in soil with 290 mg kg^{-1}. In soils with more than 150 mg K kg^{-1}, increasing the exchangeable Na concentration to approximately 90 mg kg^{-1} had little effect on fresh weight yield (Fig. 1). However, at lower soil K concentrations growth was increased significantly by the extra Na. At the lowest soil K concentration growth was almost doubled in the Na amended soil, although the yield was still not as great as that obtained in soils with high K concentrations.

Stimulation of growth by the addition of Na to a K-deficient soil was observed at all harvests in a

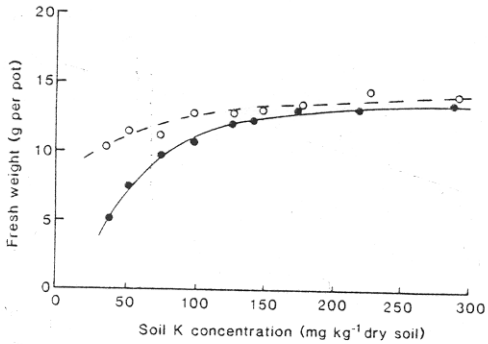

Figure 1. The relationship between soil exchangeable K concentration and the yield of barley shoots after 21 d growth. The soils contained approximately 10 mg (●) or 90 mg (○) exchangeable Na kg^{-1} dry soil.

time–course experiment (Fig. 2). The average rate of fresh weight increase in the presence of 90 mg kg^{-1} of Na was 0.49 g d^{-1} compared with 0.26 g d^{-1} for plants grown without additional Na. The differences in fresh weight were not the result of increased succulence in the plants grown with extra Na because dry matter accumulation showed similar differences (Fig. 2).

The relationship between yield and shoot K concentrations

Shoot K concentration declined with decreasing soil K content (see next section). When soil Na content was low, growth showed little response to increases in tissue K concentration above about 75 mol m^{-3} (approximately 1.2% K in dry matter) but declined below this concentration (Fig. 3). Those grown in soil with extra Na showed a steady but small response at all shoot K concentrations, but this did not change below 75 mol m^{-3} K. There was no

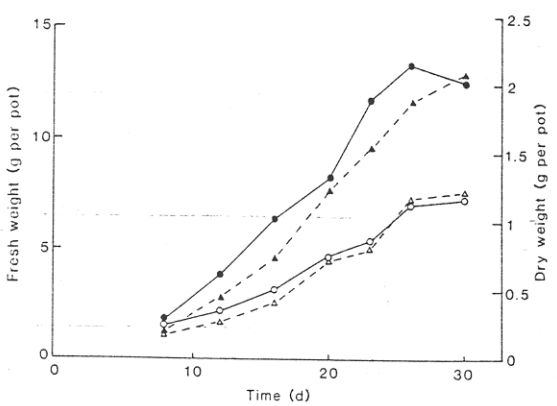

Figure 2. Time-dependent changes in the fresh weight (●, ○) or dry weight (▲, △) of shoots of barley. Plants were grown in soil with an exchangeable K concentration of 35 mg kg^{-1} dry soil and approximately 10 mg (○, △) or 90 mg (●, ▲) exchangeable Na kg^{-1} dry soil.

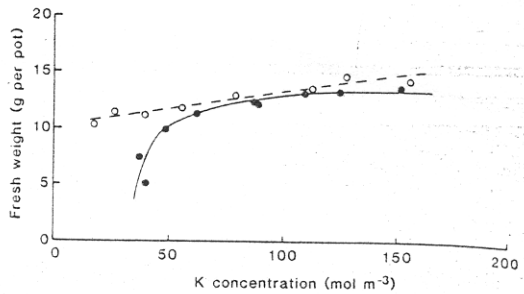

Figure 3. Relationship between shoot K concentration and the yield of barley shoots after 21 d growth in soils containing a range of exchangeable K concentrations and approximately 10 mg (●) or 90 mg (○) exchangeable Na kg^{-1} dry soil.

simple relationship between growth and tissue K concentrations because at the lowest soil K level the plants given extra Na had higher yields but lower shoot K concentrations than those grown without extra Na (Fig. 3). This suggested that at low soil K concentrations there might be a Na-dependent, inverse relationship between shoot K concentration and growth, and this was confirmed when plants were grown in soil containing 43 mg K kg^{-1} and a range of exchangeable Na concentrations (Fig. 4). As the soil Na content was increased from 8 to 95 mg kg^{-1}, the fresh weight increased linearly from 7 to 12 g per pot and was accompanied by a decrease in shoot K concentrations from 33 to 14 mol m^{-3}. Half of this reduction occurred when soil Na increased from 8 to 24 mg kg^{-1}. The decrease in K concentration was not due to increased succulence because water content was between 84% and 86% of the fresh weight in all treatments.

