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PLANT RESPONSE TO SALINE CONDITIONS

by

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General Summary

The importance of the sodium chloride effect at low nutrition has been demonstrated. The preliminary experiments with Trifolium subterraneum showed that sodium chloride reduced growth more at low nutrient (which was $^{1}/_{40}$ the concentration of the full concentration) than at full nutrient concentration. This effect on growth was due to higher uptake of sodium and chloride and greater loss of potassium from the plant tissue in low nutrient.

In Hordeum vulgare the interaction of anions and cations at $^{1}/_{10}$ full nutrient concentration with sodium chloride were studied by adding and omitting individual anions and cations in $^{1}/_{40}$ nutrient solution. The results showed that only Ca^{++} and Sr^{++} antagonized adverse effects of sodium chloride. Rapid recovery of growth was noted when plants treated first with sodium chloride and $^{1}/_{40}$ nutrient solution were transferred to the same solution containing an additional 0.6 me/l of Ca^{++}. The adverse effects of sodium chloride at $^{1}/_{40}$ nutrient solution was due to greater reduction in cell multiplication than in elongation. It is concluded that calcium-sodium antagonism is not due to absorption of essential elements, photosynthesis or mobilisation of seed reserves. In the text 0.2 me/l and 0.8 me/l of Ca^{++} in the nutrient medium with high NaCl has been referred to as low and high Ca^{++} respectively.

Growth at low Ca^{++} was reduced, probably due to increased permeability of cell membranes. Permeability was investigated by (a) feeding the labelled ions to the plants and determining their losses to the non-labelled solution, (b) determining the losses of amino acids from germinating seeds to culture solutions. The loss of amino acids, Cl^{36}, Na^{24}, Rb^{86}, labelled K, and Ca at

germination and at 1st leaf stage either from intact plants or from excised roots was higher at low than at high Ca^{++}. Concurrently the uptake of some of these ions, which was higher at low than at high Ca^{++} in the short term experiments, was more reduced than at high Ca^{++} when NaCl treatment was imposed for a longer time. This reduced uptake was insensitive to 2,4-Dinitrophenol showing that only the passive component of the uptake process remained in this treatment. The increased permeability is thought to act via a decreased organization of the protoplasm or via an increased ion balance of vital cell parts.

P^{32} uptake into the shoot at low Ca^{++} was more reduced than at high Ca^{++} but in the meristematic zone of roots P^{32} uptake was similar in control, high Ca^{++} and low Ca^{++} treatments. Phosphorus incorporation into organic form did not show significant differences between treatments, though a residual phosphorus fraction which contained nucleic acids was somewhat more reduced at low than at high Ca^{++}. Total phosphorus concentration was more reduced at low than at high Ca^{++}. It is concluded that ion unbalance due to high NaCl has little effect on phosphorus metabolism.

NaCl greatly restricted Ca^{45} movement in the plant and this restriction was more pronounced at low than at high Ca^{++} treatment, particularly in roots and young leaves. It is suggested that restricted movement at low Ca^{++} is the cause of adverse effects on growth. No structural disorganization was visible at the sub-microscopic levels in the root meristems of low Ca^{++} treatment.

DECLARATION

This is to certify that the thesis contains no material accepted for the award of any other degree.

The thesis contains no material previously published except in few of the experiments of Chapter 3 when the author collaborated with Dr. H. Greenway, the results of which were published in Plant and Soil (1965) (23 : No.2 258-260) by S. Z. Hyder and H. Greenway.

The material presented in this thesis is the work of the author except where otherwise acknowledged.

(S. Z. Hyder)
July, 1966

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CHAPTER 1

GENERAL

Introduction

Nature of Soils

Saline soils occur in arid and semi-arid regions. The accumulation of soluble salts in these soils is due to low rainfall, poor drainage and application of saline irrigation water (Kelley 1951). Ions commonly occurring in saline soils are Na^{+}, Ca^{++}, Mg^{++}, Cl^{-}, $SO_4^{=}$ and HCO_3^{-}. Salt concentrations in soils may vary from low to very high. At higher concentrations, when the diffusion pressure deficit is higher than permanent wilting, little plant growth occurs, though some halophytes survive and even grow. Many salt tolerant, non-halophytic species thrive at a moderate concentration; 100 - 250 me/l NaCl. Most workers agree that high ionic concentrations of saline soils affect the plant in several ways, e.g.

1. reduced water availability due to high osmotic pressure of the medium (Wadleigh and Ayers 1945).

2. reduced absorption of essential elements (Bernstein and Hayward 1958).
3. specific effects of some ions reaching high enough concentration in plants to give toxicities (Hayward and Bernstein 1958).

There is however considerable difference of opinion as to the relative importance of these factors. Some emphasize the reduced water availability (Majistad 1945 and Hayward and Wadleigh 1949); others believe that the relative importance of each factor depends largely on species and climatic conditions (Berg 1952). Successful agronomy on saline soils often includes drainage and leaching of salt. Use of salt tolerant species would be especially important

(a) during reclamation
(b) on soils of moderate to high salinity which are difficult to drain
(c) during use of saline irrigation water

Causes of salt tolerance

Salt tolerance of plants is best expressed by reference to the salt concentration of the root medium and may be defined as "the degree to which a crop can produce a satisfactory yield on salty land" (Bernstein 1958). Certain halophytes tolerate salts only because ion accumulation is concentrated in older tissues,

which die and absciss, so that internal salt concentrations in younger parts do not reach critical limits. Mangroves can secrete excess salts through glands (Arisz et al. 1955). Other halophytes regulate internal salt concentration by increasing their succulence i.e. their leaf thickness and moisture contents tend to dilute the salt. It is not known how well these principles of halophytism apply generally to salt tolerant crops. Some Russian workers (e.g. Shakhov 1956) emphasize the colloidal theory of salt tolerance. It is suggested that metabolism of salt tolerant plants is modified and causes an increase in protein content in hydrophily and in binding sites of the ions of protoplasm (Na^+, K^+), and a lowering of the respiratory rate.

In the literature there are many data concerning agronomical investigations of salinity on plant growth; what are lacking are precise physiological experiments under controlled conditions which could indicate effects of saline substrates on plants' structural and functional organization. Such studies would help to explain plant behaviour under saline environments and also further elucidate the mechanism of salt tolerance, particularly if they could establish effects of high salt concentration on membranes, protoplasm, enzymes etc. Especially relevant would be studies on the whole internal organization of the cell at the sub-microscopic level.

Nutritional

The important aspect of salinity which has so far not attracted the attention it deserves, is the problem of ion unbalance. Sodium is the main cation involved in salinity, its proportion being much higher than that of essential cations. Soil scientists have shown that some of the damage caused to the crops by sodium can be attributed to its deflocculating influence on soil colloids. However, the use of soil conditioners (Pearson and Bernstein 1958) has shown that sodium has an additional toxic effect, the nature of which is still unknown. Observations on the specific effects of various cations and cationic ratios on the physiochemical properties of protoplasm (Seifriz 1949) and on the activity of enzyme systems (Braverman et al. 1948) have contributed to the understanding of the role of many mineral nutrients in plant metabolism. The results of these studies support the concept of "balance".

For the normal functioning of the cell a definite balance between various ions is required. Cell cytoplasm strives for a salt concentration which regulates water absorption and buffers the cell sap. Since each kind of ion species has some nutritive role in plant growth and is used in various syntheses, unequal absorption of nutrient is likely to result in an unbalance which could reduce normal function and development of plants.

Interionic influences on uptake processes have been studied and reciprocal relationships among ions in plants have been well demonstrated; for example between Ca^{++} and Mg^{++} (Smit and Mulder 1942; van Itallie 1937), K^{+} and Mg^{++} (Carolus 1933; Boynton and Compton 1945; Emmert 1961), Ca^{++} versus Mg^{++} and K^{+} (Wadleigh and Bower 1950), Mg^{++} and nitrogen compounds (Hoblyn 1940; Mulder 1956; Holmes 1962). It has also been found that Li^{+}, Ca^{++}, Mg^{++} and other divalent and trivalent cations accelerate the uptake of ions such as K^{+}, Rb^{+}, Br^{-}, SO_4^{--} and PO_4^{---} (Viets 1944; Jacobson et al. 1950; Overstreet et al. 1952; Fawzy et al. 1954, Tanda 1955). Various explanations have been advanced to explain the stimulating effect of divalent ions on the uptake of monovalent ions e.g. Epstein (1962) has shown that H^{+} ions prevent K^{+} uptake and Ca^{++} reverses the inhibitory effects of H^{+} ions. Middleton and Russell (1958) have pointed out that divalent ions being bigger in size may compete successfully with monovalent ions replacing them from the absorption sites. Overstreet et al. (1952) suggested that divalent ions stimulate the entry of monovalent ions by partieipating in the mechanism whereby the monovalent ions are bound within the cell. As all these investigations were carried out at low electrolyte concentrations, the interpretation of the result is not applicable to saline environment. The high concentration of a single ion can reduce the uptake of other ions to the extent of causing a deficiency e.g. Boynton et al.

(1944) have reported K-induced Mg deficiency in apples. Salinity may also cause a similar deficiency by reducing the uptake of some essential element.

The saline environment with sodium chloride predominating is likely to cause nutritional disturbance, presumably because of the interaction of NaCl with other essential ions. This interaction has not been well studied. In the past NaCl was added either to full strength nutrient solution or to distilled water. Neither of these approaches could show any synergistic or antagonistic interactions of nutrient ions with NaCl. Reifenberg and Hosovsky (1947) and more recently Greenway (1963) have shown stronger effects of NaCl on growth at low than at high nutrition, demonstrating that growth reductions are due to ion unbalance, at least at low nutrient. There are still conflicting reports as to the nature of this ion unbalance. Heimann (1958) emphasizes the importance of K^+/Na^+ ratio in protecting against the salinity effects, whereas Bower and Turk (1946) believe that deficiency of Ca^{++} is involved in alkaline soils though their evidence was not conclusive because in their experiments the adverse effects could have been due to lack of other nutrients or to poor soil structure (Hyder and Greenway 1965). A better approach to this problem would be to grow plants under controlled nutrition preferably in water culture. Interaction of NaCl with nutrient ions can then be studied by

(a) varying the ratio of individual ions to NaCl

(b) measuring the uptake of various ions in the presence of high NaCl concentrations.

There is no report in the literature where a systematic study has been made with regard to varying the ratio of individual ions. A number of investigations on the unbalance of ions have been reported with differing results. These differences were mainly due to different varieties and also to different experimental conditions.

The aim of the present series of experiments was to investigate the interaction of NaCl with the essential nutrient ions in the manner outlined above and to find out the causal relationship between unbalanced conditions and growth reduction. The preliminary experiments showed that the strongly adverse effect of NaCl at low nutrition found in Hordeum vulgare (Greenway 1963) was also found in a dicotyledon (Trifolium subterraneum). For Hordeum vulgare it was later shown conclusively that the essential element interacting with NaCl was calcium. This interaction was further studied by determining the permeability of tissue to sodium, chloride, potassium and calcium. The effects of the interaction between NaCl and calcium on phosphorous metabolism, on Ca^{45} movement and on protoplasmic structure at sub-microscopic level were also investivated.

The effects of sodium and its interaction with other ions, particularly calcium, were established but on the basis of presented evidence it is difficult to arrive at a suitable hypothesis for the primary site of action on the physiology and metabolism of the plants.

CHAPTER 2

RESPONSE OF TRIFOLIUM SUBTERRANEUM TO NaCl

Introduction

Most previous experiments on plant response to salinity involved a lengthy treatment. Results of such studies are difficult to interpret because development between treatments becomes too diverse. It has been claimed by Berg (1952) and Matukhin and Boiko (1957) that the degree of salt tolerance of species was related to their ability to regulate their ion uptake. These workers used the whole shoot for determination of ion uptake. This approach is not very useful as recent studies in translocation have shown that ion content varies in different parts of the shoot and also with plant development. Furthermore Greenway and Thomas (1965) have shown that older leaves of Hordeum vulgare regulate their sodium and chloride content by limited uptake whereas younger organs have perhaps an additional control of chloride and sodium concentrations by their high relative growth rates.

Salt relations of individual plant parts may also be important in obtaining more information on the mechanism of tolerance; such studies on Hordeum vulgare have been reported (Greenway 1962).

In the present experiment the effect of sodium chloride was studied on sub-clover (Trifolium subterraneum) in a short term experiment. Ion uptake into individual plant parts, and the interaction of NaCl with nutrients of different strengths were studied. Sub-clover was taken as a test species and compared with the monocotyledon, barley, which has been used for studies on salt tolerance and ion uptake and regulation (Greenway 1962(a) (b); Greenway and Thomas 1965).

Many legumes are more salt sensitive than most cereal crops e.g. Toniolo and Poli (1958) have shown that salt tolerance decreased in the order barley > rye > wheat > oats > beans peas > lucerne. Some legumes are moderately salt tolerant such as Medicago, Melilotus alba, M. officinales, Trifolium fragiferrum, others are sensitive e.g. T. repens, T. pratense (Hayward and Bernstein 1958).

Legumes are important on saline land because of their nitrogen fixation, their improvement of soil structure and thus increasing fertility and checking of erosion.

Trifolium subterraneum is an important pasture species

of Australia. Knowledge of its salt tolerance would be useful particularly in areas where salt problems might occur.

GENERAL METHODS

In the past salt tolerance has been studied in the field or in sand cultures; as the intention was to obtain data of agronomic value, the technique used was not very satisfactory from a plant physiological view. For example, in experiments with soils neither the moisture nor the salt distribution can be known very well. Sand cultures require frequent flushings to avoid local increases in concentration of salt; hence for plant physiological studies water cultures are more suitable.

GENERAL CULTURE AND HARVESTING PROCEDURE

Seeds of Trifolium subterraneum (cv. Wenigup) were germinated in sand. After 4 days, when the cotyledons had fully emerged, they were transplanted to culture dishes of $^{1}/_{2}$ litre capacity in glasshouse at $^{1}/_{4}$ strength of Hoagland solution. When the first trifoliate leaf had unfolded, the solutions were replaced with full strength nutrient. The nutrient solution contained in me/l

Ca^{++} 8; Mg^{++} 4; K^{+} 5; NH_4^{+} 2;

NO_3^{-} 13; $SO_4^{=}$ 4; $H_2PO_4^{-}$ 2.

Microelements were added as described by Arnon and Hoagland (1940).

To minimise pH changes, and local salt concentration around the roots, solutions were aerated and replaced according to experimental condition. The plants to be harvested were transferred from glasshouse to laboratory early in the morning. Roots were rinsed in the treatment solution and dried between filter papers. After determining the fresh weight all plants were dried at 70°C. Chloride was determined by the electrotitrimetric method of Best (1950) and sodium and potassium by the EEL flame photometer.

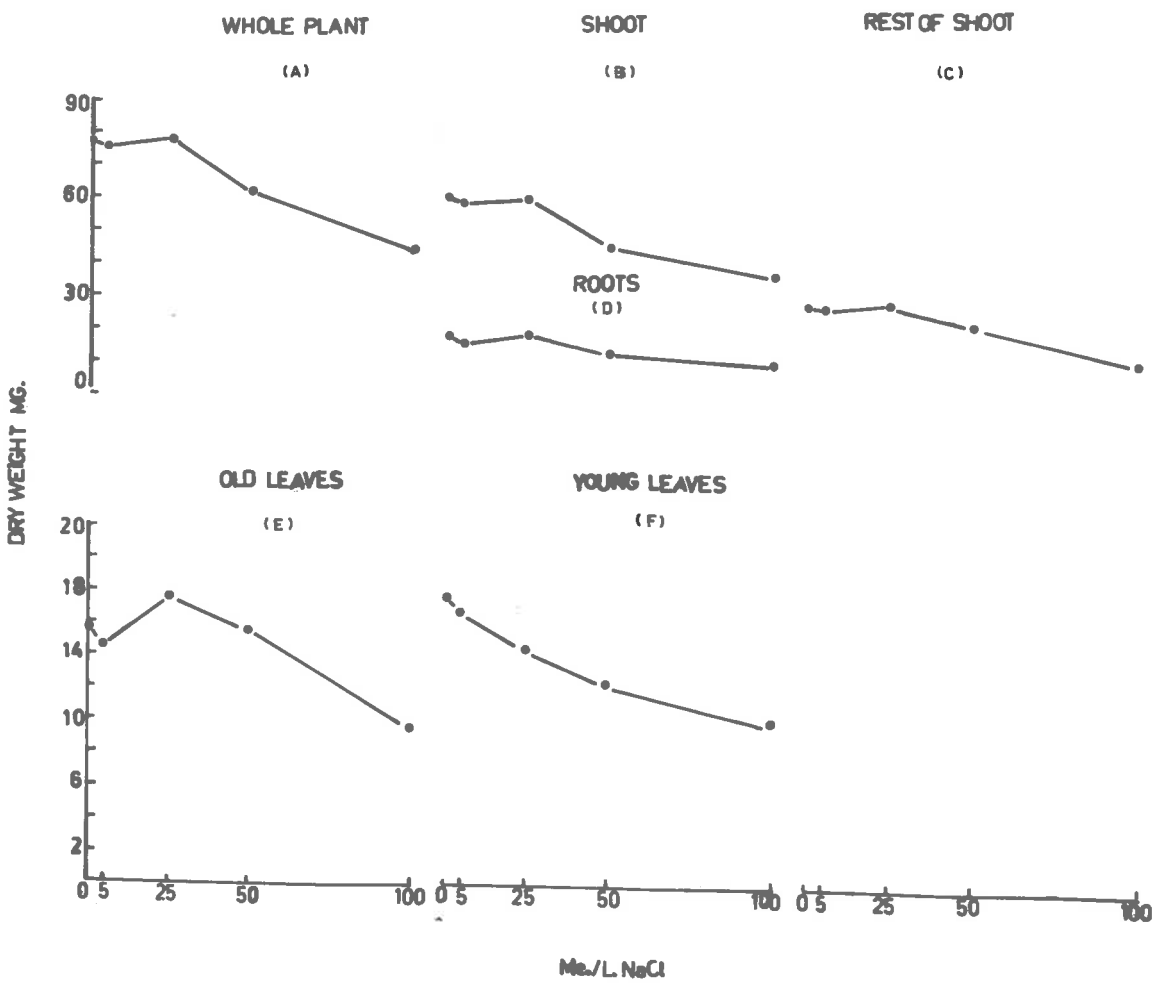
Experiment 1: NaCl was applied at 0, 5, 25, 50 and 100 me/l. To avoid the sudden effect of too great an increase in salt concentration, the 100 me/l treatment received 50 me/l on the first day and was raised to 100 me/l the next day. Solutions were replaced every 4th day. There were two harvests; H_1 was taken prior to NaCl application and H_2 13 days later. Plant parts were separated into young leaves, old leaves, rest of shoot and roots. There were four replicates in each treatment.

Experiment 2: The two nutrient levels were full strength Hoagland and $^1/_{40}$ strength Hoagland. At each nutrient level there were 0,

FIGURE 2:1

Dry weight of whole plant and its individual parts of *Trifolium subterraneum* as affected by different NaCl concentrations in the medium.
(Expt. 1 of Chapter 2).

FIG. 2.1


WHOLE PLANT
(A)
SHOOT
(B)
REST OF SHOOT
(C)
ROOTS
(D)
OLD LEAVES
(E)
YOUNG LEAVES
(F)
DRY WEIGHT MG.
90
60
30
0
20
18
14
10
6
2
0 5 25 50 100
Me./L. NaCl

25, 50 me/l NaCl and there were 7 replicates. Solutions were replaced every 24 hours. H_1 was on the day of NaCl application and H_2 11 days later.