The effect of Na on the composition of barley grown in soils with different K concentrations

Potassium concentration in the shoots declined linearly with decreasing soil K concentration, irrespective of the soil Na concentration (Fig. 5). In

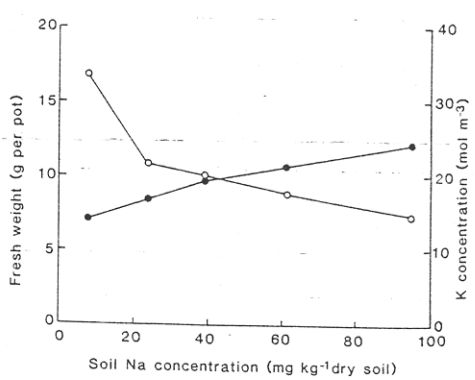

Figure 4. The effect of soil Na concentration on the yield (●) and K concentrations (○) of shoots of barley after 20 d growth in a soil containing 43 mg exchangeable K kg^{-1} dry soil.

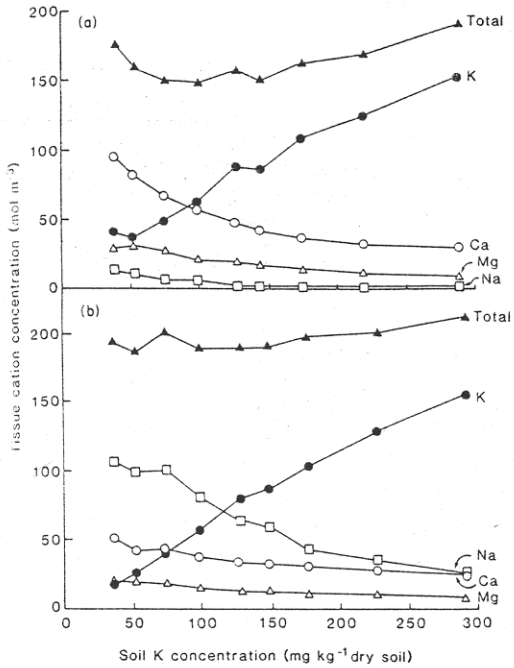

Figure 5. The effect of soil K concentration on the cation concentrations in shoots of barley. The soil contained approximately 10 mg (a) or 90 mg (b) exchangeable Na kg^{-1} dry soil.

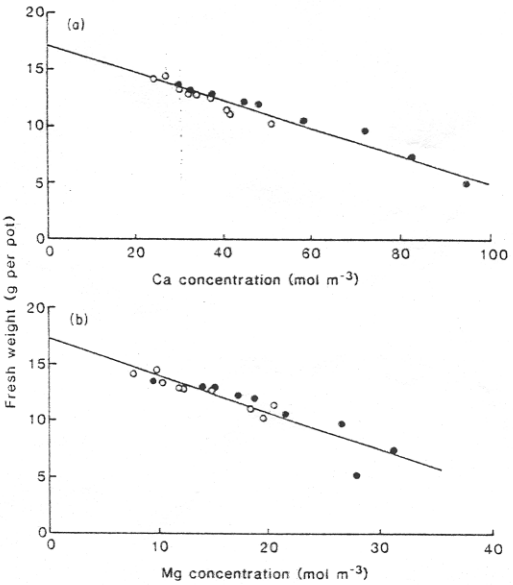

Figure 6. The relationship between yield and the Ca (a) or Mg (b) concentrations in shoots of barley. Plants were grown in soils containing a range of K concentrations and approximately 10 mg (●) or 90 mg (○) exchangeable Na kg^{-1} dry soil. The lines indicate the best fit to the data. (a) $y = -0.12x + 17.1$, $r = -0.975$; (b) $y = -0.32x + 17.2$, $r = -0.930$.

plants grown without extra Na, decreases in K were accompanied by the accumulation of Ca, which increased from a concentration of 30 mol m^{-3} in high K plants to 95 mol m^{-3} in low K plants (Fig. 5a). Concentrations of Mg and Na increased slightly in these plants but were always much lower than those of Ca. As a result of these changes, total cation concentration declined by a maximum of only 40 mol m^{-3}, even though K concentration decreased by 110 mol m^{-3} (Fig. 5a).