Plant parts separated were

(1) First unifoliate and first trifoliate leaf

(2) Second and third trifoliate leaves

(3) Young trifoliate leaves

(4) Rest of shoot

(5) Roots

Experiment 3: Nutrient was at $^1/40$, $^1/20$, $^3/40$ and $^1/10$ strength of Hoagland. NaCl treatments were at 0 and at 50 me/l and there were 5 replicates. Plant parts were separated in the same way as in Expt. 2.

RESULTS

(a) Effects of different NaCl Concentrations. Expt. 1.

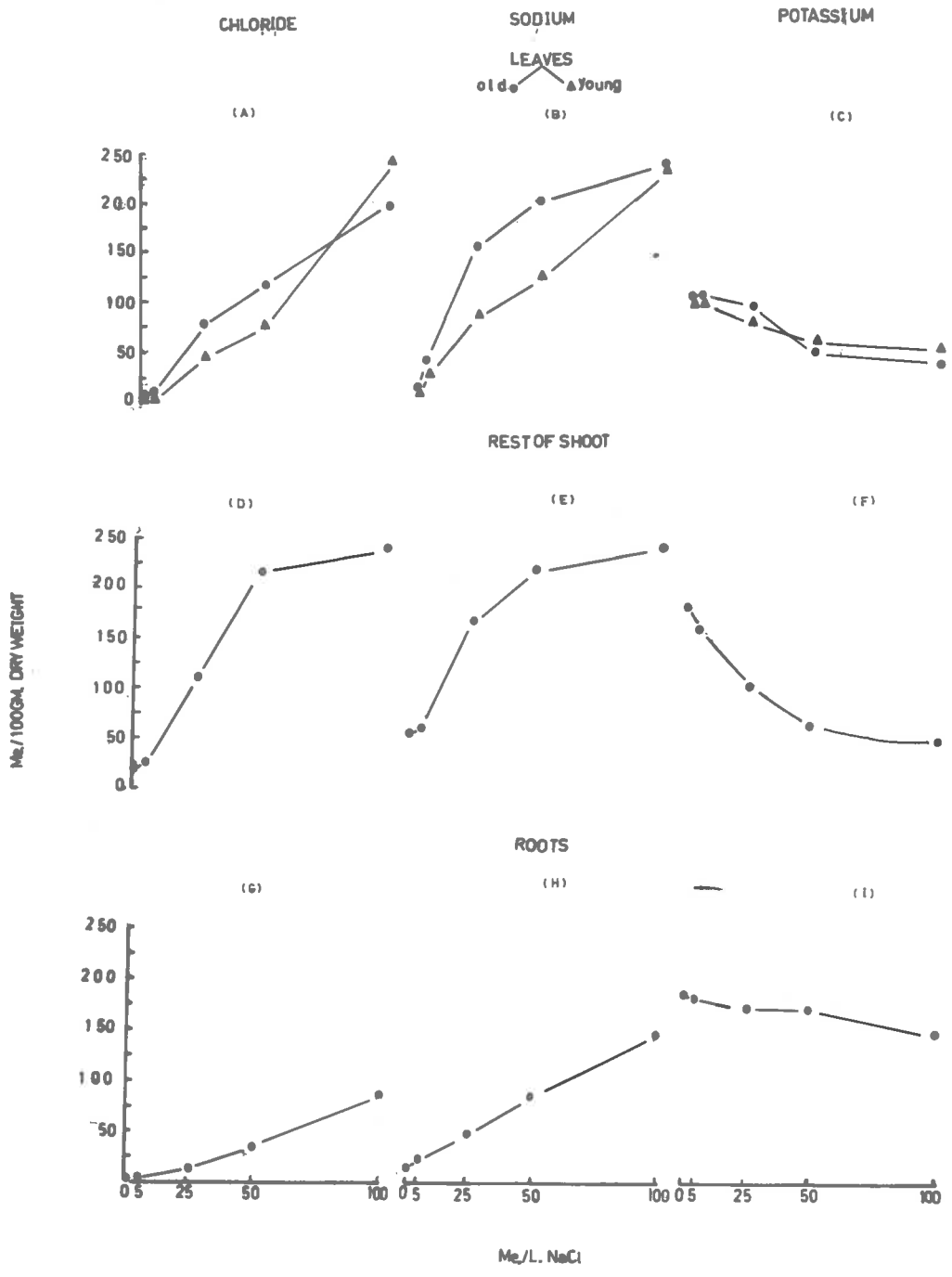
Dry Weight: For the whole plant, the first adverse effect of NaCl was found at 50 me/l with a more pronounced decrease in growth at 100 me/l. A similar response was found for all individual parts except roots but the growth of young leaves was reduced even at 25 me/l NaCl in the medium (Fig. 2:1).

FIGURE 2:2

Chloride, sodium and potassium concentrations in individual plant parts of *Trifolium subterraneum* as affected by different NaCl concentrations in the medium.

(Expt. 1 of Chapter 2).

FIG. 2.2


CHLORIDE
SODIUM
POTASSIUM
LEAVES
old
young
(A)
(B)
(C)
250
200
150
100
50
0
REST OF SHOOT
(D)
(E)
(F)
ROOTS
(G)
(H)
(I)
0 5 25 50 100
Me./L. NaCl
Me./100GM. DRY WEIGHT

Chloride, Sodium and Potassium Concentration: Chloride and sodium concentrations in both control and 5 me/l NaCl were low. Above 5 me/l, chloride and sodium usually increased markedly with NaCl concentration of the medium (Fig. 2:2). There were large increases in old leaves and in the rest of the shoot (Fig. 2:2B,D,E) but chloride and sodium increases in young leaves were smaller than in old leaves except at 100 me/l (Fig. 2:2B). In all treatments above 1 me/l NaCl, the roots contained less chloride, but more sodium, than the medium (me/l plant water, Table 2:1). Potassium concentrations of all parts of the shoot decreased in 25 me/l NaCl and further decreased at higher concentrations (Fig. 2:2C,F). Potassium concentration of roots was not appreciably affected by NaCl in the medium (Fig. 2:2I).

Increases in sodium were higher than increases in chloride in old leaves and roots (Fig. 2:2B,A), but the younger leaves contained more chloride than sodium at the highest NaCl concentration (Figs. 2:2A,B). With increasing NaCl in the medium all parts of shoot showed a greater increase in sodium concentration than concurrent decrease in potassium concentration.

(b) Effects of NaCl at low and high nutrient strengths. Expt. 2.

NaCl at both 25 and 50 me/l reduced growth of the whole plant and the effects were more pronounced at low than at high

TABLE 2:1

CHLORIDE, SODIUM AND POTASSIUM IN PLANT WATER* (me/l) OF OLD LEAVES AND ROOTS AS AFFECTED BY DIFFERENT NaCl CONCENTRATIONS.

	Chloride		Sodium		Potassium	
Treatments	Old leaves	Roots	Old leaves	Roots	Old leaves	Roots
Control	11	1.4	28	11	215	135
5 me/l NaCl	12	2.3	84	20	202	141
25 me/l NaCl	157	11	294	38	191	133
50 me/l NaCl	276	22	350	70	131	143
100 me/l NaCl	-	-	-	-	-	-

* This was calculated in the following way

$$me/l = me/100 \text{ gm dry wt.} \times \frac{100}{\text{Relative water}}$$

$$\text{Relative water} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Dry weight}}$$

FIGURE 2:3

Dry weight of whole plants and its individual parts of Trifolium subterraneum (as affected by two different NaCl concentrations in the medium at high (full nutrient) and at low ($^{1}/40$ nutrient) nutrients.

(Expt. 2 of Chapter 2).

Fig. 2.3

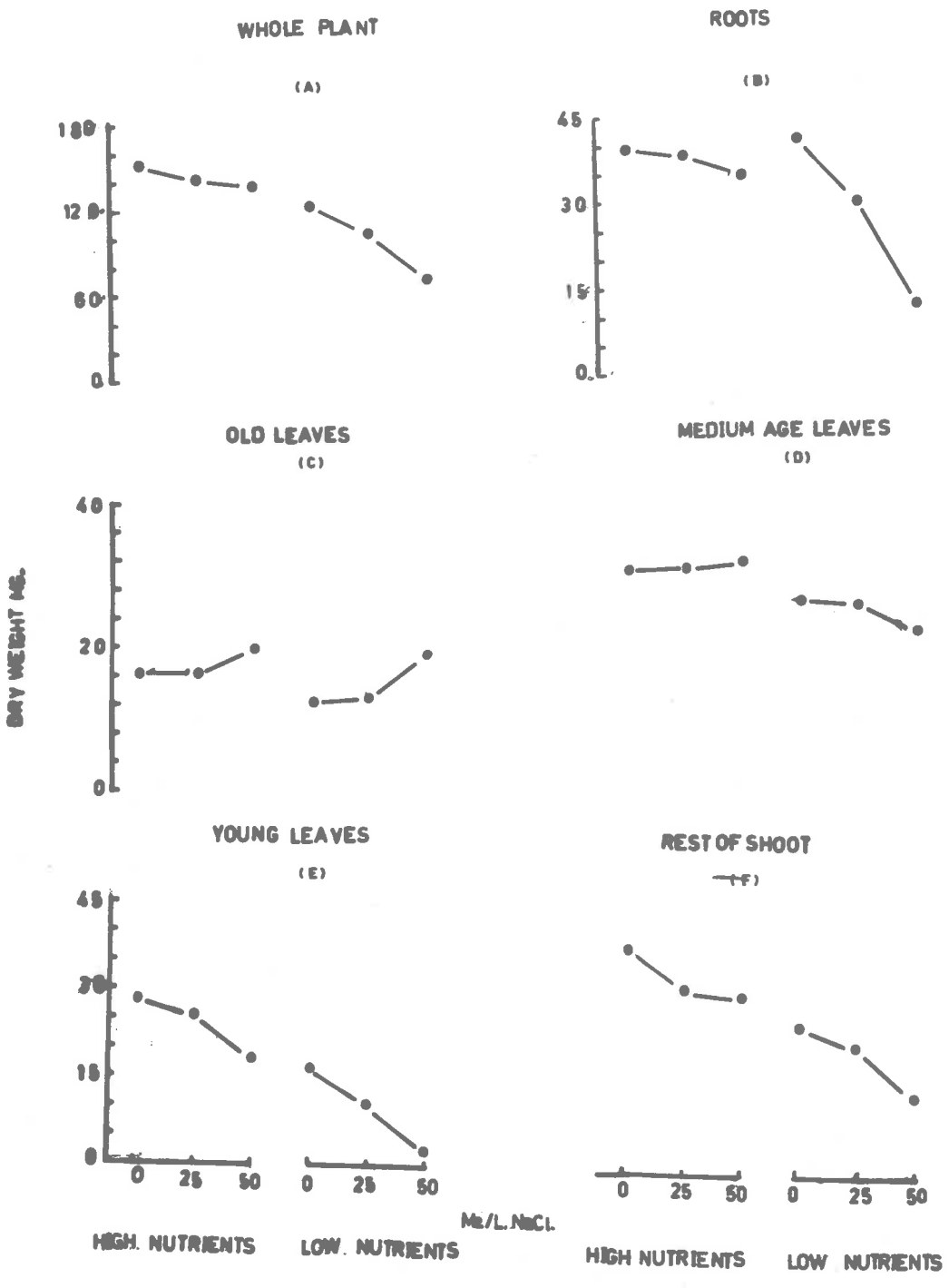

WHOLE PLANT
(A)
180
120
60
0
ROOTS
(B)
45
30
15
0
OLD LEAVES
(C)
40
20
0
MEDIUM AGE LEAVES
(D)
DRY WEIGHT MG.
YOUNG LEAVES
(E)
45
30
15
0
0 25 50
0 25 50
REST OF SHOOT
(F)
0 25 50
0 25 50
Mg/L. NaCl.
HIGH. NUTRIENTS
LOW. NUTRIENTS
HIGH NUTRIENTS
LOW NUTRIENTS

FIGURE 2:4

Chloride, sodium and potassium concentrations in different aged leaves of Trifolium subterraneum as affected by different NaCl concentrations in the medium at high (full nutrients) and low ($^{1}/_{40}$ nutrients) nutrients.

Expt. 2 of Chapter 2.

FIG. 2.4

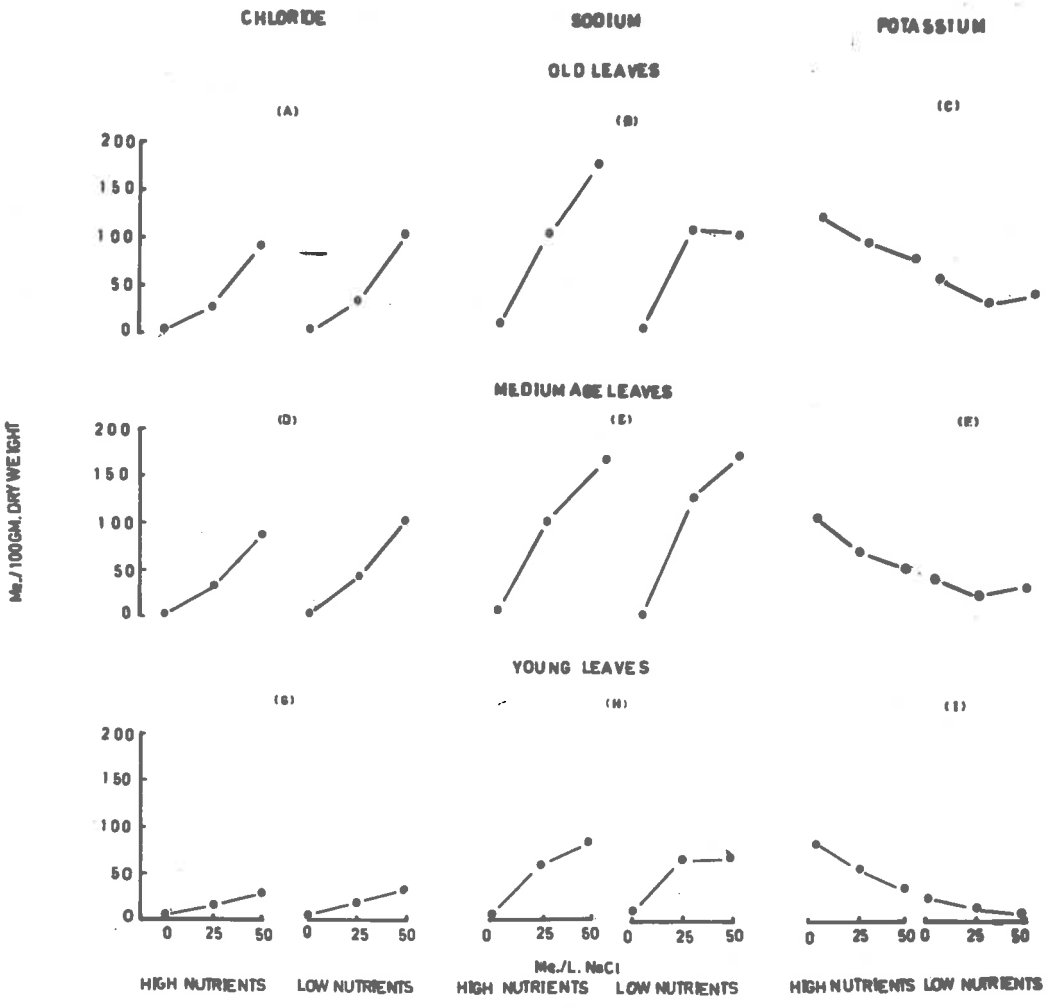
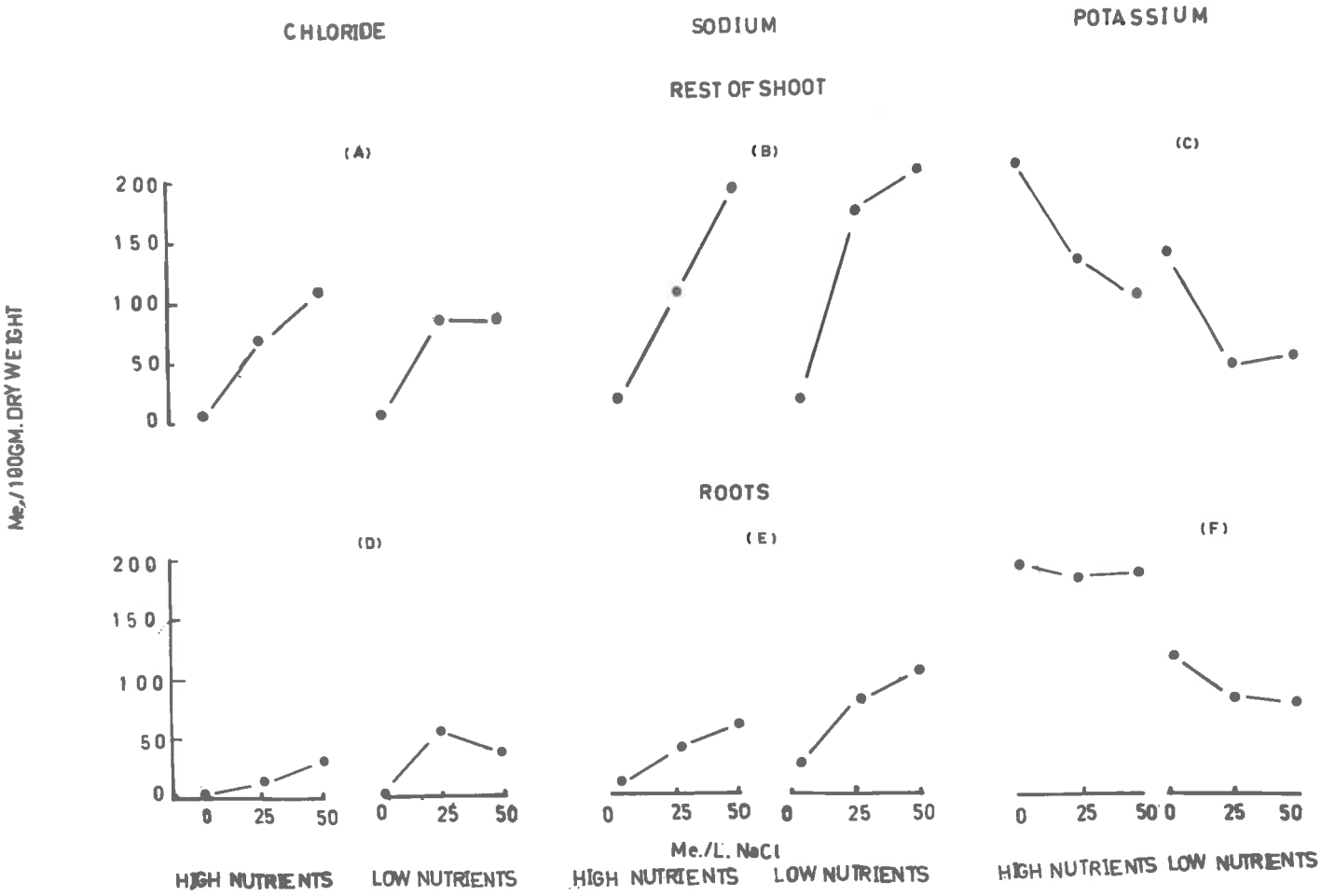

CHLORIDE
SODIUM
POTASSIUM
OLD LEAVES
(A)
(B)
(C)
MEDIUM AGE LEAVES
(D)
(E)
(F)
YOUNG LEAVES
(G)
(H)
(I)
200
150
100
50
0
0
25
50
Me./100GM. DRY WEIGHT
Me./L. NaCl
HIGH NUTRIENTS
LOW NUTRIENTS
HIGH NUTRIENTS
LOW NUTRIENTS
HIGH NUTRIENTS
LOW NUTRIENTS

FIGURE 2:5

Chloride, sodium and potassium concentrations in shoot and roots of Trifolium subterraneum as affected by different NaCl concentrations in the medium at high (full nutrient) and low ($^{1}/40$ nutrient) nutrients.
(Expt. 2 or Chapter 2).

FIG. 2.5


CHLORIDE
SODIUM
POTASSIUM
REST OF SHOOT
ROOTS
(A)
(B)
(C)
(D)
(E)
(F)
200
150
100
50
0
Me./100GM. DRY WEIGHT
0
25
50
Me./L. NaCl
HIGH NUTRIENTS
LOW NUTRIENTS

nutrients (Fig. 2:3A). These trends were particularly pronounced in the youngest leaf, rest of shoot and roots (Figs. 2:3E,F,B). The 2nd and 3rd trifoliate leaves were reduced only at 50 me/l NaCl low nutrient (Fig. 2:3D). NaCl treatment even increased the dry weight of the oldest leaves (Fig. 2:3C).

At high nutrient, NaCl reduced root and shoot growth to the same extent. However, at low nutrient NaCl resulted in very poor root growth so that there was pronounced difference in the root weight ratio between high and low nutrient. Results are presented in Table 2:2.

It will be noted in Table 2:2 that 50 me/l NaCl at high nutrient did not decrease root weight ratios compared with control plants, whereas at low nutrient, a decrease is noted even at 25 me/l NaCl and this becomes more pronounced at 50 me/l NaCl.

Ion Concentration: Sodium and Chloride: At high nutrient, chloride and sodium concentrations rose with increasing concentrations of the medium and the increases were close to linearity (Figs. 2:4; 2:5). However, at low nutrient, the sodium concentrations in the shoot organs did not increase much between 25 and 50 me/l NaCl (Fig. 2:4B,H; Fig. 2:5B). Similarly chloride concentration in the roots at low nutrient did not increase between 25 and 50 me/l (Fig. 2:5D). At 25 me/l NaCl in the medium,

TABLE 2:2

ROOT WEIGHT RATIO $\left(\frac{\text{weight of root}}{\text{weight of whole plant}}\right)$

NaCl me/l	High Nutrient	Low Nutrient
0	0.260	0.336
25	0.262	0.286
50	0.252	0.177

sodium concentrations of roots and rest of shoot were higher at low than at high nutrient and the same was found for chloride in the roots. This rather complicated situation is best interpreted by assuming that low nutrient induces two opposing effects on chloride and sodium uptake (Greenway 1963, 1965).

(1) An increase due to less competition by other ions

(2) A decrease due to reduced health of the plants.

It is assumed that at 25 me/l, the first tendency of increased uptake would be still apparent in some cases, but at 50 me/l the decreased health would become the dominant factor, with the result that chloride and sodium concentrations are not much higher than at 25 me/l low nutrient and 50 me/l high nutrient.

In most cases chloride and sodium (me/l 100 g dry weight) within the plant decreased in the order - rest of shoot, old leaves, medium age leaves, young leaves and roots. A rather similar pattern was found when ions were expressed as me/l plant water but then old leaves and medium leaves contained higher chloride and sodium concentrations than rest of shoot (Table 2:3). At high nutrient, chloride concentration of the roots was considerably lower than of the medium i.e. chloride was excluded in this treatment. A distinct accumulation of chloride was found however in low nutrient NaCl roots. Sodium concentration of the roots was higher than the concentration of the medium in both low and high nutrient treatment.

TABLE 2:3

CHLORIDE, SODIUM AND POTASSIUM IN PLANT WATER* (me/l) IN DIFFERENT PLANT PARTS AS AFFECTED BY NaCl AT LOW AND HIGH NUTRIENT

TREATMENTS	CHLORIDE					SODIUM					POTASSIUM				
	L_1	L_2	L_3	S	R	L_1	L_2	L_3	S	R	L_1	L_2	L_3	S	R
Full Nutrient Control	6.5	5.8	4.9	3.9	3.1	41.7	33	36.7	36.7	14.3	285	237	264	236	147
" " + 25 me/l NaCl	79	66.7	27.2	68.2	14	254	186	167	132	39	227	133	139	140	141
" " + 50 me/l NaCl	173	193	56	131	35.5	344	405	195	221	60	161	140	101	87	143
1/40 Nutrient Control	7.9	13.8	22	16	5.3	31	33	36.7	26	37	153	146	117	196	119
" " + 25 me/l NaCl	100	123	60	116	80	245	318	224	235	119	55.4	59	137	42	90
" " + 50 me/l NaCl	289	450	105	137	61	318	540	293	357	141	107	145	67	60	91

L_1 = leaf 1 L_2 = leaf 2 L_3 = leaf 3 S = Rest of shoot R = Roots

* Calculated similarly as in Table 2:1

Potassium concentration: Addition of NaCl to the medium decreased potassium content of all shoot organs and particularly large decreases were found in shoots and roots of low nutrient treatment (Figs. 2:4, 2:5). At high nutrient NaCl treatment reduced potassium of the shoot but had little effect on root content. A similar situation is found in barley at high NaCl (Greenway 1963), though at lower sodium and chloride in the medium, selectivity of shoot is usually most pronounced (Sutcliffe 1957 and Pitman 1965).

In both nutrients, potassium decreased in the order - roots, shoots, leaf 1, leaf 2 and leaf 3. However, on a plant water basis (me/l), shoots and roots were lower in potassium than leaves. Low nutrient plants contained considerably less potassium than high nutrient plants (Table 2:3).

(c) NaCl effects over a range of nutrient strengths. Expt. 3.

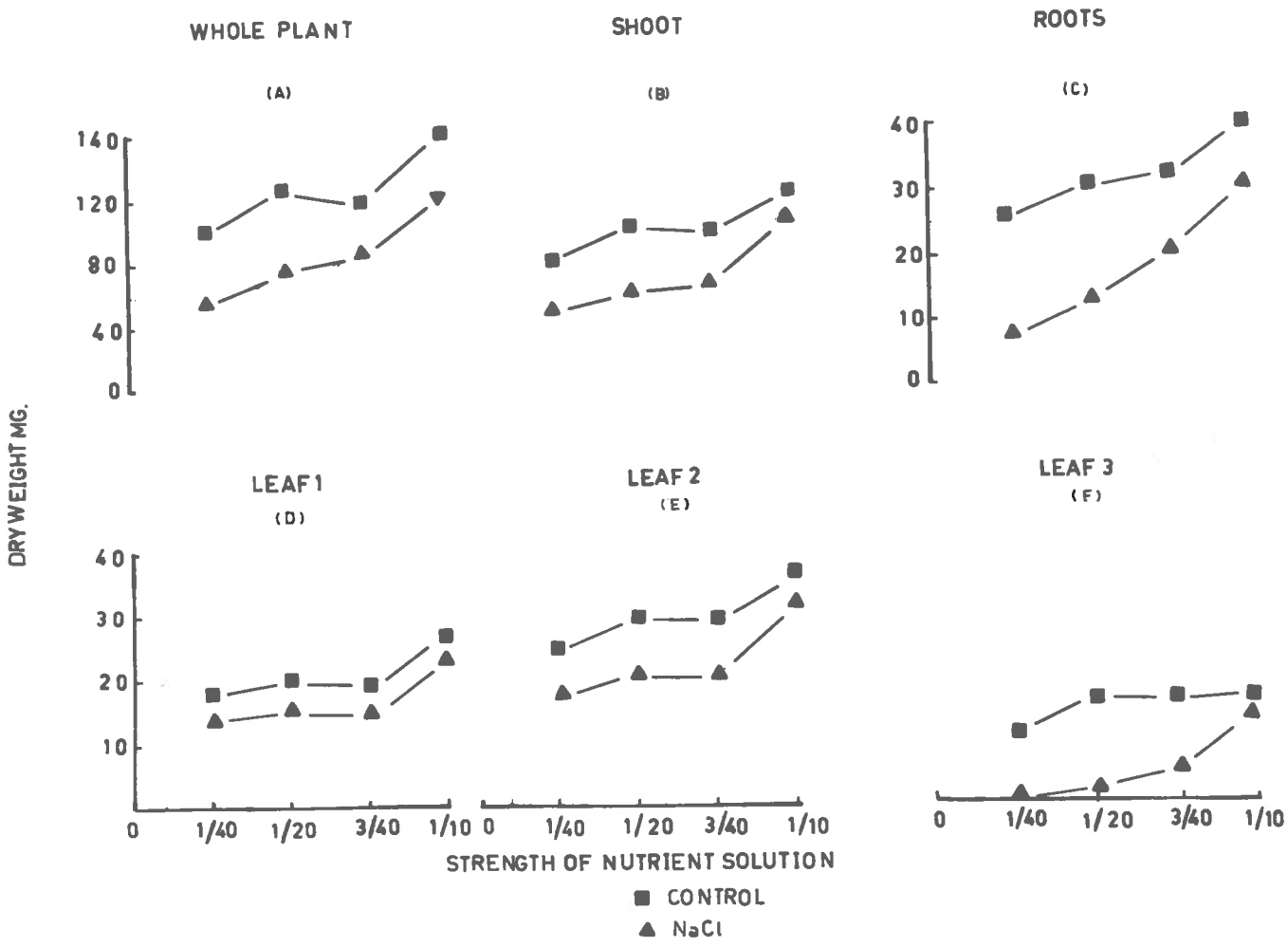
Various dilutions of nutrients were used to establish the lowest concentration at which the nutrients can counterbalance the severe NaCl effects at low nutrient.

Dry weight: NaCl reduced the dry weight of the whole plant and these effects of NaCl become progressively more pronounced at lower nutrient concentration in the medium (Fig. 2:6). NaCl reduced root growth more than shoot growth and adverse effects on root growth were particularly pronounced at low nutrient. The

FIGURE 2:6

Dry weight of whole plant and its individual parts of *Trifolium subterraneum* as affected by NaCl (50 me/l) over a range of nutrient strength. (Expt. 3 or Chapter 2).

FIG. 2.6


WHOLE PLANT
(A)
SHOOT
(B)
ROOTS
(C)
LEAF 1
(D)
LEAF 2
(E)
LEAF 3
(F)
DRY WEIGHT MG.
140
120
80
40
0
40
30
20
10
0
1/40
1/20
3/40
1/10
STRENGTH OF NUTRIENT SOLUTION
CONTROL
NaCl

strong effect of NaCl at low nutrient was found in the growth of all shoot organs particularly in the development of youngest leaf (L_3). The exception was the oldest leaf which was increased in dry weight due to NaCl treatment at both low and high nutrient.

DISCUSSION

There is very little information on salt tolerance of sub-clover. Salt tolerance of a crop is generally based on the relationship between growth and electrical conductivity of the saturation extract (ECs). The growth of a salt sensitive crop is reduced to 50 per cent at an ECs value of 4 mmho/cm (in the saturation extract) (U.S. Salinity Lab. Staff 1954). At field capacity the moisture content of the soil is about half of that at "saturation", the growth of a salt sensitive crop would be reduced to 50 per cent at 8 mmho/cm which is approximately 80 me/l of chloride. In the present results, growth of sub-clover was reduced at 50 me/l NaCl and that of youngest leaf even at 25 me/l; thus sub-clover is a very salt sensitive species. The present data do not permit a definite conclusion on the cause of reduced growth; however ionic balance was changed strongly at higher NaCl levels i.e. at 50 me/l most parts of the shoot contained more than 150 me/100 grams dry weight of sodium and chloride and had very low potassium concentration. Greenway (1962a) has also concluded that

growth of a salt sensitive variety of Hordeum was reduced due to high chloride and sodium and low potassium uptake.

Although growth of sub-clover was better at high nutrient, better root development occurred at low nutrient; this is in agreement with Reinberge and Rosovsky (1947) and May et al. (1964). NaCl effects on growth varied according to nutrient status of the medium, being more adverse at low than at high nutrient (Expts. 2 and 3). Similar effects were found for Hordeum vulgare by Greenway (1963). At low nutrient, addition of 25 me/l NaCl, i.e. an increase of only 1 atmosphere in osmotic pressure, decreased growth of the youngest leaf. This emphasises the importance of nutrient strength of the medium in salinity studies. It was noted that at low nutrient, the roots became brown after a few days of NaCl application. This browning intensified further and these roots appeared collapsed at the end of the experiment. Thus ion unbalance at low nutrient disturbed cell organization as well as reducing growth. It would be of interest to study the nature of this ion unbalance further.

In studying the interaction of NaCl at 4 different nutrient concentrations (Expt. 3, Table 2:4) the result showed a linear relationship between nutrient strength of the medium and growth in NaCl solution. Between $^{3}/_{40}$ and $^{1}/_{10}$ nutrient solution this might be due to improved growth even in the control treatments. However, the youngest leaf and roots, the most sensitive indicators of pot-

ential growth in a short term experiment, showed a strong improvement in growth between 3/40 and 1/10 nutrient in NaCl treatment but not in the control treatments. At 1/10 nutrient, NaCl reduced growth to a small extent showing that this nutrient concentration was antagonizing nearly all the specific unbalance effects observed at the lower nutrient levels. In Table 2:4 growth (dry weight) in NaCl treatments is expressed as percentages of the corresponding nutrient with no NaCl (Expt. 3).

All these experiments demonstrated a pronounced ion unbalance effect at low nutrition. The nature of this unbalance effect is unknown. Perhaps there were increased uptakes of chloride and sodium, but these were apparent only in some instances of the present experiments (Figs. 2:4, 2:5). However poor health of the plants in the later part of the experiment might have reduced uptake i.e. uptake and concentration might have been much higher at low nutrient before the health decline. This would be particularly so for the roots, where ions could be lost again to the solution. Another contributory factor might be a reduced uptake of essential elements. Such a reduced uptake was demonstrated for potassium, at least of the youngest leaf, where potassium concentrations were low enough to suspect a detrimental effect on growth. The other elements might be even more important than potassium and this can be established only by varying the concentration of individual essential element in the nutrient solution. This was done in the

TABLE 2:4

GROWTH (DRY WEIGHT) IN NaCl (50 me/l) AS A PERCENTAGE OF THE GROWTH IN CORRESPONDING NUTRIENT WITH NO NaCl

Nutrient strength	1/40	1/20	3/40	1/10	Full
L_1	112	117	112	114	116
L_2	66	72	84	96	104
L_3	6	12	27	90	63
Rest of shoot	61	58	67	80	82
Roots	25	40	67	67	80
Whole plant	55	59	72	86	89

subsequent experiments. However, sub-clover has certain disadvantages as test species, especially in its variable growth. It was therefore decided to continue the work with Hordeum vulgare as the test species. The experiments are described in the following chapters.

CHAPTER 3

RESPONSE OF *HORDEUM VULGARE* TO NaCl AT DIFFERENT NUTRIENT BALANCES

Introduction

In Chapter 2 it was observed that adverse effects of NaCl were more pronounced at low than at high nutrient content of the medium. In a medium of 50 me/l NaCl, growth of a salt tolerant variety of *Hordeum vulgare* was strongly reduced at $^{1}/_{40}$ strength of Hoagland but not at $^{1}/_{10}$ strength of Hoagland (Greenway 1963). There are some similarities between a high NaCl, low nutrient treatment, and soils with a high exchangeable sodium percentage i.e. in both, the medium has a high ratio of sodium and the plant roots show a distinct brown discolouration. There is little information on the causes of adverse effects of high NaCl and it has been suggested that these effects might have been due to high O.P. of the medium, increased NaCl content of the tissues or to an unbalance of ions i.e. monovalent/divalent cation ratio. Lagerwerrf and Ogata (1960) showed that in *Phaseolus vulgaris* growth was related to the ratio $\frac{Na^{+}}{\frac{Ca^{++} + Mg^{++}}{2}}$ of the solution.

More information is available on the growth response of unicellular algae to ion unbalance of the medium (Curtis and Clark Page 348, 1950). Optimum growth of algae was obtained in a mixture of monovalent and divalent cations (Mazia 1940) and it was concluded by these authors that the toxicity of a single-salt solution was due to permeability changes.

Previous work has not shown which ion of the nutrient culture was responsible for the unbalanced condition so this was studied in the present experiments. H. vulgare was used, usually during the first five days after germination. Barley was chosen because a lot is known about its salt tolerance, it is genetically uniform and is easy to handle during early stages of germination. In the past most of the germination experiments were in soils (Ayer 1953; Ayers et al. 1952) or on moistened filter paper in petri dishes (Uhvits 1946). However the composition of the solution was not exactly known because seeds absorb more water than sodium and chloride leading to high concentrations of salts around the seeds. In the present experiments such local salt accumulation was avoided by constant stirring and frequent replacement of the solution. Experiments were also done when the first leaf had fully developed.

When this work was begun, there were no published studies on the effect of salinity on growth as analysed by cell size and cell number. Recently Niemann (1965) has shown that both of these

features were affected by salinity in Phaseolus leaves. In the present work effects of salinity on cell size and cell number in roots were investigated at different nutrient balances.

METHODS

Germination experiments. H. vulgare seeds were germinated in the dark on nylon mesh suspended over the medium. Solutions were aerated and replaced every 24 hours to ensure that solutions surrounding the seeds were of the desired composition. The growth room was at 22°C and the atmosphere around the seeds was kept humid by placing petri dishes over the culture vessels. These dishes were removed when root development had satisfactorily advanced. Material was collected 5 days after the start of the experiment; seedlings were separated into coleoptile, roots and seeds. Fresh weight and dry weight were determined and in some experiments sodium, potassium and chloride were also determined. Full nutrient solution used in the experiment was of the same composition as in Chapter 2. NaCl was at 150 me/l unless otherwise stated. There were 3 or 4 replicates, each consisting of 10 to 15 seeds. Chloride was determined by the method described by Best (1950) and the "ELL" Flamephotometer was used for the determination of sodium and potassium.

Determination of Cell Length and Cell Number in Roots

Bürstrom's (1941) technique with modification was followed. This technique involves the determination of cell and root length and the computation of cell number, since increase in root length depends upon the size of individual cells and their number. A description of the technique is as follows:

After recording the length in millimeters, roots were placed in a clearing solution of chloral hydrate, phenol and lactic acid in the ratio of 1:1:3 (w:w:v) (Bisalputra 1960). These roots were examined under the microscope and the length of the epidermal cells was measured at the end of the zone of elongation. Each series of measurements included 180 cells. The ratio, $\frac{\text{increase in root length}}{\text{cell length}}$ was used to give a measure of cell number in the longitudinal direction of the roots.

(b) Experiment during 1st - 2nd leaf stage: Treatments were imposed when the 1st leaf had fully developed. There were 3 harvests.

H_1 = start of treatment with oldest leaf fully developed

H_2 = 7 days after H_1

H_3 = 5 days after H_2

The design of the experiment was as follows:-

	H_1	H_2	H_3
Start of the following treatments			
(1)	$^1/_{40}$ nutrient + NaCl (50 me/l)	++ Continued	+++++ Harvested
(2)	" " " "	++ Harvested	----
(3)	" " " "	++ Ca^{++} of $^3/_{40}$ nutrient added and continued	+++++ Harvested
(4)	$^1/_{40}$ nutrient + Ca^{++} of $^3/_{40}$ nutrient + NaCl (50 me/l)	++ Continued	+++++ Harvested
(5)	" " "	+ Harvested	-----

Each of the above treatments had a corresponding control without NaCl. Plant parts were separated into shoot, Leaf 1, Leaf 2 and roots.

RESULTS

(a) Germination stage. Effect of different NaCl concentrations on growth. Expt. 1.

Response of growth to different NaCl concentrations was established first. In this experiment NaCl at concentrations of 50, 100, 150 and 250 me/l was added to distilled water. The length of the coleoptile is shown in Table 3:1.