Total cation concentration also remained relatively constant in the plants grown in the Na-amended soil (Fig. 5b). However, Ca concentration increased only from 24 to 51 mol m^{-3} in response to the decrease in K whereas Na increased from 25 mol m^{-3} in the high K plants to over 100 mol m^{-3} in the low K plants. Increases in Mg concentration were again small.

Sodium appeared to have little effect on sugar concentrations, except in the plants grown with high K and high Na (Table 3). In this treatment, concentrations of reducing sugars were decreased significantly but in all other treatments sugar concentrations were unaffected by the soil cation concentration. Thus, there was no evidence that increased sugar concentrations were specifically associated with the treatments that decreased growth. This is important because Leigh & Wyn Jones (1984) suggested that diversion of fixed-C, particularly reducing sugars, into an osmotic role might account for decreased growth of some K-deficient plants.

Instead, the most obvious compositional change in

Table 3. Solute concentrations and sap osmotic pressure in barley grown in soils with low and high K concentrations and with or without extra Na

Soil exchangeable cation concentration (mg kg^{-1} dry soil)		Tissue solute concentration (mol m^{-3})						Sap osmotic pressure (mosm kg^{-1})	
K	Na	K	Ca	Mg	Na	Reducing sugar	Sucrose	Measured	Calculated*
42	9	34	95	39	15	93	35	426	570
43	84	28	46	21	116	90	27	453	562
290	4	193	40	12	2	74	31	471	494
285	78	175	31	9	30	15	22	455	431

*Using tables in Weast (1974) and assuming Cl^- was the major counteranion.

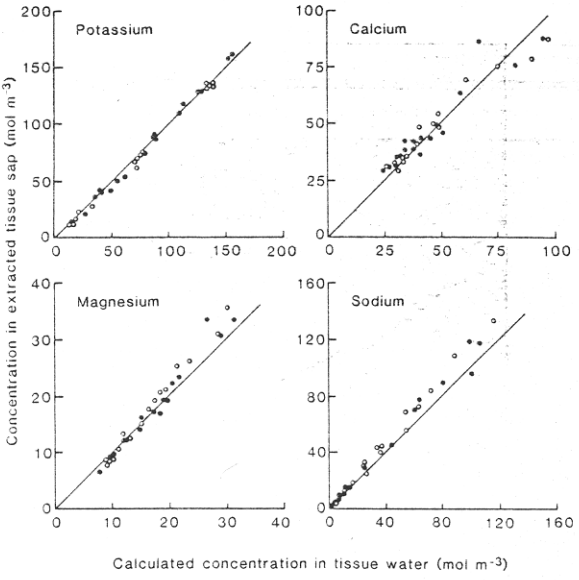

Figure 7. The relationships between the concentration of cations measured in sap extracted by freezing and thawing and the concentrations in tissue water calculated from the amounts of acid-extractable cations. The lines show the relationship expected if the concentrations in the sap were equal to those in the tissue water. Open and closed symbols represent results from different experiments.

plants in which growth was decreased was their greater concentration of Ca (Fig. 5a). As shown in Fig. 6a there was a highly significant inverse correlation between the Ca concentration in the shoots of the barley plants and their fresh weight yield. Further, this relationship seemed to hold for all treatments, irrespective of the Na or K concentrations in the soil. Magnesium concentrations were also inversely related to fresh weight yield (Fig. 6b) even though changes in Mg concentrations were smaller than those of Ca (Fig. 5). The relationships in Fig. 6 were not artefacts of using fresh weight yields and aqueous concentrations of cations in the tissue. Similar relationships were obtained when these parameters were expressed on a dry matter basis, although correlation coefficients were slightly smaller ($r = -0.950$ and -0.912 for Ca and Mg, respectively). Similar correlations between yield and Ca and Mg concentrations were also obtained in an experiment in which soil Na concentrations were varied at three different soil K levels (not shown). There were no obvious relationships between Na concentrations in the crops and their yield (not shown).

Osmotic activity of the Ca and Mg accumulated in response to K deficiency

Despite the large decrease in K concentration in the plants grown on low K soil, there was no corresponding decrease in sap osmotic pressure, which was between 425 mosm kg^{-1} and 475 mosm kg^{-1} in plants from all treatments (Table 3). Since reducing sugar accumulation was not induced by K deficiency, this suggested that the cations accumulated as tissue K declined were replacing K in its osmotic role. This was supported by the observation that concentrations of cations in sap extracted by freezing and thawing were very similar to total (acid extractable) cation concentrations expressed on a tissue water basis (Fig. 7). Quantitative extraction by freezing and thawing is expected for K and Na because their presence as soluble salts is well established. That the same is true for Ca and Mg strongly suggests that these were also present as soluble salts. However, errors might arise, particularly for Ca, if large amounts of crystalline salts, such as Ca oxalate, were recovered in the sap and, hence, mistakenly assumed to be soluble.

To test whether this was occurring, the measured and calculated sap Ca concentrations were compared in two plants (sugar beet and spinach) known to contain high concentrations of Ca oxalate (Gallagher, 1975). Allaway *et al.* (1984) have shown that in oxalate-containing plants, sap Ca concentrations are less than those expected from acid extraction of dried plant material. Sugar beet and spinach contained total Ca concentrations of 51 and 17 mol m^{-3}, respectively, but in both plants nearly 90% was present as Ca oxalate. Saps extracted from them by freezing and thawing contained 0.3 mol m^{-3} Ca or less, which accounted for less than 1% of the tissue Ca. In contrast, the majority of K in these plants was extracted by freezing and thawing. A simultaneous comparison using barley with low and high Ca concentrations showed that 5% or less of the Ca was present as oxalate and, as expected, measured and calculated sap Ca concentrations were not significantly different. These results support the conclusion that oxalate is not extracted by the freeze/thaw technique and suggest that the majority of Ca and Mg in barley is soluble and, hence, osmotically active.

The counteranion accumulated with the extra Ca and Na in K-deficient barley plants is not known. Calculation of the sap osmotic pressure expected if the anion was monovalent, e.g. Cl^- or NO_3^- resulted in an over-estimation of the measured value in K-deficient plants but not in those with sufficient K (Table 3). This probably indicates that a substantial proportion of the counteranions in K-deficient plants are polyvalent, but no attempt has been made to confirm this.

Location of cations in K-sufficient and K-deficient barley leaves using X-ray microanalysis

Plants used for X-ray microanalysis were grown in Hoagland's nutrient solution containing 0.02 or 2.5 mol m^{-3} K. At the lower concentration, growth was reduced by 75% and the shoot K concentration