TABLE 3:1

COLEOPTILE LENGTH AT DIFFERENT CONCENTRATIONS OF NaCl AFTER 5 DAYS OF TREATMENT

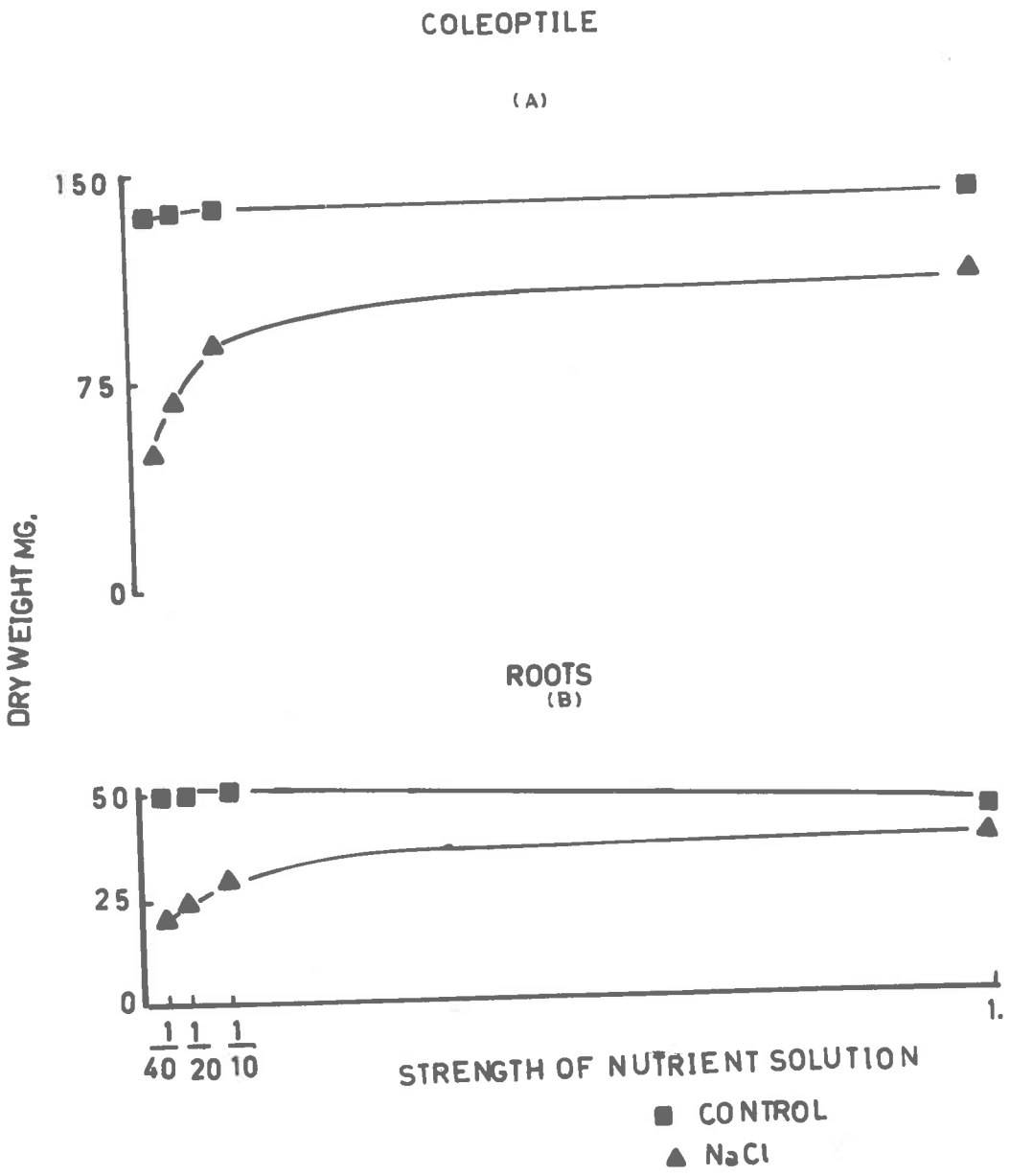
Treatment	Length in mm
Distilled water	31.2
NaCl (50 me/l)	27.8
" (100 me/l)	22.6
" (150 me/l)	18.4
" (250 me/l)	8.1

FIGURE 3:1

Dry weight of Coleoptile (A) and roots (B) of Hordeum vulgare at germination stage as affected by NaCl (150 me/l) over a range of nutrient strength.

(Expt. 2 of Chapter 3)

FIG.3.1


COLEOPTILE
(A)
150
75
0
DRY WEIGHT MG.
ROOTS
(B)
50
25
0
1.
1/40
1/20
1/10
STRENGTH OF NUTRIENT SOLUTION
CONTROL
NaCl

Although reduced at the rather low concentration of 50 me/l, growth also proceeded slowly at 250 me/l. These results are in agreement with the reported high salt tolerance of Hordeum vulgare during germination (Millington et al. 1951; Ayers 1953). The level of 150 me/l NaCl was chosen for most of the subsequent experiments.

Effect of NaCl at high and low nutrient. Expt. 2.

To 1, 1/10, 1/20 and 1/40 strength of nutrient solution, NaCl (150 me/l) was added and the results are presented in Fig. 3:1. It is shown that, under full nutrient conditions, NaCl reduced growth, particularly of the shoot. The effect of NaCl increased slightly when nutrient strength was reduced to 1/10. However, the effect of NaCl became more pronounced below 1/10 nutrient (Fig. 3:1). Root growth was particularly reduced so that at 1/40 nutrient, coleoptile and root growth were reduced by about the same percentage as compared with control (Fig. 3:1A and B).

Chloride, Sodium and Potassium contents:

Table 3:2 shows the concentrations of chloride, sodium and potassium in the seedlings. In the roots, sodium and chloride contents were high perhaps because the roots were washed in treatment solution and rinsing and drying of the surfaces had not been

TABLE 3:2

CHLORIDE, SODIUM AND POTASSIUM CONCENTRATIONS (me/100 gm Dry wt) IN COLEOPTILE AND ROOTS AT DIFFERENT NUTRIENT LEVELS AS AFFECTED BY NaCl (150 me/l)

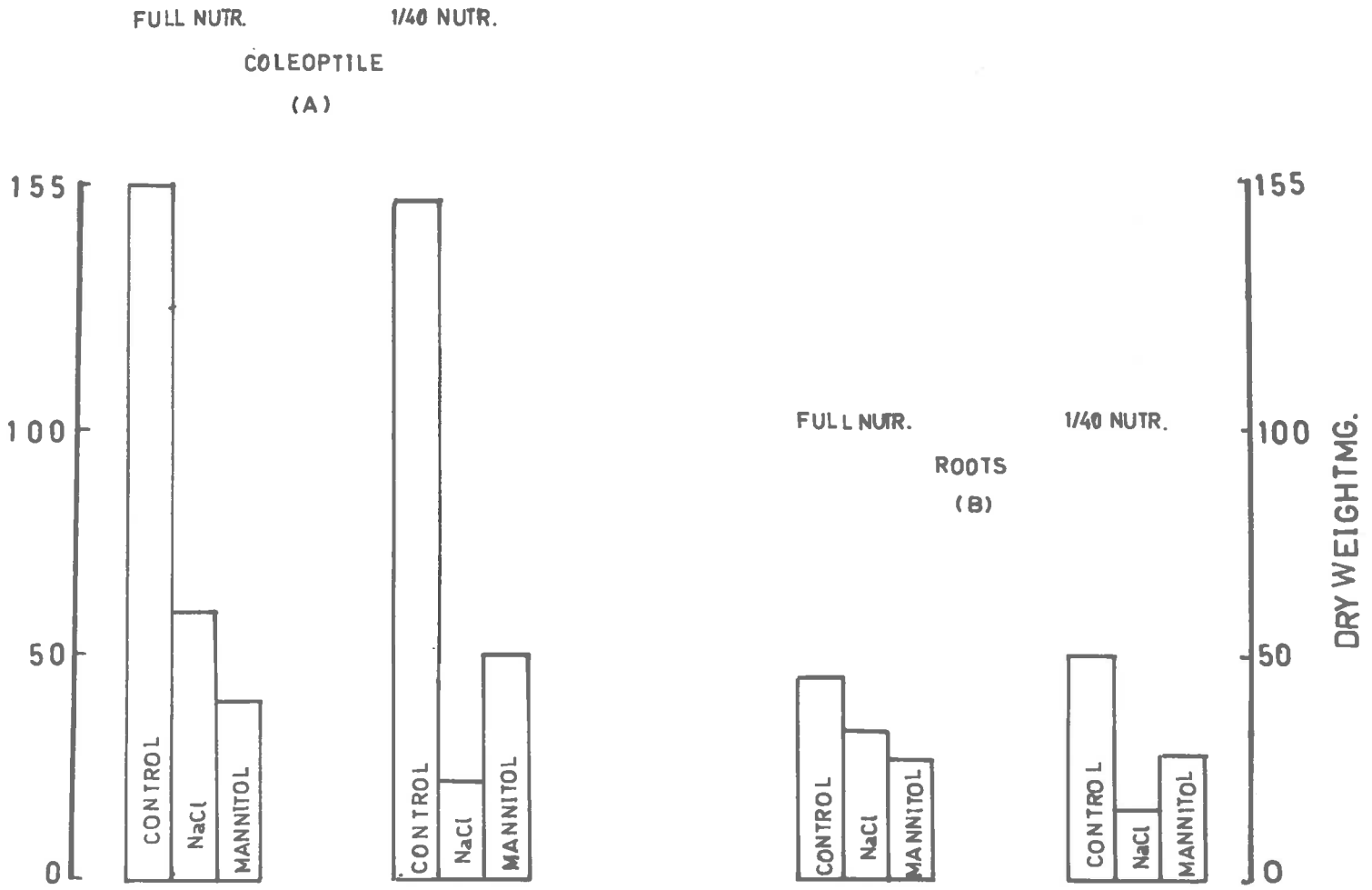
	Coleoptile			Roots		
Nutrients	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+
1/40 Control	5.6	10.6	57	12	22	17
1/20 "	3.5	9	79	3.6	16	26
1/10 "	4.0	9.2	102	2.0	18	39
1 "	5.0	9.6	153	3.9	16	132
1/40 + NaCl	16	27	39	198	200	15
1/20 + "	40	45	40	205	188	12
1/10 + "	60	63	41	183	200	15
1 + "	54.5	45	82	200	143	114

FIGURE 3:2

Dry weight of Coleoptile (A) and roots (B) of Hordeum vulgare at germination stage as affected by mannitol and NaCl at 6 atmospheres at low ($^{1}/_{40}$ nutrient) and high (full nutrient)

(Expt. 3 of Chapter 3)

FIG. 3.2


FULL NUTR.
1/40 NUTR.
COLEOPTILE
(A)
155
100
50
0
CONTROL
NaCl
MANNITOL
CONTROL
NaCl
MANNITOL
FULL NUTR.
1/40 NUTR.
ROOTS
(B)
CONTROL
NaCl
MANNITOL
CONTROL
NaCl
MANNITOL
155
100
50
0
DRY WEIGHTMG.

adequate. In the NaCl treated roots, sodium, potassium and chloride content as well as the potassium/sodium ratio were similar at all (except full) nutrient levels. In the coleoptile from NaCl treatment the sodium and chloride contents increased from $^{1}/_{40}$ nutrient to $^{1}/_{10}$ nutrient but decreased again somewhat at full nutrient. Potassium was very similar at all nutrient levels except at full nutrient where a high level of potassium was found. In order to know whether the sodium, chloride and potassium ions are excluded from roots or seeds at $^{1}/_{40}$ and full nutrient when subjected to high NaCl, the concentration of these ions was calculated as me/l in the next experiment at these two levels of nutrient. The results are presented in Table 3:3 and will be discussed subsequently.

Effect of Mannitol and NaCl at high and low nutrient. Expt. 3.

At full and $^{1}/_{40}$ nutrient, mannitol and NaCl at 6 atmospheres were compared (Fig. 3:2). As in Experiment 2, NaCl effects were more pronounced at $^{1}/_{40}$ than at full nutrient but mannitol effects were independent of nutrient strength. At full nutrient, growth in NaCl was better than in mannitol (Fig. 3:2) but at $^{1}/_{40}$ nutrient growth was depressed more in NaCl. Increased diffusion pressure deficit of the medium is often counterbalanced by organic acid accumulation by plant tissues, usually with potassium as the accompanying cation. It is therefore possible that the low potassium uptake at $^{1}/_{40}$ nutrient (Table 3:2) led to inadequate DPD

TABLE 3:3

CHLORIDE, SODIUM AND POTASSIUM CONCENTRATION IN PLANT WATER* IN ROOTS AND SEEDS AT 1/40 AND FULL NUTRIENT AS AFFECTED BY NaCl (150 me/l)

	Roots			Seeds		
	Cl^-	Na^+	K^+	Cl^-	Na^+	K^+
1/40 nutrient	175	250	510	40	66	80
Full nutrient	160	230	270	62	95	92

* Calculated similarly as in Table 2:1 (Chapter 2)

adjustment. However, as stated before, when mannitol of isosmotic concentration was used reductions of growth were equal at full and $^{1}/_{40}$ nutrient. The results of the above experiment showed that adverse effects of NaCl at low nutrient conditions were due to ion unbalance.

To investigate the nature of this ion unbalance the following experiments were designed.

NaCl effects at different cation to anion ratios. Expt. 4.

In this experiment the composition of the nutrient solutions in me/l was as follows.

Treatment	Ca^{++}	Mg^{++}	K^{+}	NO_3^{-}	$HPO_4^{=}$	$SO_4^{=}$
(1) $^{1}/_{40}$ nutrient	0.2	0.1	0.125	0.325	0.376	0.1
(2) $^{1}/_{10}$ "	0.8	0.4	0.5	1.3	0.15	0.4
(3) $^{1}/_{40}$ " + Ca, Mg and K of $^{3}/_{40}$ nutrient added as chloride	0.8	0.4	0.5	0.325	0.0376	0.1
(4) $^{1}/_{40}$ nutrient + PO_4, SO_4 and NO_3 of $^{3}/_{40}$ nutrient added as sodium	0.2	0.1	0.125	1.3	0.15	0.4

The above treatments constituted the controls and were compared with corresponding treatments containing NaCl (150 me/l).

FIGURE 3:3

Dry weight of coleoptile and roots of Hordeum vulgare at germination stage as affected by NaCl (150 me/l) at 1/40 nutrient with and without addition of ions of 3/40 nutrient strength.

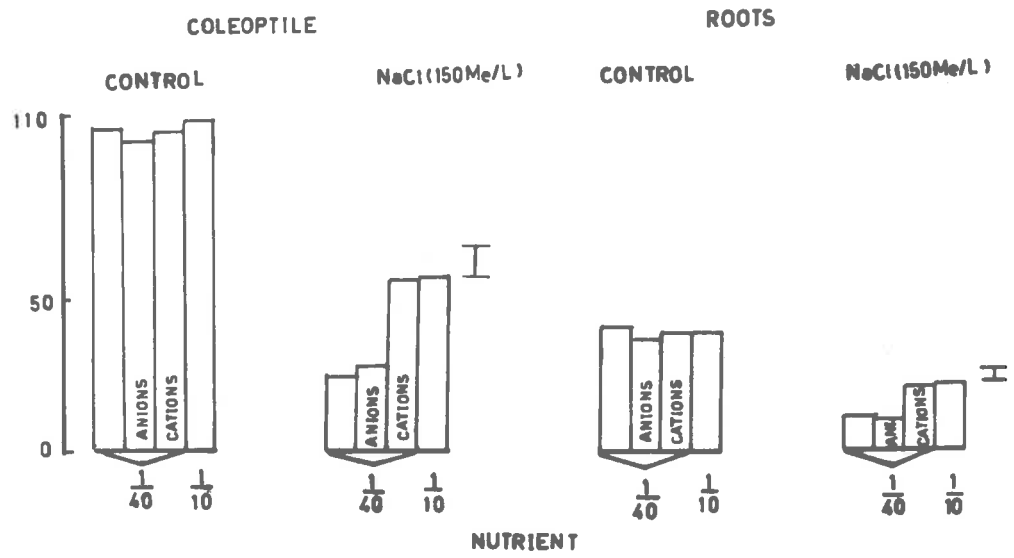
(A) To 1/40 nutrient containing NaCl cations and anions of 3/40 nutrient were added and compared.
(Expt. 4 of Chapter 3)

(B) To 1/40 nutrient containing NaCl calcium and magnesium and potassium of 3/40 nutrient were added and compared.
(Expt. 5 of Chapter 3)

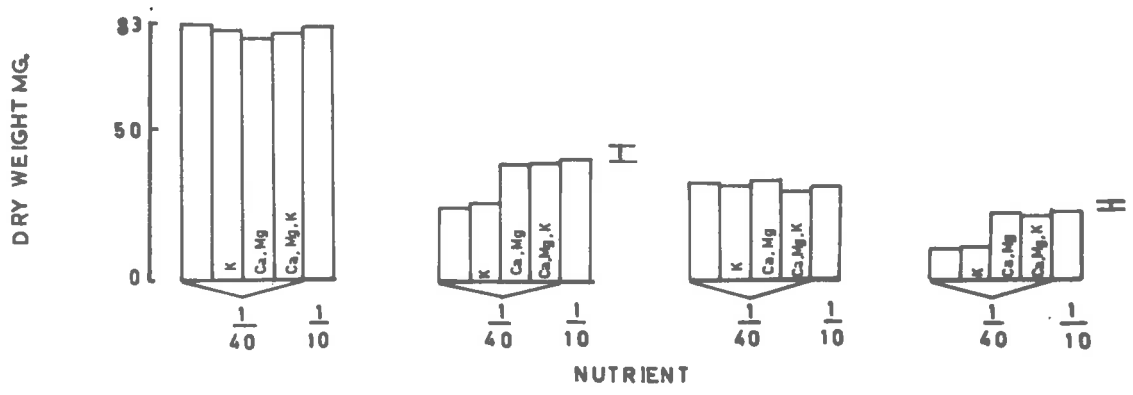
(C) To 1/40 nutrient containing NaCl calcium and magnesium of 3/40 nutrient were added and compared.
(Expt. 6 of Chapter 3)

FIG. 33

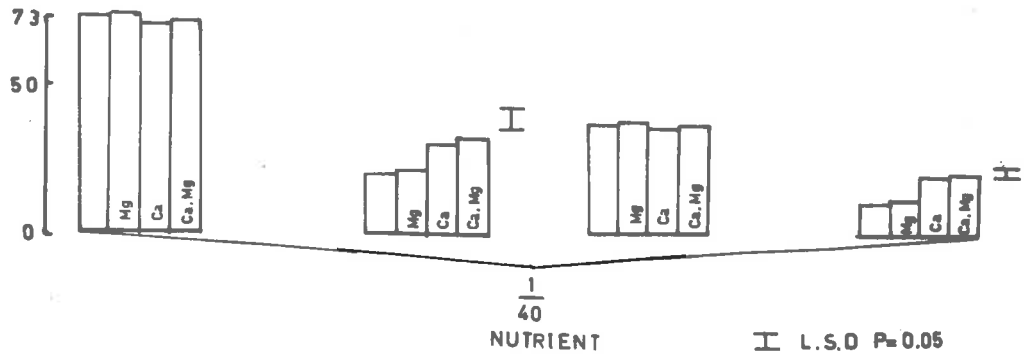
(A) COMPARISON BETWEEN CATIONS AND ANIONS



(B) COMPARISON BETWEEN K AND Ca, Mg



(C) COMPARISON BETWEEN Ca AND Mg



In the controls addition of cations also increased chloride concentration of the solution and anion addition increased sodium concentration. In NaCl treatment however, these additions of chloride and sodium respectively were negligible compared with 150 me/l NaCl.

There was no significant difference between the effects of these solutions on the growth of control plants (Fig. 3:3A). As in other experiments, high NaCl at $^1/_{40}$ nutrient reduced growth more than at $^1/_{10}$ nutrient. Addition of nutrient anions did not improve growth but addition of cations increased growth to the level of $^1/_{10}$ nutrient (Fig. 3:3A).

Effects of NaCl with other cations varied. Expt. 5.

(i) K^+ compared with Ca^{++} and Mg^{++}

The composition of the nutrient solutions in the different treatments in me/l was as follows

Treatment	Ca^{++}	Mg^{++}	K^+	NO_3^-	$HPO_4^=$	$SO_4^=$
(1) $Ca^{++} + Mg^{++} + K^+$	0.8	0.4	0.5	0.325	0.0376	0.1
(2) $Ca^{++} + Mg^+$	0.8	0.4	0.125	0.325	0.0376	0.1
(3) K^+	0.2	0.1	0.5	0.325	0.0376	0.1
(4) $^1/_{10}$ nutrient	0.8	0.4	0.5	1.3	0.15	0.4
(5) $^1/_{40}$ nutrient	0.2	0.1	0.125	0.325	0.0376	0.1

The above treatments constituted the controls and were compared with corresponding treatments containing NaCl (150 me/l).

Growth in the control plants was not significantly different in the various treatments (Fig. 3:3B). In the NaCl treatment, addition of potassium did not improve growth but addition of calcium and magnesium to the $^{1}/_{40}$ nutrient improved growth to the level of $^{1}/_{10}$ nutrient (Fig. 3:3B).

(ii) Ca^{++} compared with Mg^{++}. Expt. 6.

The composition of the nutrient solutions in the different solutions in me/l was as follows:

Treatment	Ca^{++}	Mg^{++}	K^{+}	NO_3^{-}	$HPO_3^{=}$	$SO_4^{=}$
(1) $^{1}/_{40}$ nutrient	0.2	0.1	0.125	0.325	0.0376	0.1
(2) Mg^{++}	0.2	0.4	0.125	0.325	0.0376	0.1
(3) Ca^{++}	0.8	0.1	0.125	0.325	0.0376	0.1
(4) Ca^{++} + Mg^{++}	0.8	0.4	0.125	0.325	0.0376	0.1

The above treatments constituted the control and were compared with corresponding treatments containing NaCl (150 me/l).

Growth in the control plants was similar in all the treatments (Fig. 3:3C). In the NaCl treatment addition of magnesium did not improve growth over $^{1}/_{40}$ nutrient but addition of calcium with or without magnesium increased growth over $^{1}/_{40}$ nutrient (Fig. 3:3C).

FIGURE 3:4

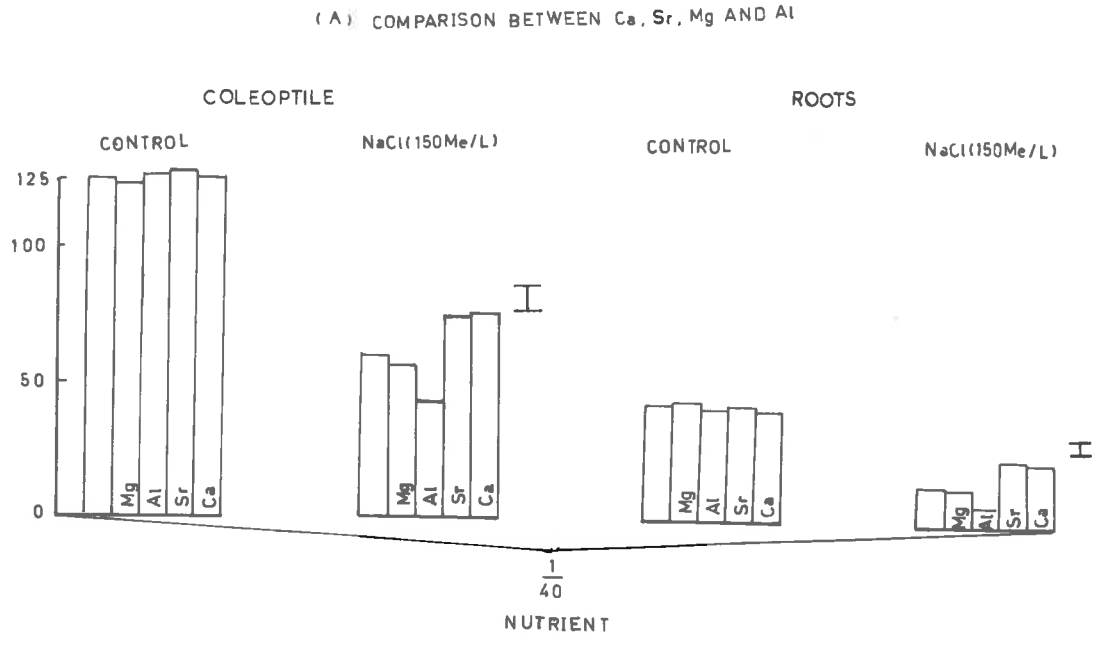
Dry weight of Coleoptile and roots of Hordeum Vulgare at germination stage as affected by NaCl (150 me/l) at $^{1}/_{40}$ nutrient

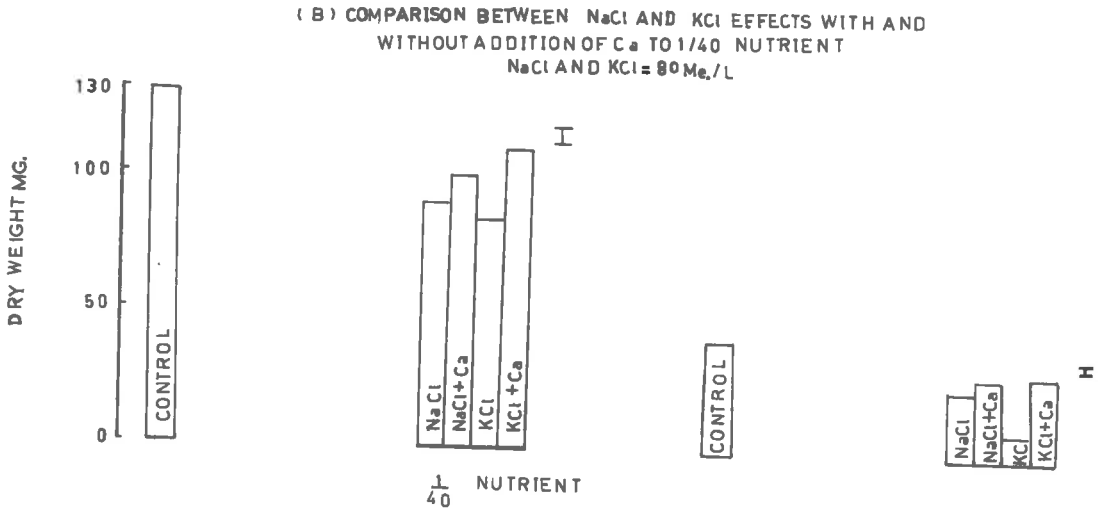
(A) with and without addition of Calcium, Strontium, Magnesium and Aluminum of $^{3}/_{40}$ nutrient.
(Expt. 7 of Chapter 3)

(B) Dry weights of Coleoptiles and roots in KCl (80 me/l) and NaCl (80 me/l) at $^{1}/_{40}$ nutrient were compared when Calcium of $^{3}/_{40}$ nutrient was added and omitted from these solutions.
(Expt. 8 or Chapter 3)

(C) Dry weights of Coleoptile and roots in NaCl (150 me/l) added to distilled water were compared when Calcium with or without other ions of $^{1}/_{10}$ nutrients was added.
(Expt. 9 of Chapter 3).

FIG 3.4


(A) COMPARISON BETWEEN Ca, Sr, Mg AND Al
COLEOPTILE
ROOTS
CONTROL
NaCl(150Me/L)
CONTROL
NaCl(150Me/L)
125
100
50
0
Mg
Al
Sr
Ca
1/40
NUTRIENT


(B) COMPARISON BETWEEN NaCl AND KCl EFFECTS WITH AND WITHOUT ADDITION OF Ca TO 1/40 NUTRIENT
NaCl AND KCl = 80 Me./L
DRY WEIGHT MG.
130
100
50
0
CONTROL
NaCl
NaCl+Ca
KCl
KCl+Ca
CONTROL
NaCl
NaCl+Ca
KCl
KCl+Ca
1/40 NUTRIENT


(C) DEPENDENCE OF Ca EFFECTS ON THE NUTRIENT LEVEL.
Ca AND NUTRIENTS = 1/10 OF FULL NUTRIENT SOLUTION NaCl = 150Me/L
140
100
50
0
1
2
3
4
5
6
D. WATER + NUTRIENTS
D. WATER
D. WATER + NUTRIENTS + NaCl + Ca
D. WATER + NaCl + Ca
D. WATER + NUTRIENTS + NaCl
D. WATER + NaCl
L.S.D P = 0.05

(iii) Ca^{++}, Sr^{++}, Mg^{++} and Al^{+++} compared. Expt. 7.

At $^{1}/40$ nutrient the above salts as chloride at 0.8 me/l were compared for their antagonism against NaCl (150 me/l) effects. At this NaCl concentration addition of calcium and strontium improved growth equally, whereas magnesium did not (Fig. 3:4A). Aluminium decreased growth of the NaCl-treated seedlings below that of $^{1}/40$ nutrient (Fig. 3:4A) but was without effect in the controls (i.e. without NaCl).

Effects of Ca^{++} on high KCl and high NaCl levels. Expt. 8.

It has been shown that NaCl toxicity is greatly antagonized by the addition of small amounts of calcium (Expt. 6, Fig. 3:3C). High concentrations of KCl may also reduce growth strongly. An experiment was designed to establish this point and to see whether calcium antagonized KCl as well as NaCl effects. Sodium and potassium were determined in the NaCl-treated and control plants, but not in the KCl-treated plants.

Treatments

(1) $^{1}/40$ nutrient

(2) $^{1}/40$ " + NaCl (80 me/l)

(3) $^{1}/40$ " + NaCl (80 me/l) + Ca^{++} (0.6 me/l)

(4) $^{1}/40$ " + KCl (80 me/l)

(5) $^{1}/40$ " + KCl (80 me/l) + Ca^{++} (0.6 me/l)

TABLE 3:4

SODIUM AND POTASSIUM CONCENTRATION (me/100 gm dry wt)
IN COLEOPTILE AND ROOTS AS AFFECTED BY
NaCl (80 me/l) AT 0.2 AND 0.8 me/l Ca^{++}

Treatments	Coleoptile		Roots	
	Na^+	K^+	Na^+	K^+
(1) *1/40 nutrient	15	85	20	24
(2) " " + NaCl (80 me/l)	53	42	165	14
(3) " " + " " + Ca (0.6 me/l)	84	46	170	26

* 1/40 of nutrient contains 0.2 me/l of Ca^{++}

KCl reduced growth more than NaCl at the $^{1}/_{40}$ nutrient level (Fig. 3:4B). Addition of calcium recovered growth in KCl back to higher level than it recovered growth in NaCl, i.e. the unbalance in KCl had been higher than in NaCl at $^{1}/_{40}$ nutrient. However KCl treatment did not show the brown discolouration of roots, which was always observed in the NaCl-treated plants.

Sodium and Potassium uptake

NaCl treatment increased sodium concentration in coleoptiles and there were high concentrations in the roots. In shoots and roots sodium concentrations were higher at 0.8 me/l of calcium than at 0.2 me/l of calcium (Table 3:4).

NaCl with 0.8 me/l calcium did not reduce the potassium concentration of the root below $^{1}/_{40}$ nutrient control (Table 3:4) though there was a considerable decrease in potassium concentration in the coleoptile. Omission of calcium in NaCl treatment reduced potassium concentration, particularly in the roots (Table 3:4).

Dependence of Ca^{++} effects on the nutrient level. Expt. 9.

Though the experiments were done during germination, adverse effects of NaCl at low nutrient might conceivably be due to reduced absorption of essential elements in the absence of calcium. This experiment was designed to establish whether the presence of calcium increased the availability of the nutrients for growth.

Treatment

(1) Distilled water + NaCl (150 me/l) + Ca^{++} (0.8 me/l)

(2) " " + NaCl (150 me/l)

(3) " "

(4) " " + $PO_4^{\equiv}$, NO_3^-, $SO_4^{\equiv}$, Mg^{++}, K^+, of $^1/10$ nutrient + NaCl (150 me/l) + Ca^{++} 0.8 me/l)

(5) " " + $PO_4^{\equiv}$, NO_3^-, $SO_4^{\equiv}$, Mg^{++}, K^+, of $^1/10$ nutrient + NaCl (150 me/l)

(6) " " + $PO_4^{\equiv}$, NO_3^-, $SO_4^{\equiv}$, Mg^{++}, K^+, of $^1/10$ nutrient + Ca^{++} (0.8 me/l)

In control plants growth of coleoptile was better in the presence of nutrient but better root development occurred in distilled water (Fig. 3:4C). There were no differences between distilled water and $^1/10$ nutrient as a basal medium for the calcium-induced recovery of growth in NaCl-treated plants (Fig. 3:4C).

(b) Recovery at germination and at 1st and 2nd leaf stage. Expt. 10.

Germination

In previous experiments it has been shown that at low nutrient, calcium protects growth against NaCl toxicity. The well known effects of calcium on membrane permeability may in part contribute to better growth by preventing leakage of intermediates from the roots. Before making metabolic studies it is important

to establish how quickly addition of calcium results in growth recovery and this was studied in the following experiments.

Treatments

(1) $^{1}/40$ strength nutrient + Ca^{++} (0.6 me/l) + NaCl (150 me/l) throughout the experiment.

(2) $^{1}/40$ strength nutrient + NaCl (150 me/l) for 2 days then + Ca^{++} (0.6 me/l) added.

Harvests H_1 = 2 days after Ca^{++} addition to treatment 2

H_2 = 3 days after H_1

H_3 = 3 days after H_2

Results - At H_1 growth was strongly reduced in low calcium treatment (Table 3:5) showing a strong adverse effect of the previous $^{1}/40$ nutrient i.e. the combined effects of NaCl and $^{1}/40$ nutrient had been very pronounced even after only 2 days of treatment. Recovery in this treatment after day 4 (H_1) was very rapid; dry weight increments of coleoptile and roots were higher than in the continuous 0.8 me/l calcium.NaCl treatment (Table 3:5).

The 1st - 2nd Leaf Stage

The design of this experiment is described in part (b) of the Methods.

During the 1st - 2nd leaf stage recovery upon addition of calcium was also studied. It was thought that at this stage of growth the calcium in the seed reserves would have become exhausted and the growth would depend upon the external source of supply.

TABLE 3:5

DRY WEIGHT INCREMENTS (Milligrams) OF COLEOPTILE AND ROOTS OF H. VULGARE AT 0.8 AND 0.2 me/l OF Ca^{++} AS AFFECTED BY NaCl (150 me/l) AT DIFFERENT PERIODS AFTER TREATMENT APPLICATION

	Treatment 1 (0.8 me/l Ca^{++})			Treatment 2 (0.2 me/l Ca^{++})		
Harvest Intervals	Coleoptile	Roots	Total	Coleoptile	Roots	Total
H_1	34	22	56	20	10	30
Between H_1 and H_2	39	2	41	40	8	48
Between H_2 and H_3	17	5	2	21	4	25
Total	90	29	119	81	22	103
Recovery between H_1 and H_3	56	7	63	61	12	73

Dry weight in treatment 2 as percentage of dry weight in treatment 1 at different harvests.

	H_1	H_2	H_3
Coleoptile	58	82	90
Roots	49	75	75

FIGURE 3:6

Dry weight of whole plant and its individual parts of Hordeum vulgare at 1st leaf stage as affected by NaCl (50 me/l) with and without addition of Calcium of $^{3}/_{40}$ nutrient to $^{1}/_{40}$ nutrient at different harvest intervals.

(Expt. 10 of Chapter 3)