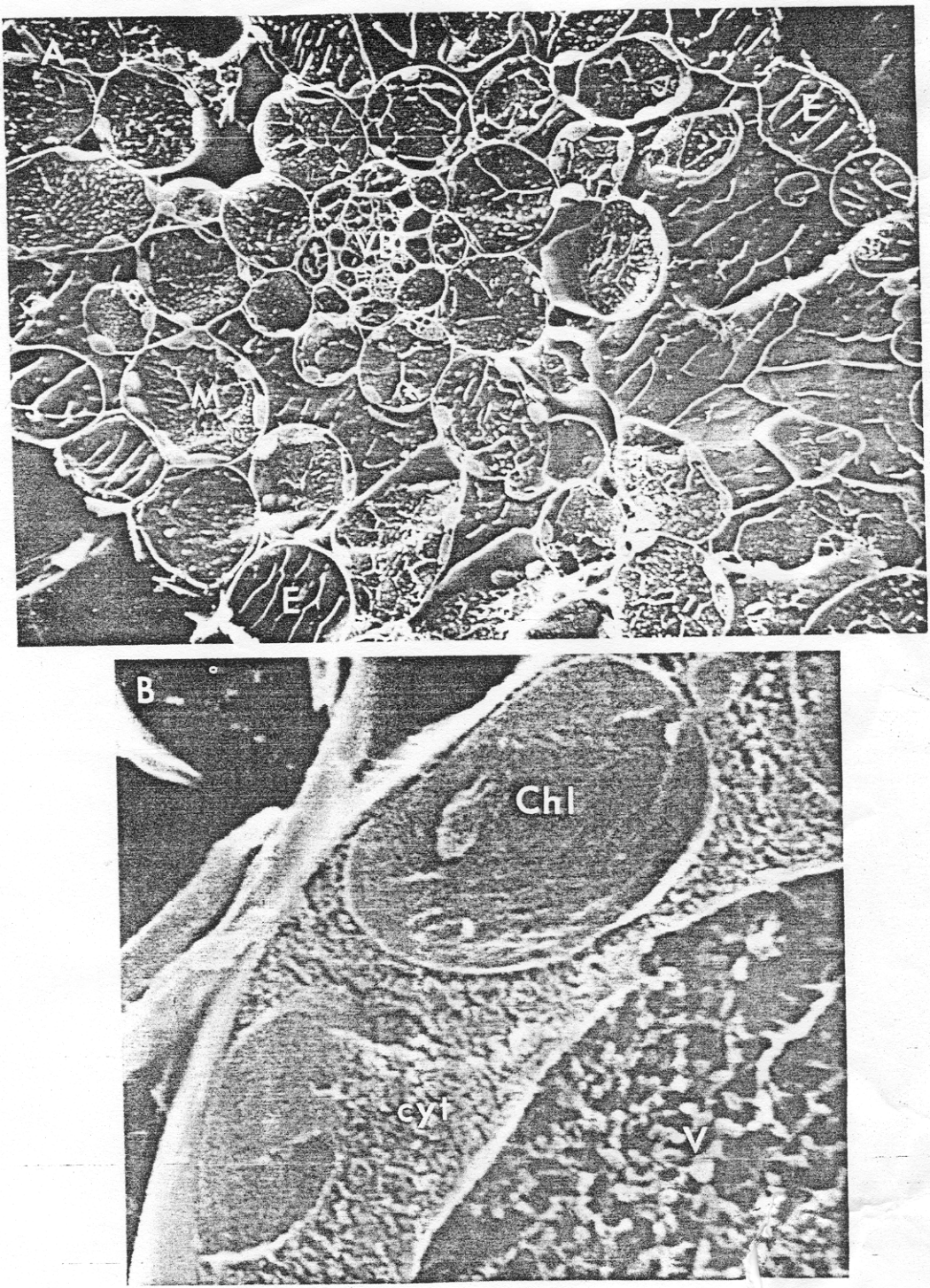

Figure 8. Appearance of cells and cell compartments in leaves of barley prepared for X-ray microanalysis. A: transverse section oof leaf with epidermal (E) and mesophyll (M) cells and a vascular bundle (VB) (× 800). B: Part of mesophyll cell with vacuple (V), cytoplasm (cyt) and chloroplasts (Chl) (× 12,000).

Table 4. Fresh weight and cation concentrations of barley shoots grown for 13 d in Hoagland's nutrient solution containing 0.02 or 2.5 mol m^{-3} K

Potassium concentration in nutrient solution (mol m^{-3})	Fresh weight (g per three plants)	Shoot cation concentration (mol m^{-3})			
		K	Ca	Mg	Na
0.02	0.76	94	52	40	47
2.5	3.00	281	19	10	2

decreased from 281 to 94 mol m^{-3} (Table 4). However, unlike the plants grown in soil, lack of K was compensated by substantial increases in the concentrations of all three other major cations rather than specific increases in Ca or Na. This is probably a reflection of the different availabilities of the cations in solution compared with the soil.

After preparation of tissue for X-ray microanalysis, all major cell types in a barley leaf could be identified (Fig. 8a). In mesophyll cells, vacuole and cytoplasm could be distinguished, but in epidermal cells only the vacuole could be identified. Cytoplasm was identified as structureless areas between the plasmalemma and tonoplast but outside the chloroplasts (Fig. 8b). In addition to cytosol it probably also included endoplasmic reticulum, mitochondria and other small organelles.

In the plants grown in 2.5 mol m^{-3} K, the major cation in both the vacuole and cytoplasm of the mesophyll cells was K (Fig. 9). Both compartments also contained substantial concentrations of P, but there were no detectable concentrations of Ca, Mg or Na. In contrast the vacuoles of epidermal cells of these plants contained significant quantities of Ca. In some epidermal cells this was the only detectable cation, but in others K was also present. In leaves of plants grown with 0.02 mol m^{-3} K there was no detectable K in the vacuoles of mesophyll cells, which accumulated Na and Mg instead (Fig. 9). In contrast, K was still present in detectable amounts in the cytoplasm of these cells. There were no obvious increases in Ca concentrations in any compartments of the mesophyll cells of the low K plants. Instead, as in the high K plants, substantial concentrations of Ca were present in epidermal vacuoles (Fig. 9). At present, it is not possible to convert the peak heights of the spectra into concentrations because calibration has not yet been achieved. However, preliminary results suggest that major differences in peak height can be equated with substantial changes in concentration.