FIG. 3.6

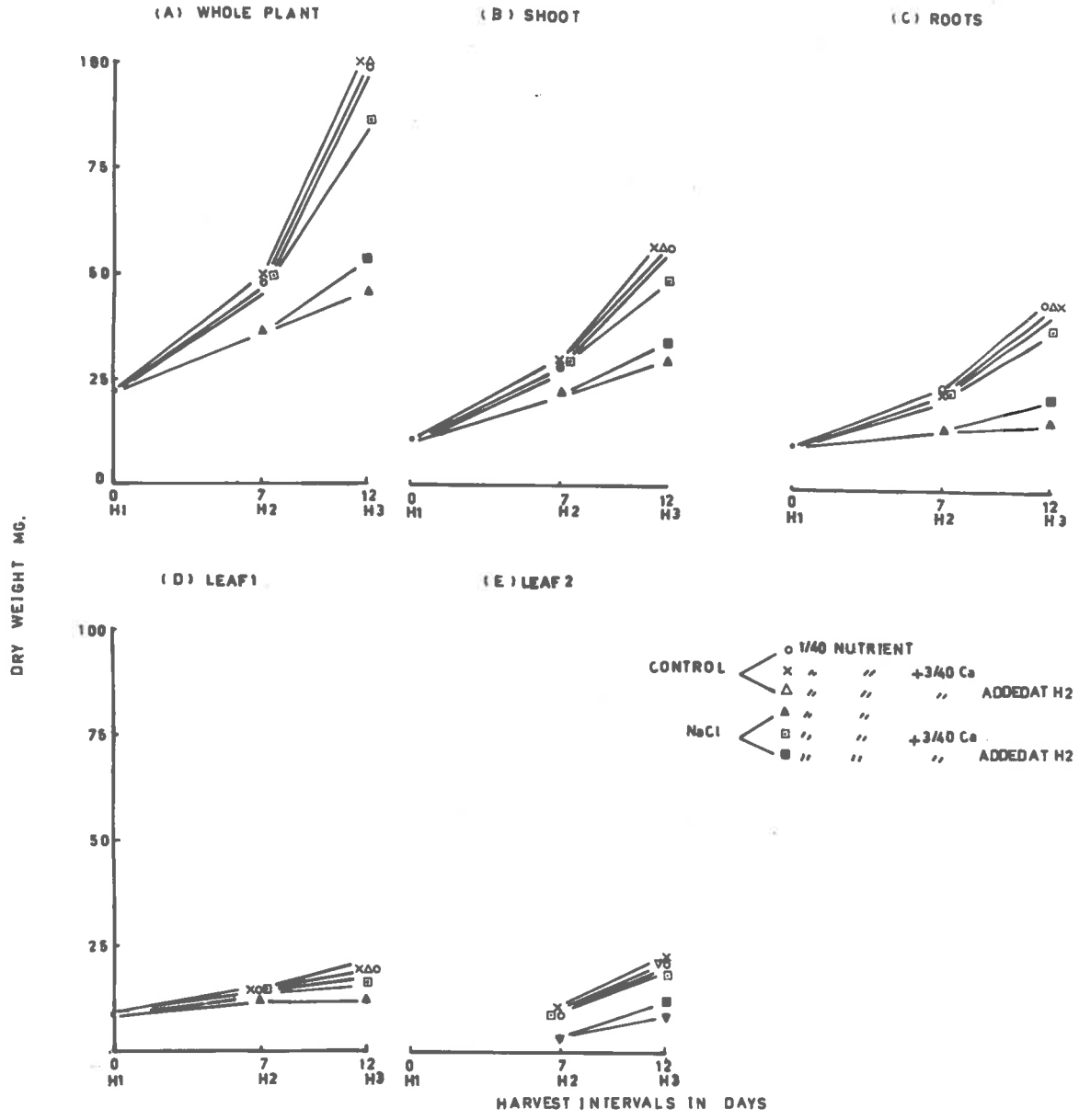
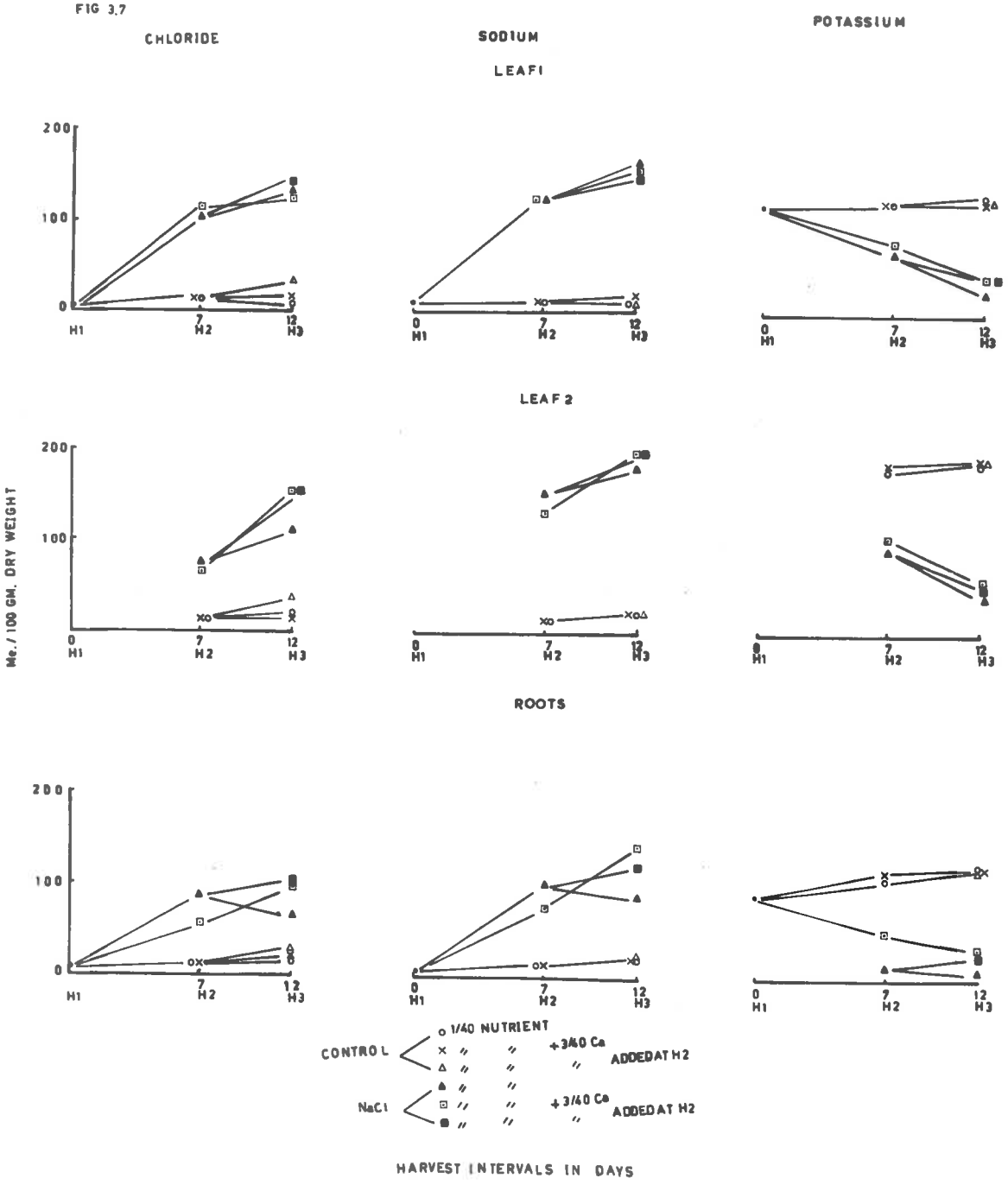

(A) WHOLE PLANT
(B) SHOOT
(C) ROOTS
(D) LEAF1
(E) LEAF 2
DRY WEIGHT MG.
100
75
50
25
0
0 H1
7 H2
12 H3
HARVEST INTERVALS IN DAYS
CONTROL
o 1/40 NUTRIENT
× " " +3/40 Ca
△ " " " ADDEDAT H2
NaCl
▲ " "
⊡ " " +3/40 Ca
■ " " " ADDEDAT H2

FIGURE 3:7

Chloride, sodium and potassium concentrations in leaf 1, leaf 2 and roots as affected by NaCl (50 me/l) with and without addition of calcium of $^{3}/_{40}$ nutrient to $^{1}/_{40}$ nutrient at different harvest intervals.

(Expt. 10 of Chapter 3)

FIG 3.7


CHLORIDE
SODIUM
POTASSIUM
LEAF1
LEAF 2
ROOTS
Me./100 GM. DRY WEIGHT
200
100
0
H1
H2
H3
0
7
12
1/40 NUTRIENT
CONTROL
NaCl
+3/40 Ca
ADDEDAT H2
HARVEST INTERVALS IN DAYS

The effect of 50 me/l NaCl alone was very pronounced and root growth virtually ceased after 6 days (H_2) of treatment (Fig. 3:6C). Supplying 0.6 me/l of calcium to $^1/_{40}$ nutrient during the whole experiment removed nearly all the adverse effects of NaCl (Fig. 3:6). When after 7 days of treatment 0.6 me/l of calcium was added to $^1/_{40}$ nutrient, growth recovered pronouncedly (Figs. 3:6A to D).

Ion Concentration

Analysis of plants subjected to NaCl treatment at high calcium, showed that sodium and chloride concentrations of all plant parts usually increased with time and that younger leaves had lower chloride concentration than older leaves (Fig. 3:7, Leaf 2 compared with Leaf 1). Similar results for Hordeum vulgare were reported by Greenway (1965).

At H_2 sodium and chloride concentrations of roots subjected to NaCl were higher at low than at high calcium (Fig. 3:7 Roots)and a similar though less pronounced difference was found for sodium of leaf 2 (Fig. 3:7 Leaf 2). However at H_5 both leaf 2 and roots contained less sodium and chloride at low than at high calcium i.e. both sodium and chloride content of the root and leaf 2 increased strongly at high but very little at low calcium.

Potassium

Potassium concentrations of plant tissues in all the control treatments were similar and did not change with time (Fig. 3:7). In

the NaCl treatments on the other hand, potassium in these tissues decreased at the subsequent harvest intervals, the decrease being more pronounced at low than at high calcium, particularly in roots (Fig. 3:7 Roots). Similarly in the NaCl treatment, addition of extra calcium after 7 days to low calcium treatment increased potassium in roots to the same level as high calcium plant (Fig. 3:7 Roots).

Effects of NaCl on cell size and cell number at low and high Ca^{++}. Expt. 11.

In Experiment 2 it was noted that high NaCl at low nutrient had inhibitory effects on growth which could not be explained on osmotic considerations. Addition of 0.5 me/l of calcium to the medium reduced the inhibitory effects of NaCl as well as recovering growth of plants previously treated with low calcium.

Since growth depends on cell division and elongation, the next experiments were designed to see if inhibitory effects of NaCl at low calcium could be explained by either a reduction of cell size or cell number. This was investigated together with the effect of high NaCl at low and high calcium on the action of added indole acetic acid (I.A.A.) which is known to affect cell elongation.

Treatment (a) Seeds were soaked in distilled water for 24 hours, after which a single primary root per seed was re-

tained and rest removed. These seeds were then transferred to the following treatment solutions.

1. $^{1}/_{40}$ nutrient
2. $^{1}/_{40}$ " + NaCl (30 me/l)
3. $^{1}/_{40}$ " + NaCl (30 me/l) + Ca (0.6 me/l)

There were two culture dishes containing 50 seeds each for each treatment. Six seeds from each treatment were harvested on each day for root and cell length measurements and the experiment was continued for 4 days. Treatments 2 and 3 will be referred to as low and high calcium.

Treatment (b) To test the effect of NaCl on the action of I.A.A. at low and high calcium, treatments were as above except that solutions contained indole acetic acid at the concentration of 10^{-11}M. This concentration of I.A.A. has been found to stimulate root growth (Bonner and Koepfli 1939). Harvest was after 5 days of treatment. Dry weights of coleoptile and root were determined.

RESULTS

Treatment (a) The results have been expressed as % of control for

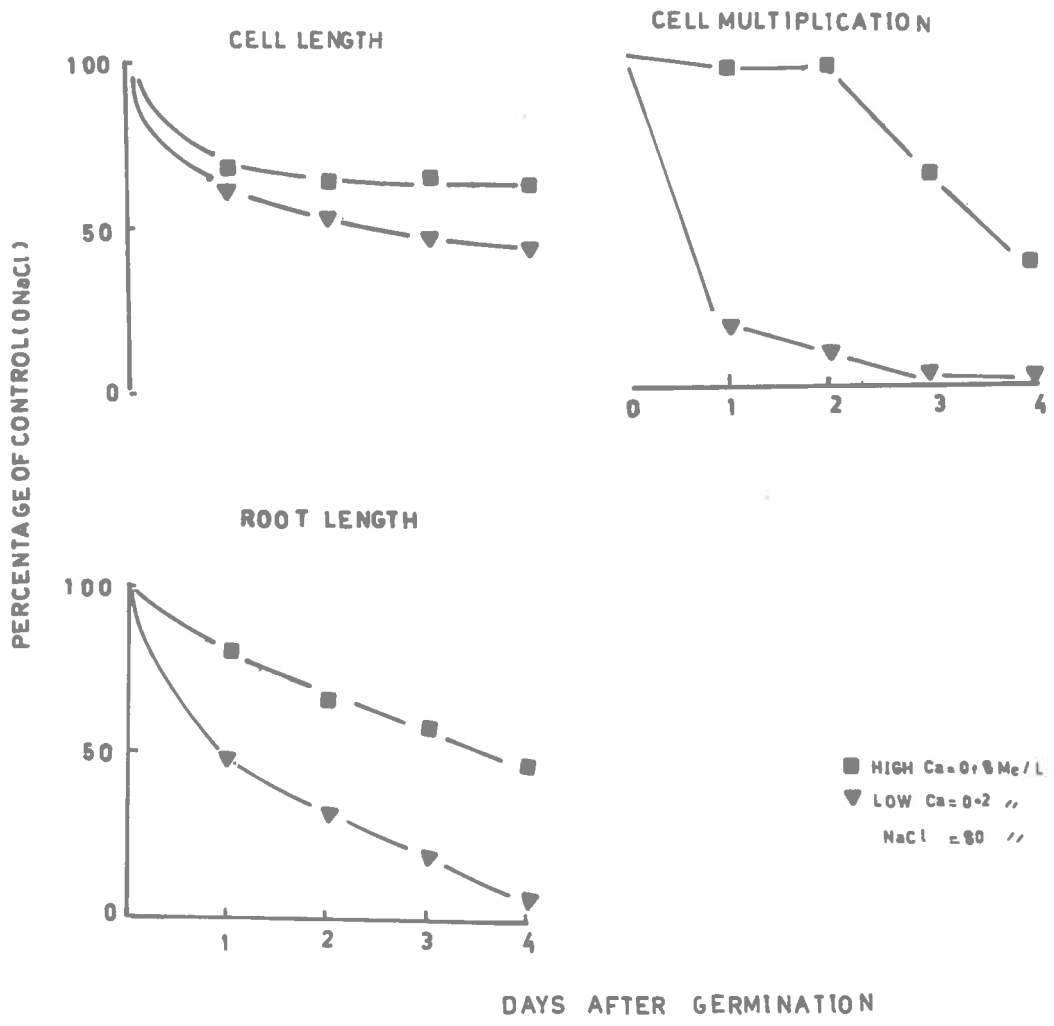
FIGURE 3:8

Effect of NaCl (80 me/l) on root length, cell length and cell multiplication of Hordeum vulgare at germination stages during 4 days at $^{1}/_{40}$ nutrient (low calcium) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrient (high calcium). Results are expressed as percentage of control (0 NaCl).

(Expt. 11 of Chapter 3).

FIG. 3.8

EFFECT OF NaCl ON ROOT LENGTH CELL LENGTH
AND CELL MULTIPLICATION AT LOW
AND HIGH Ca



root length, cell length and cell number and are shown in Fig. 3:8.

Root length: Reduction in the root length due to NaCl was greater at low than at high calcium throughout the experimental period. At high calcium the decline in root length as percentage of control was linear with time but a slow and constant growth was maintained. In low calcium treatment the root length which dropped to 50 per cent of control on first day ceased elongation after the second day. This is reflected by a continuous decrease in percentage length of control in Fig. 3:8.

Cell length: On the first day of the experiment, reduction in cell length at both calcium levels was similar but on subsequent days reduction at low calcium was greater than at high calcium. Cell length at high calcium did not decrease during the experimental period but did decrease at low calcium level.

Cell number: Cell multiplication was greatly reduced at low calcium even after the first day of the experiment and on the last 2 days cell formation in this treatment had virtually ceased. At high calcium however, cell multi-

TABLE 3:6

ANALYSIS OF VARIANCE FOR EFFECT OF Ca^{++} AND I.A.A. ON THE DRY WEIGHT OF COLEOPTILE OF H. VULGARE

Source of Variance	Degree of freedom	F	Sum of Squares	Mean square variance
Between Ca^{++} treatments	1	77.0***	7700	7700
Between I.A.A. "	1	12.8 **	1278	1278
Interaction Ca^{++} and I.A.A.	1	1.04	105	105
Total between treatments	3	30.0***	9083	3024
Residual	12	-	1205	1004
Total	15	-	10288	686

*** Significant at 0.1% level

** " " 1% "

plication was not affected for the first 2 days but during the last 2 days a sharp decrease must have occurred.

Treatment (b)

Effect of hormone: Addition of I.A.A. either to low or high calcium treatment increased the dry weight of the plants. The difference in the dry weights of the coleoptile and roots in the above two treatments was not statistically significant from the difference in the dry weights of the corresponding treatments without I.A.A. The results for the coleoptile are presented in Table 3:6.

Discussion

Ion Uptake

Hordeum vulgare, in the first and second leaf stages, shows a pronounced ion regulation when grown on media of high NaCl concentration; for example concentrations are much lower in the roots than in the medium (Greenway 1963). In the present germination experiment, at low nutrient, chloride and sodium concentrations were higher in the roots than in the medium (Table 3:3).

Table 3:3 shows that ion exclusion has been demonstrated by seeds both at high and low nutrient level and it is more pronounced at low nutrient. In interpreting this ion exclusion one possibility is that at germination when seeds are full of endospermic reserves of starch, free space for diffusion may be very restricted, offering little chance for ions to diffuse although the seed coat may be permeable. If such were the case, higher values for chloride and sodium ions (me/l) would have been expected in full nutrient NaCl than in $^{1}/_{40}$ nutrient NaCl because better growth of seedlings at full nutrient would have depleted the seed reserves and thus allowed entry of ions. Since lower values for sodium and chloride (me/l) were found at $^{1}/_{40}$ nutrient where, due to poor growth most of the seed material still remains undepleted, lack of diffusion, or slow diffusion could be a cause of apparent ion exclusion in the seed.

Ion regulation is presumably as essential for the seed during germination as it is for the whole plant during later growth stages. It is therefore surprising that no information on the ion unbalance of germinating seeds is available. Particularly relevant would be the ion balance of different parts of seed i.e. endosperm and developing embryo.

In the germination experiments (Expt. B, Table 3:4) NaCl treatment reduced the uptake of sodium into coleoptiles at the low

calcium level of the medium but root uptake was not reduced. This reduced uptake into the shoot appears to be due to impairment of the translocation system in low calcium-high NaCl treatment. These sodium concentrations in the coleoptiles were greater at $^{1}/10$ than at $^{1}/40$ nutrient (Expt. 8, Table 3:4). Despite this the growth in the $^{1}/10$ nutrient was better than at $^{1}/40$ nutrient so the higher concentrations of sodium and chloride were obviously not responsible for the specific adverse effects of NaCl at $^{1}/40$ nutrient.

In the 1st and 2nd leaf stage recovery experiment it is noted that at H_2 low calcium led to higher chloride and sodium accumulation than at high calcium. Similar trends in accumulation of these ions were noted in sub-clover (Chapter 1). This must be a real effect since the low chloride and sodium of low calcium at H_3 is most likely to be due to injury similar to that noted in the germination stage. Effects on chloride uptake described by Greenway (1963) in a short term experiment (i.e. that chloride uptake decreased continuously with increasing nutrient) are now shown to be due to the level of calcium.

In previous experiments, at $^{1}/40$ nutrient, high NaCl resulted in a very low potassium concentration of roots and shoots (Greenway 1963). He implied that this was due to a low sodium/potassium ratio in the medium. However the present experiments

show that low potassium concentrations were primarily due to the low calcium status of the 1/40 nutrient, i.e. addition of 0.6 me/l of calcium to this medium resulted in higher potassium concentrations which can also be attained when calcium is added subsequent to a treatment at 1/40 nutrient high NaCl (Fig. 3:7).

(b) Growth

At high nutrient, NaCl reduced growth less than an isosmotic concentration of mannitol (Expt. 3) and this was presumably due to a different type of diffusion pressure deficit adjustment. Slatyer (1961), working with tomatoes, showed high relative turgidities in NaCl treatments due to rapid electrolyte absorption, while mannitol-treated plants, increased their DPD by waterloss. In barley, wilting was also more severe in mannitol than in NaCl (Groenewegen et al. 1960).

Low nutrient in the medium increased the adverse effects of NaCl on growth of H. vulgare during the 1st - 2nd leaf stage (Greenway 1963) and similar effects were found in the present experiments for the 5 days following commencement of germination. This was due to ion unbalance as shown by the much better growth in isosmotic concentration of mannitol (Expt. 3). Uhvits (1946) also showed much stronger effects of NaCl than of mannitol during treatment of germinating lucerne seeds. Uhvits used tap water as the basal medium, so the specific ion unbalance at low nutrient, shown

in this thesis, would also have been involved in the lucerne experiments.

At high nutrient, NaCl suppressed cell enlargement and cell division proportionately in the first trifoliate leaf of Phaseolus vulgaris (Niemann 1965). Present results at low nutrition show that depression of root elongation in NaCl treatments was particularly due to inhibition of cell multiplication, cell elongation being affected to a lesser extent. It has also been established that the adverse effects of NaCl at low nutrient on cell multiplication and elongation were due to limiting levels of calcium in the medium because at a higher calcium level reduction in cell elongation or multiplication was not as pronounced as at low calcium (Fig. 3:3). The results of hormone experiments show that the adverse effect of NaCl on root elongation at low calcium level is not due to interaction with an I.A.A. requiring mechanism since I.A.A. did not interfere with the calcium effects and calcium did not interfere with the I.A.A. effects.

Heiman and Ratner (1961) attributed the adverse effects of NaCl at low nutrient to changes in the potassium/sodium ratios of the plant tissues. Ion unbalance has also been noted for plants grown on media with a high exchangeable sodium percentage (Bernstein and Pearson 1956). Bernstein and Pearson using ionic composition of the shoot to interpret their results suggested that

calcium was not responsible for the adverse effects.

In the present experiments, calcium was found to be the only nutrient responsible for the larger difference in NaCl effects at $^{1}/40$ and $^{1}/10$ nutrient, other ions having no effect. Growth was also greatly increased by supplying extra calcium to $^{1}/40$ nutrient, both during the 1st and 2nd leaf stage at high NaCl and during germination in media of high KCl concentrations (Expt. 8).

In the germination stage, antagonising effects of calcium could be replaced by equivalent amounts of strontium. Walsh (1945) has reported that in oats, strontium carbonate could replace calcium carbonate for better stem production and increase the calcium content of the straw. From the germination experiment it appears that strontium might have replaced calcium from non specific binding sites, thus releasing calcium for specific functions. This possibility needs further investigation.

(c) Antagonistic effects of calcium

The adverse effect of low calcium, high sodium was not primarily due to the absorption of essential elements from the medium as shown by the improved growth when calcium was added to distilled water during the germination stage (Expt. 8). Germination experiments were carried out in the dark, hence photosynthesis was not involved, and seed reserves were at a high level till the

end of the experiment.

Another possibility is an effect on mobilisation of seed reserves. However, this is not likely because similar responses were obtained during the 1st - 2nd leaf stage, when seed reserves were depleted. Moreover, ion balance of the seed was not adversely affected and a reduction in calcium concentration would presumably have altered at least the potassium concentration of the seeds.

The other two possible causes for the reduced growth may be a change in permeability of membranes or a direct effect on metabolism, presumably during synthesis.

CHAPTER 4

LOSSES AND UPTAKE OF IONS AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}

Introduction

In Chapter 3 it was shown that at high concentration of NaCl, growth was reduced severely. The addition of calcium prevented or restored this inhibited growth. The dry weight of plants in low calcium (conc.) treatment was 77 per cent of high calcium (conc.) (Fig. 3:4A). Ion concentration in plant tissues at low calcium were also less than at high calcium, so that sodium in coleoptiles and potassium in roots were 65 per cent and 50 per cent respectively of high calcium treated plants (Table 3:4).

Ion concentration was not directly correlated with dry weight. The following two factors might have been responsible for lower than expected ionic concentrations in low calcium plants:

1. Retention of absorbed ions was reduced due to increased losses from plants.
2. Active ion uptake was reduced.

Both of these possibilities were studied in the present series of experiments. Losses of ions from plants will be dealt with first and this will be followed by the discussion of their uptake.

LOSSES OF IONS AS RELATED TO PERMEABILITY

Ion retention in plants is reduced when membranes become more permeable (Marschner et al. 1964). Another effect of increased permeability would be the loss of important metabolites, resulting in reduced growth. It is therefore possible that growth reductions observed in high NaCl-low calcium plants might have been due to increased permeability. It is still unknown how the nutritional status of the cell affects membrane permeability (Collander 1959). This information is particularly important for the understanding of the plant responses to saline environments. In the older literature (Höber 1945 and Heilbrunn 1952) the concept was developed that univalent ions increase cell permeability whereas divalent ions decrease it. The evidence on which this hypothesis was based was not conclusive e.g. greater uptake of bromide from potassium bromide solutions than from calcium bromide solutions does not prove that calcium decreases cell permeability. The result can also be explained by one or more of the following: ion antagonism; suitability of accompanying ions; or competition for carriers.

Permeability studies on unicellular organisms, tissue discs and excised roots, using radio isotopes have considerably advanced the knowledge of electrolyte fluxes in these organs (Briggs, Hope and Robertson 1961), but these studies are not directly applicable to intact plants because the situation in intact plants is more complicated.

In the present experiments possible differences in permeability at low and high calcium plants were measured by:

1. Feeding the labelled ions to the plants and determining their losses to the non-labelled solution.
2. Determining the losses of naturally occurring ions (amino acids) from the seed to the culture solution.

The radioactive isotopes used were Na^{24}, Cl^{36} and Rb^{86}.

GENERAL METHODS

Experiments were done with Hordeum vulgare both during germination and 1st leaf stage. Culture methods were the same as described in Chapters 3 and 5. The basic solution was $^{1}/_{40}$ strength of full nutrient and NaCl concentrations were 0, 50 or 80 me/l and calcium concentrations were 0.2 and 0.8 me/l. These two calcium levels with NaCl will be referred to as low and high calcium respectively.

(i) Sampling of plant material: After the period of tracer absorption, plants were rinsed for 3 minutes in cold (non-labelled) culture solution, and separated into shoots and roots. Roots were dried between tissue papers and fresh or dry weights determined. Further details are given in the appropriate sections.

(ii) Measurement of radioactivity: Radioactive tracers were obtained from the Australian Atomic Energy Commission. Na^{24} and Rb^{86} were applied as chloride salts and Cl^{36} as the sodium salt. Cl^{36} was counted both as liquid and solid. For solid counting samples were ground and spread uniformly on planchets and counted with a mica end-window counter. Liquid counting of Cl^{36}, Na^{24} and Rb^{86} was done in a MX 124/Cl liquid Geiger Muller tube. The plant tissues were treated with dilute nitric acid and boiling distilled water to extract Rb^{86} and Na^{24} and the total volume made to 15 ml. Total counts for each sample were minimally 1000 disintegrations, and at high counting rates counting was continued for at least 5 minutes. The counts were converted to micro-equivalents per gram of fresh or dry weight.

(iii) Chemical: Calcium was determined with an Atomic absorption spectrophotometer (David 1959) and sodium and potassium with an EEL flame photometer.

Individual Experiments

Amino acid loss from germinating seeds. Expt. 1.

Culture dishes contained 125 ml of solution. Each dish had 50 seeds and there were 2 dishes for each treatment. NaCl was at 0 and 80 me/l at low and high calcium and the seeds were germinated in the treatment solution. Loss of amino acids was determined to the culture solution in which the seeds had grown for 24 hours. This solution was sampled and then reduced to 2 ml after which amino acids were determined by the ninhydrin method (Yemm and Cocking 1955). The experiment was continued for 5 days after commencement of treatment.

Cl^{36} loss. Expt. 2.

This experiment was done during the 1st leaf stage. NaCl at 50 me/l at low and high calcium was labelled with Cl^{36} and treatments were continued for 80 hours. There were 3 replicates of 4 plants each in each treatment. Seventy two hours after tracer application one group of plants was harvested (this harvest will be referred to as H_1), another group was transferred to non-labelled solution and Cl^{36} loss from these plants was measured at intervals of 1, 3, 5 and 8 hours. At the conclusion of loss measurements (80 hours after tracer application) this group was also harvested. (This harvest will be called H_2).

Cl^{36} uptake at low and high Ca^{++} as affected by NaCl. Expt. 3.

This experiment was done at the 1st leaf stage. Cl^{36} uptake was determined when NaCl treatment was imposed for short and long durations. In the short term experiment plants were treated with NaCl at low and high calcium for 2 days and then transferred to Cl^{36} labelled solution for 1 hour for uptake measurements.

In the long term experiment pretreatment with NaCl was for 5 days and Cl^{36} uptake was measured over 6 hours. There were 2 replicates of 4 plants each in each treatment.

Na^{24} loss and uptake at germination. Expt. 4.

Treatments were the same as for the amino acids (Expt. 1). Control treatment i.e. without NaCl had to be omitted since the basic nutrient solution had no sodium that could be labelled. Culture dishes had 16 seeds, and there were two dishes for each treatment. After 4 days of NaCl treatment the solutions in the dishes were replaced with solutions of the same composition but containing Na^{24}. Seedlings were allowed to absorb tracer for 20 hours and then transferred to non-labelled solutions. At the time of tracer removal plants were sampled for uptake measurements. The loss of tracer was determined by counting the culture solutions as well as by tissue analysis at 2, 3, 5, 8 hours after tracer removal.

^{24}Na uptake at 1st leaf stage. Expt. 5.

This experiment was done at the 1st leaf stage. NaCl was at 0 and 50 me/l at low and high calcium. There were two replicates of 4 plants each for each treatment. The plants were first treated with NaCl for 4 days and then transferred to fresh treatment solutions which were labelled with Na^{24}. Na^{24} uptake was measured in each treatment by sampling plants at intervals of 1, 5 and 8 hours.

K^+ loss and uptake from intact plants. Expt. 6.

This experiment was done at the 1st leaf stage. NaCl was at 0 and 50 me/l at low and high calcium. There were two replicates of 4 plants each for each treatment. Plants were treated with NaCl for 5 days before transferring them to the tracer solution. Three replicates of each treatment were harvested prior to tracer application. This harvest will be known as H_1. The rest of the plants were transferred to the respective treatment solution in which potassium was labelled with Rb^{86}. The uptake was measured by harvesting 2 replicates of each treatment at intervals of 4, 16 and 24 hours. After the absorption period, the loss of tracer was determined by transferring 3 replicates from each treatment to non-labelled solutions. These non-labelled solutions were replaced at 2, 5, 16 and 24 hours. Potassium concentration in all harvested samples was determined on the flame photometer.

K loss and uptake from excised roots. Expt. 7.

In order to know how quickly permeability changes are induced by NaCl treatment, plants at 1st leaf stage were treated with 50 me/l of NaCl at low and high calcium for a period of 1 to 4 days. There were 2 replicates of 4 plants each for each treatment. The excised roots of these pretreated plants were transferred to aerated treatment solution at 25°C in which potassium was labelled with Rb^{86}. Uptake measurements were done by taking samples at 24 hours after Rb^{86} application. The loss of tracer was measured by transferring the plants to non-labelled solutions and the loss was measured after 2, 4, 6 and 8 hours.

K uptake by excised leaf tissues. Expt. 8.

Plants at the 1st leaf stage were treated with 0 and 50 me/l of NaCl at low and high calcium for a period of 4 days. There were 2 replicates of 4 plants each for each treatment. The leaves of these pretreated plants were cut into slices 500 u thick according to the method of Smith and Epstein (1964). The slices were transferred to aerated treatment solution at 25°C in which potassium was labelled with Rb^{86}. Rb^{86} was measured by taking samples at ½, 1, 1½, 2 and 2½ hours.

Na^{+}, K^{+} and Ca^{++} concentration in intact plants as affected by NaCl at low and high Ca^{++}. Expt. 9.

The design of the experiment was identical to the excised roots experiment (Expt. 7). Sodium, potassium and calcium concentrations in plants were determined chemically by analysing roots and shoots for these ions.

Effect of DNP on Na^{24} uptake at low and high Ca^{++}, as affected by NaCl. Expt. 10

This experiment was done at germination stage. Plants at low and high calcium were treated with NaCl (80 me/l) for 4 days. They were then transferred to Na^{24} labelled treatment solutions with and without DNP (10^{-5}M). The number of replications was the same as in Expt. 3. Na^{24} uptake in the shoots and roots was measured at 6 (H_1) and 12 (H_2) hours after tracer application.

RESULTS

The loss of inorganic ions from plant tissues has been shown in the Figures by calculating the percentage of ions retained at different time intervals after the removal of tracer. The ion content at the time of tracer removal (time 0) was taken as 100 per

TABLE 4:1

Cl^{36} LOSS IN U.EQUIV/GM DRY WEIGHT MEASURED OVER 1 TO 8 HOURS FROM PLANTS AT LOW AND HIGH Ca^{++} AS AFFECTED BY NaCl (50 me/l)

Treatments	1 hour	3 hours	5 hours	8 hours
High Ca^{++}	3	5.9	6.02	14.5
Low Ca^{++}	2.78	6.68	12.78	19.16

FIGURE 4:1

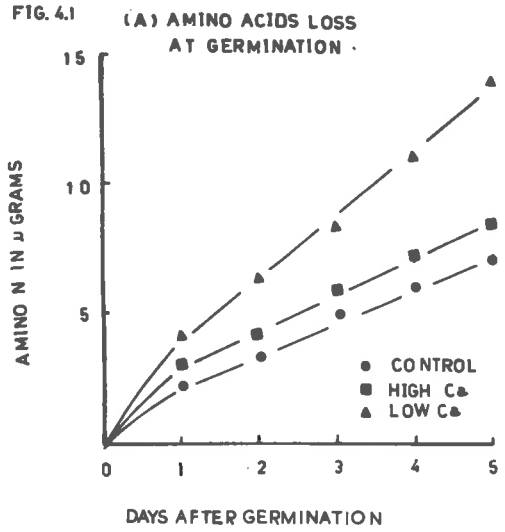
Effect of NaCl (50 me/l) in Hordeum vulgare at $^{1}/_{40}$ nutrient (low Ca^{++}) and at $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrients (high Ca^{++}) on

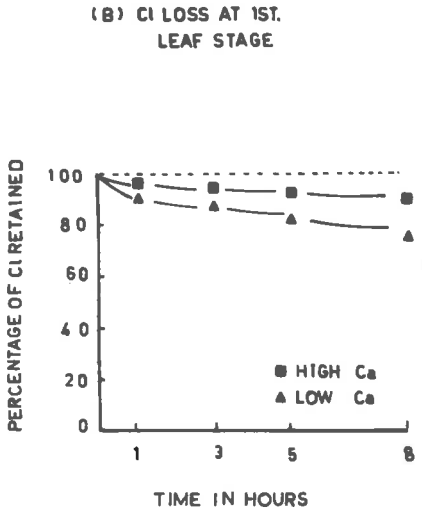
(A) Amino acid loss at germination stages (Expt. 1 of Chapter 4)

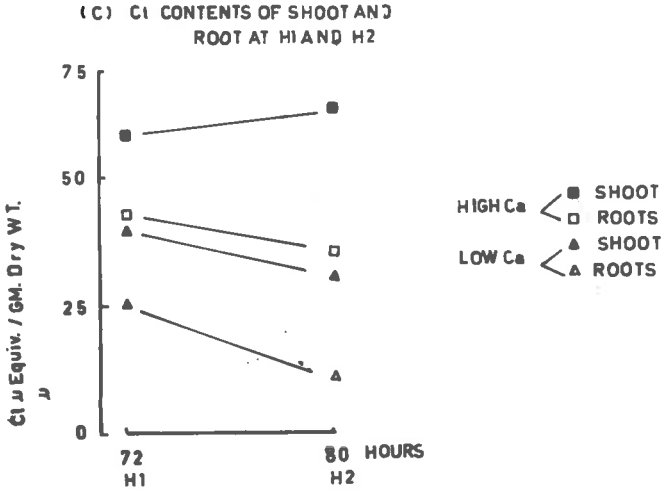
(B) Cl^{36} loss of 1st leaf stage (Expt. 2 of Chapter 4).

(C) Cl^{36} content of shoot and roots at first and final harvests. (Expt. 2 of Chapter 4).

FIG. 4.1


(A) AMINO ACIDS LOSS AT GERMINATION
AMINO N IN µ GRAMS
15
10
5
0
1
2
3
4
5
CONTROL
HIGH Ca
LOW Ca
DAYS AFTER GERMINATION


(B) Cl LOSS AT 1ST. LEAF STAGE
PERCENTAGE OF Cl RETAINED
100
80
60
40
20
0
1
3
5
8
HIGH Ca
LOW Ca
TIME IN HOURS


(C) Cl CONTENTS OF SHOOT AND ROOT AT H1 AND H2
Cl µ Equiv. / GM. Dry WT. µ
75
50
25
0
72
H1
80 HOURS
H2
HIGH Ca
SHOOT
ROOTS
LOW Ca
SHOOT
ROOTS

cent and the percentage of ions retained was obtained from the differences in ion content at subsequent periods from that at time 0.

Amino acid loss at germination. Expt. 1.

In all treatments amino acid losses were high at first but decreased with time after the first day. Losses of amino acid were much higher from low than from either control or high calcium (Fig. 4:1A).

Cl^{36} loss. Expt. 2.

At both levels of calcium, Cl^{36} loss to the solution after 3 days of labelling was greater in the first hour than during the subsequent hours (Fig. 4:1B). Low calcium plants lost more Cl^{36} than plants in high calcium. Table 4:1 gives the absolute amount of Cl^{36} loss at low and high calcium. In order to know whether Cl^{36} loss occurred from shoot or roots, Cl^{36} concentration of these organs at 72 (H_1) and 80 (H_2) hours were plotted (Fig. 4:1C). From this figure it appears that only roots of high and low calcium plants and shoots of low calcium plants lose Cl^{36} but the loss from shoots was not statistically significant.

FIGURE 4:2

Effect of NaCl (50 me/l) in Hordeum vulgare at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrient (high Ca^{++}) on:-

(A) Cl^{36} uptake at 1st leaf stage in short and long term experiment. (Expt. 3 of Chapter 4).

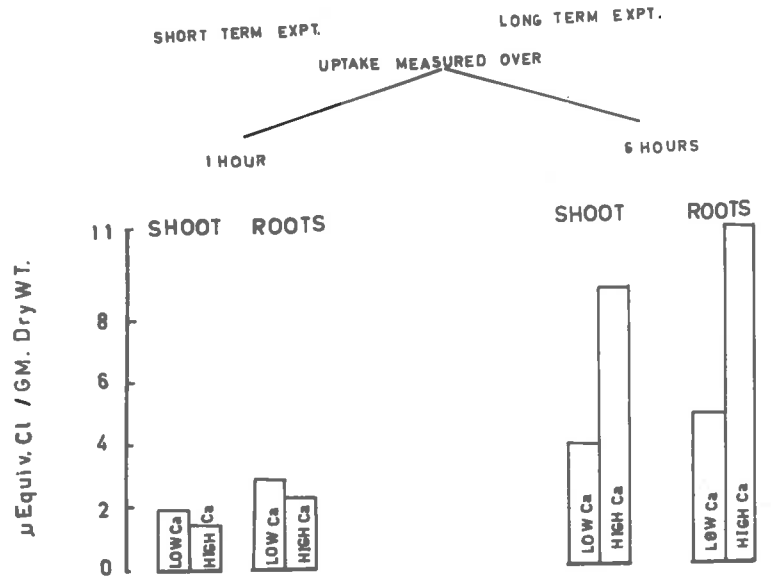
(B) Na^{24} loss at germination measured over 3 hours

(a) in non-labelled solution.

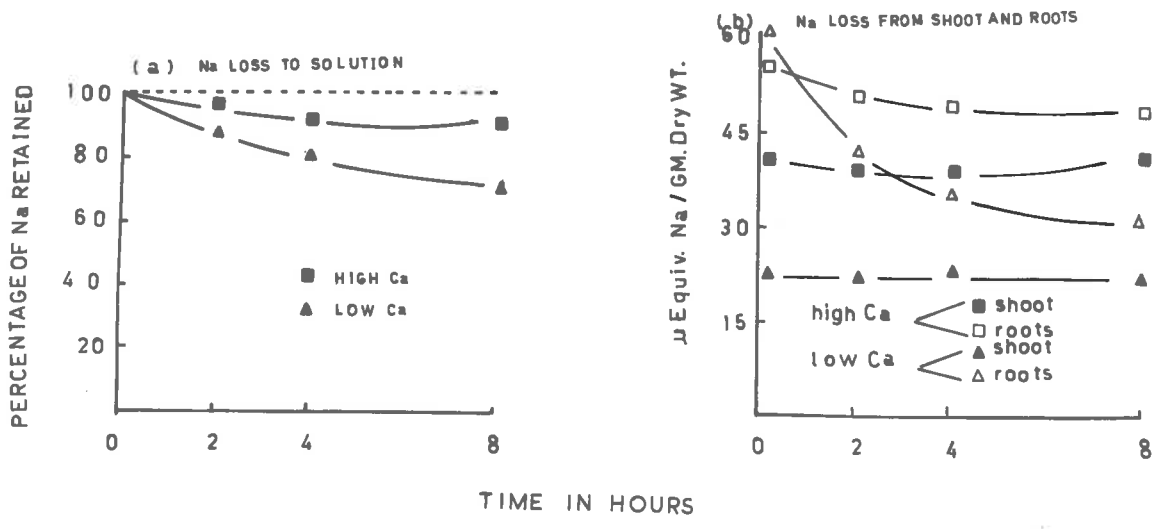
(b) by counting plant tissues.

(Expt. 4 of Chapter 4).

FIG.4.2 (A) Cl UPTAKE AT 1ST. LEAF STAGE.



(B) Na LOSS AT GERMINATION



Cl^{36} uptake at low and high Ca^{++} as affected by NaCl. Expt. 3.