Discussion

A major aim of the work described in this paper was to determine whether decreases in the growth of K-deficient barley could be related to changes in the composition of the plants. The intention was to test some of the assumptions that underlay a recently proposed model that attempted to explain growth responses to K in terms of intracellular compartmentation of solutes (Leigh & Wyn Jones, 1984). To ensure that the results were relevant to field conditions, most of the plants used in this study were grown in soil so that the availabilities of cations were as similar as possible to those in the field. This seems to have been worthwhile because barley grown in K-deficient soil without extra Na accumulated Ca (Fig. 5a), as observed in the field at Rothamsted (Leigh & Johnston, 1983), whereas in nutrient solution they accumulated more Na and Mg (Table 4).

Two assumptions underlying the model proposed by Leigh & Wyn Jones (1984) were, firstly, that growth would be independent of the nature of the cation accumulated to replace K in vacuoles. It was assumed that growth reductions in response to changes in vacuolar composition would only occur if organic solutes were diverted into an osmotic role. Secondly, it was assumed that changes in the composition of whole tissue were spread evenly over all cell types, i.e. that there was no significant

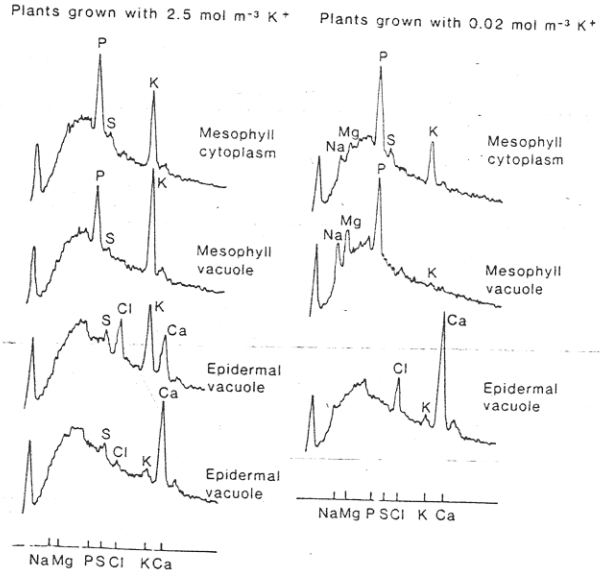

Figure 9. X-ray microanalysis spectra for cell compartments in leaves of barley grown in Hoagland's nutrient solution containing 2.5 or 0.02 mol m^{-3} K.

intercellular compartmentation of solutes. Neither of these seems to be true for barley, indicating that understanding growth responses to K may be more complicated than proposed by Leigh & Wyn Jones (1984).

The results presented here clearly show that K-deficient barley that contains high concentrations of Na grows faster than barley that accumulates Ca instead (Figs 3 & 5). These growth responses may be linked directly to the concentrations of Ca or Mg in the shoots (Fig. 6). Apparently, Na ameliorated the effects of K-deficiency because it decreased the Ca and Mg concentrations in the shoot. These results suggest that the decreased growth of K-deficient barley in the field at Rothamsted is probably caused by the high concentrations of Ca they accumulate (Leigh & Johnston, 1983).

It is obvious that the inverse correlations between fresh weight and divalent cation concentrations (Fig. 6) do not prove a causal relationship. None the less, a possible explanation for the decreases in yield is that the tonoplast is unable to maintain large concentration gradients of divalent cations resulting in a significant leakage of these ions into the cytoplasm where the inhibition of some essential metabolic process occurs. This would suggest that if Ca directly causes the decreased growth of K-deficient barley then the sensitive process may be in the epidermis, but if high Mg concentrations are the cause then the process may be in the mesophyll. However, the concentration of free Ca^{2+} in the cytoplasm is probably 1 mmol m^{-3} or less (Clarkson & Hanson, 1980) and that of Mg^{2+} less than 5 mol m^{-3} (based on the Mg^{2+} requirement of protein synthesis *in vitro*; Wyn Jones *et al.*, 1979; Gibson, Speirs & Brady, 1984). These concentrations are well below the detection limits for these elements by X-ray microanalysis. Therefore, inhibitory concentrations of these ions could be reached in the cytoplasm before either could be detected by X-ray microanalysis. Hence, it is possible that the relationships in Fig. 6 are caused by increases in cytoplasmic Ca^{2+} or Mg^{2+} levels in cells other than those with the highest vacuolar concentrations of these ions. The X-ray microanalysis does not provide conclusive evidence that growth is limited by a Ca-sensitive process in the epidermis or a Mg-sensitive process in the mesophyll.

Whatever the relationship between divalent cation concentrations in cell compartments and the growth of K-deficient barley, it is clear that substantial intercellular compartmentation of cations occurs. Therefore, changes in tissue composition cannot be interpreted in terms of the 'average' cell (cf. Leigh & Wyn Jones, 1984). As a consequence, any explanation that seeks to describe growth responses in terms of solute compartmentation must take account of this added level of complexity. Such considerations are probably also relevant to other nutrient deficiences because van Steveninck *et al.* (1982) showed unequal distribution of P and S between epidermal and mesophyll cells in leaves of *Lupinus luteus*.

Although responses to K are more complicated than envisaged by Leigh & Wyn Jones (1984), the X-ray microanalysis data (Fig. 9) support the fundamental assumption of their model that K concentrations in the vacuole and cytoplasm respond differently to changes in tissue K concentrations. Thus, whereas K concentrations in the mesophyll cell vacuoles of K-deficient plants were at or below the limit of detection of this ion (about 20 mol m^{-3}), those in the cytoplasm were still detectable (Fig. 9). These results confirm the behaviour of vacuolar and cytoplasmic K concentrations that were observed in low and high salt barley roots by Pitman, Läuchli & Stelzer (1981). A full description of the changes in concentrations in each of these compartments as tissue K concentration declines must await a more detailed and quantitative study.

The available evidence suggests that the majority of Ca accumulated in barley leaves is soluble (Fig. 7). Thus, in barley this ion can have an important osmotic role—a function not often ascribed to it (e.g. Kirkby & Pilbeam, 1984). This appears to be because it is usually assumed that the majority of Ca is present as pectate in the cell wall or as insoluble oxalate, and that the requirement for a low cytoplasmic free Ca^{2+} concentration limits the concentration accumulated within cells (Clarkson & Hanson, 1980). The compartmentation of Ca in epidermal vacuoles indicates that it can be accumulated to high concentrations within specific cells. It presumably reaches the epidermis via the transpiration stream. Its restriction to the epidermis may be a protective mechanism preventing inhibition of Ca-sensitive processes in other cell types. However, in K-deficient plants with high Ca : K ratios, the capacity of this mechanism may be exceeded, leading to disruption of Ca-sensitive processes in other cells or compartments.

The observation that Ca concentration is decreased in leaves of plants grown in soil with extra Na is similar to observations by Lynch & Läuchli (1985). They found decreases in shoot Ca concentrations in barley seedlings when the external NaCl concentration was increased from 1 to 30 mol m^{-3}. This was apparently due to Na inhibition of Ca transport from root to shoot. This presumably also underlies the effects observed in K-deficient barley.

Finally, the alteration of growth responses to K by the addition of Na to the soil has implications for the use of critical K concentrations for assessing the K requirements of barley. The critical concentration is defined as the tissue K concentration at which 90% of maximum yield is obtained (Ulrich & Hills, 1967). Above this concentration growth shows little response to increases in tissue K but at lower concentrations it declines rapidly (Bates, 1971; Leigh & Wyn Jones, 1984). However, it is clear from the

data in Fig. 3 that this response is very dependent on the availability of Na to the crop. When this is low, the decline in growth is likely to be much more severe than when Na is present (see also Hylton, Ulrich & Cornelius, 1967). Therefore, in practice, the critical K concentration alone is unlikely to be a good indicator of the response of crops to K. Account must also be taken of the availability of alternative cations and the effect of their accumulation on crop growth. As the availability of these other cations may differ in soils and nutrient solution, the latter may be of limited use in predicting responses in the field.

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