In the short term experiment i.e. when NaCl treatment was imposed for 2 days, Cl^{36} uptake into plants was higher at low than at high calcium treatment, but when plants were treated with NaCl for longer periods Cl^{36} uptake into plants at low calcium was reduced more than into plants at high calcium treatment (Fig. 4:2A).

Na^{24} loss and uptake at germination. Expt. 4.

Loss: Sodium loss to the solution after 4 days of NaCl treatment was greater at low than at high calcium in all sampling occasions (Fig. 4:2B(a)). This loss at low calcium increased rapidly during the first 4 hours but decreased during the last 4 hours of loss measurements. At high calcium on the other hand Na^{24} loss to the solution which increased slowly during the first 4 hours did not increase further in the later period. Similarly to Cl^{36} loss, the only detectable Na^{24} loss at low calcium occurred from roots. The losses measured in solutions were in good agreement with those measured by counting of the tissues (Fig. 4:2B(b)).

Uptake: After 20 hours labelling, Na^{24} uptake in the coleoptiles of low calcium plants was reduced to $^1/_2$ that of the plants at high calcium (Fig. 4:2B(b)) but root uptake of Na^{24} was higher at low than at high calcium. It is quite probable that 4 days pretreatment with

FIGURE 4:3

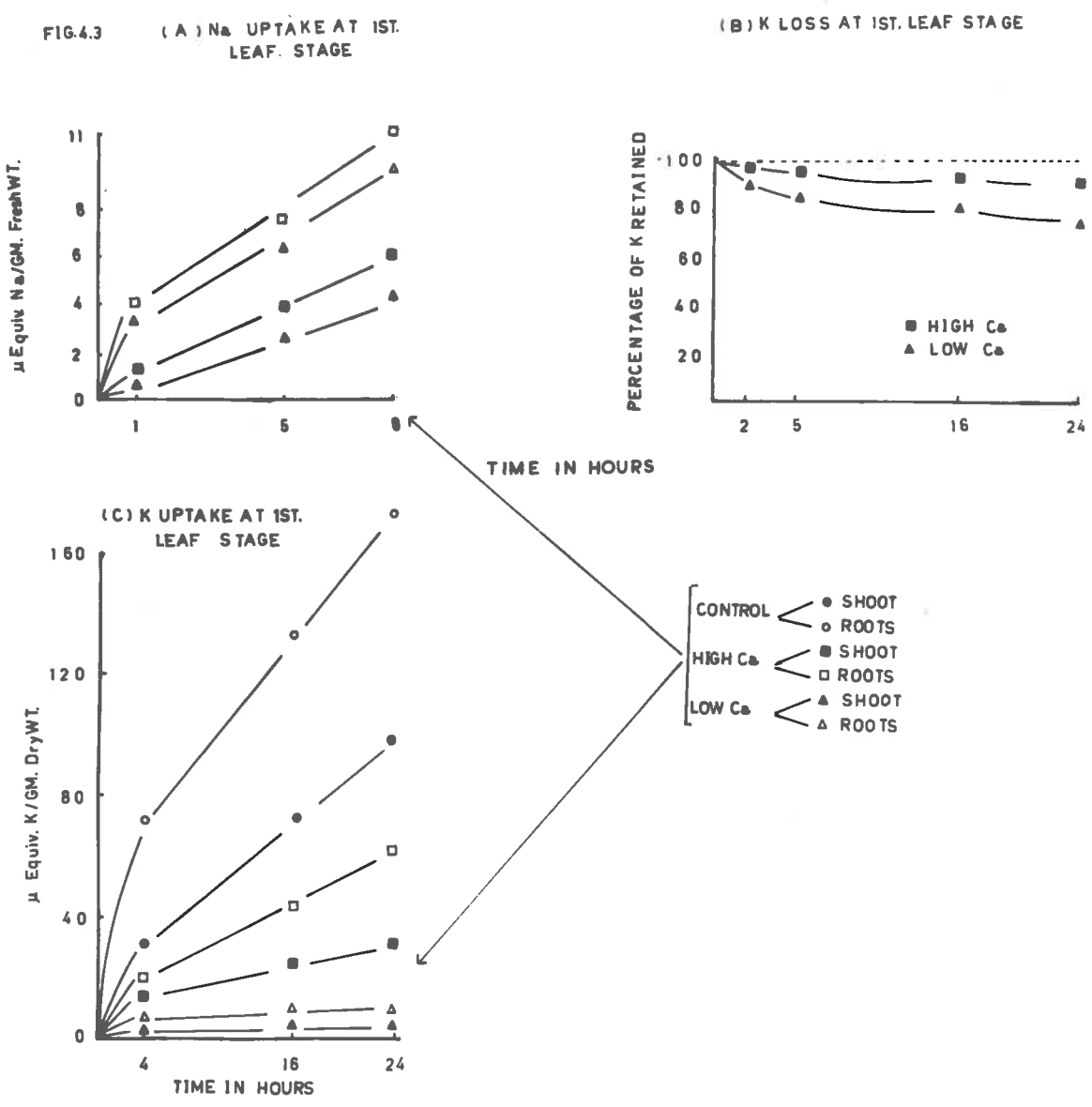
Effect of NaCl (50 me/l) in *Hordeum vulgare* at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrient (high Ca^{++}) on

(A) Na^{24} uptake at 1st leaf stage, measured for 8 hours. (Expt. 5 of Chapter 4).

(B) Rb^{86}-labelled K loss at 1st leaf stage over 24 hours. (Expt. 6 of Chapter 4).

(C) Rb^{86}-labelled K uptake measured for 24 hours. (Expt. 6 of Chapter 4).

FIG.4.3


(A) Na UPTAKE AT IST. LEAF. STAGE
µ Equiv. Na/GM. Fresh WT.
11
8
6
4
2
0
1
5
8
(B) K LOSS AT IST. LEAF STAGE
PERCENTAGE OF K RETAINED
100
80
60
40
20
HIGH Ca
LOW Ca
2
5
16
24
TIME IN HOURS
(C) K UPTAKE AT IST. LEAF STAGE
µ Equiv. K/GM. Dry WT.
160
120
80
40
0
4
16
24
TIME IN HOURS
CONTROL
SHOOT
ROOTS
HIGH Ca
SHOOT
ROOTS
LOW Ca
SHOOT
ROOTS

NaCl might have increased the permeability of the root cells in the low calcium treatment resulting in higher intake (passive) of Na^{24} in these roots.

Na$^+$ uptake at 1st leaf stage. Expt. 5.

After 4 days of pretreatment with NaCl, Na^{24} uptake at low calcium was more reduced than at high calcium on all the sampling occasions. The differences in Na^{24} content between plants at high and low calcium were already apparent after 1 hour and became more pronounced with time (Fig. 4:3A).

K$^+$ loss and uptake from intact plants. Expt. 6.

Loss: The rate of loss of potassium from plants, as measured by Rb^{86} labelling, after treating plants with NaCl for 5 days, was higher initially at both levels of calcium, but slowed later and became linear with time (Fig. 4:3B). This pattern was similar to the pattern of loss of amino acids and of Cl^{36}. The loss at low calcium was greater than at high calcium on all the sampling occasions. Specific activity i.e. ratio of labelled to non-labelled potassium was calculated in the plants from which Rb^{86} loss was already determined. The specific activities at low and high calcium were 0.025 and 0.16 respectively, again showing that the loss of tracer was greater in low calcium plants. Table 4:2 gives the potassium concentrations determined by flame photometry prior to tracer application.

FIGUREE 4:4

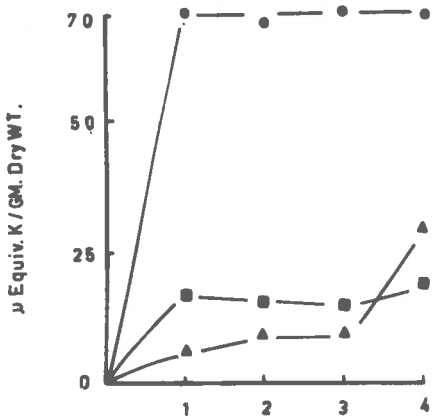
Effect of NaCl (50 me/l) in Hordeum vulgare at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + calcium of $^{3}/_{40}$ nutrient (high Ca^{++}) on

(A) Rb^{86}-labelled K uptake by excised roots. Plants were treated at low and high calcium for 1 to 4 days before excising roots.

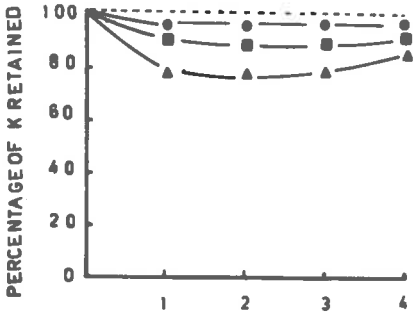
(B) Rb^{86}-labelled K loss by the excised roots. Treatments were same as above. (Expt. 7 of Chapter 4).

(C) Rb^{86}-labelled K uptake by excised leaves. Plants were treated at low and high Ca^{++} for 4 days before excising leaves. (Expt. 8 of Chapter 4).

FIG.44 (A) K UPTAKE BY EXCISED ROOTS

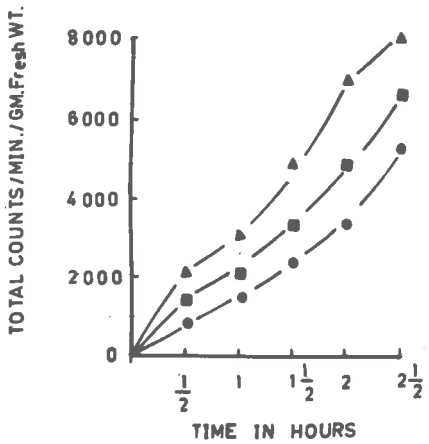


(B) K LOSS BY EXCISED ROOTS



DAYS AFTER NaCl APPLICATION

(C) K UPTAKE BY EXCISED LEAVES



● CONTROL

■ HIGH Ca

▲ LOW Ca

Uptake: NaCl treatment reduced potassium uptake more strongly in plants at low than at high calcium. At low calcium potassium uptake did not increase with time either in roots or shoots. At high calcium however, potassium uptake in both of these organs increased linearly after the initial period (Fig. 4:3C).

K^+ loss and uptake from excised roots. Expt. 7.

Loss: Potassium loss, as determined by Rb^{86} labelling, was greater from plants at low than from high calcium or control plants, on all the days of loss measurements (Fig. 4:4B). In low and high calcium the loss was constant for the first 3 days of treatment with NaCl but slowed down on the last day. However, in the controls, the rate of potassium loss remained unchanged throughout the experiment. The absolute amount of potassium loss in the two treatments is given in Table 4:3.

Uptake: The ability of excised roots to accumulate potassium was more reduced at low than at high calcium for the first three days of treatment with NaCl, but on day 4 there was a rapid increase in potassium uptake in low but not in high calcium (Fig. 4:4A). This could have been due to increased permeability at low calcium on day 4 of pretreatment with NaCl which enhanced potassium intake into roots.

TABLE 4:2

K^+ CONCENTRATIONS IN ME/100 mg DRY WEIGHT IN PLANTS AT CONTROL, LOW AND HIGH Ca^{++} AFTER 5 DAYS OF NaCl APPLICATION, PRIOR TO Rb^{86} APPLICATION

Treatments	Shoots	Roots
Control	90 ± 5	150 ± 5
High Ca^{++}	62 ± 4	35 ± 3
Low Ca^{++}	39 ± 3.5	12 ± 1

TABLE 4:3

K^+ LOSS AS MEASURED BY Rb^{86} LABELLING IN U.EQUIV/GM DRY WEIGHT FROM EXCISED ROOTS AT LOW AND HIGH Ca^{++} AS AFFECTED BY NaCl (50 me/l) TREATMENT FROM 1 TO 4 DAYS

Treatments	1 day	2 days	3 days	4 days
High Ca^{++}	1.6	1.5	1.7	1.5
Low Ca^{++}	1.4	1.6	1.9	3.6

K^+ uptake in excised leaf tissues. Expt. 8.

The effects of high NaCl on the uptake of ions by leaf tissues is still unknown. Under saline conditions ions, along with high NaCl, are expected to be transported in the transpiration stream. The mechanism of their entry into leaf cells would be helpful to the study of plant responses to saline substrate. In the present experiment, as the plants were pretreated with NaCl for 4 days, potassium intake was higher at low than high calcium (Fig. 4:4C). This higher intake could have been due to increased permeability as noted for excised roots after 4 days of NaCl pretreatment. This result is in agreement with the conclusion of Smith and Epstein (1964) that leaf tissues behave similarly to root tissues in ion absorption.

Na^+, K^+ and Ca^{++} concentrations in intact plants treated with NaCl at low and high Ca^{++}. Expt. 9

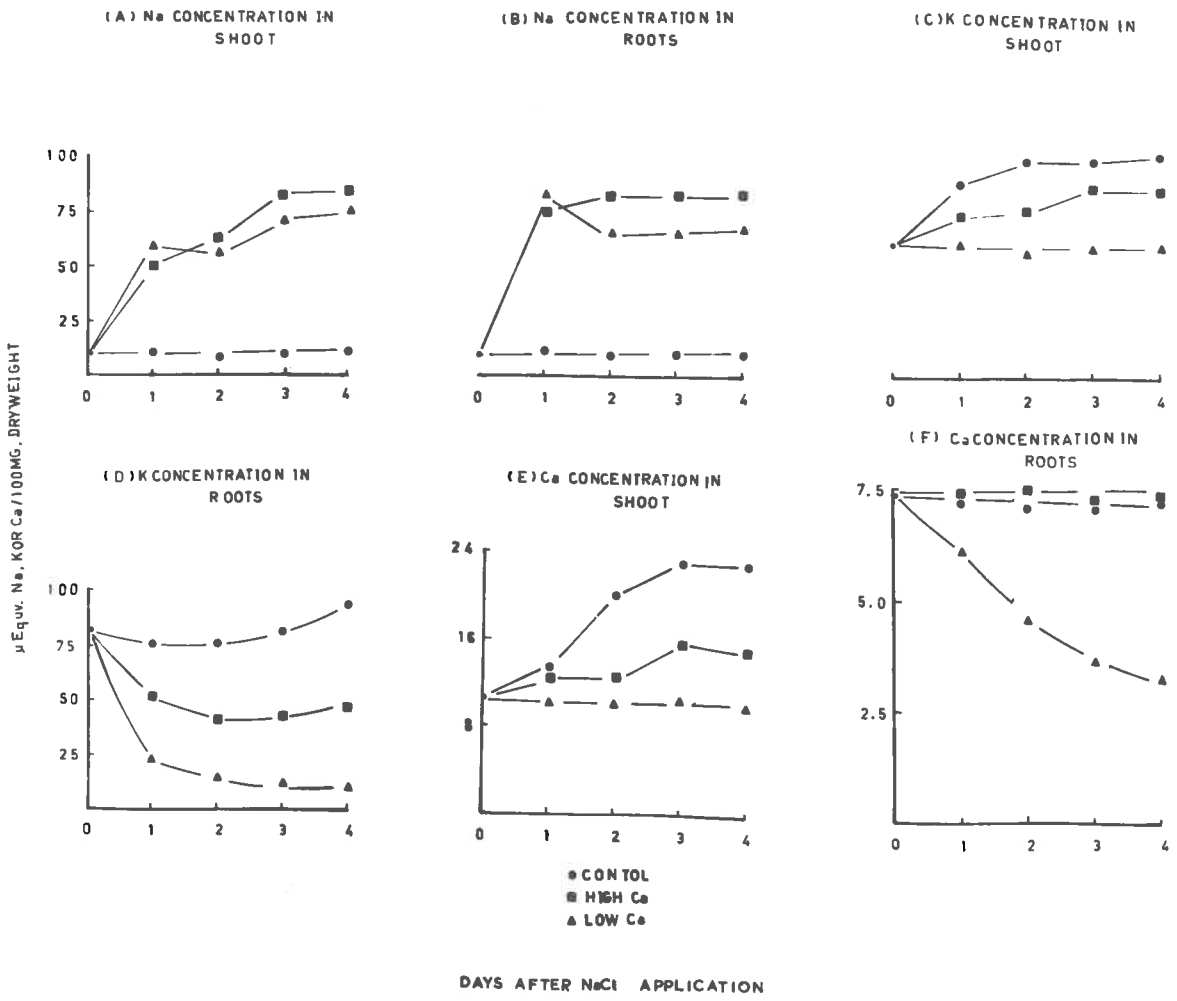
Sodium: Sodium uptake into roots and shoots of control plants was low throughout the experiment. In the NaCl treatment sodium concentrations of shoots were higher in low than in high calcium on the first day of NaCl application but on subsequent days, sodium concentrations in high calcium treatments were more than in low calcium (Fig. 4:5A). Similar trends were noted for the sodium concentration of roots (Fig. 4:5B).

FIGURE 4:5

Effect of NaCl (50 me/l) in *Hordeum vulgare* at 1st leaf stage at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrient (high Ca^{++}) on sodium, potassium and calcium concentrations of shoot and roots when the plants were treated at low and high Ca^{++} for 1 to 4 days.

(Expt. 9 of Chapter 4).

FIG.4-5


(A) Na CONCENTRATION IN SHOOT
(B) Na CONCENTRATION IN ROOTS
(C) K CONCENTRATION IN SHOOT
(D) K CONCENTRATION IN ROOTS
(E) Ca CONCENTRATION IN SHOOT
(F) Ca CONCENTRATION IN ROOTS
μEqu. Na, K OR Ca/100MG. DRY WEIGHT
100
75
50
25
24
16
8
7.5
5.0
2.5
0
1
2
3
4
● CONTOL
■ HIGH Ca
▲ LOW Ca
DAYS AFTER NaCl APPLICATION

Potassium: Potassium concentrations in plants in decreasing order were - control > high calcium > low calcium. The potassium concentration in the shoots increased rapidly in controls at first but did not change two days after NaCl application. In NaCl treatment, potassium concentrations in shoots increased with time at high but not at low calcium (Fig. 4:5C).

In roots, on the other hand, there was a rapid decrease of potassium at both levels of calcium on the first and second day of NaCl application and this decrease was more pronounced at low than at high calcium. After this period, however, potassium concentrations did not decrease in high calcium roots and at low calcium the decrease was not pronounced (Fig. 4:5D).

Calcium: Calcium concentrations in shoots after 1 day of NaCl application were similar in control and high calcium plants and were greater than in plants at low calcium (Fig. 5:4E). The calcium concentration in control plants increased rapidly between 1 to 3 days of NaCl application and the increase at high calcium was less pronounced. Calcium concentration of shoot at low calcium and that of roots at high calcium and of control treatments did not change with time. On the other hand calcium concentraion of roots of low calcium plants decreased linearly throughout the experimental period (Fig. 4:5F).

TABLE 4:4

EFFECT OF DNP ON UPTAKE OF Na^{24} AT LOW AND HIGH Ca^{++} AS AFFECTED BY NaCl (50 me/l)

Uptake was measured at 6 and 12 hours after DNP application

Na u.equiv/GM Fresh Weight

H_1 (6 hours)

	No DNP	DNP
High Ca^{++} Shoot	6.7	2.8
" " Roots	14.5	6.8
Low Ca^{++} Shoot	2.8	3.2
" " Roots	15.5	14.8

H_2 (12 hours)

High Ca^{++} Shoot	17	10
" " Roots	25.3	22.3
Low Ca^{++} Shoot	5.9	6.5
" " Roots	29.6	27.5

Effect of DNP on Na^{24} uptake at low and high Ca^{++} as affected by NaCl. Expt. 10.

In the plants pretreated with NaCl for 4 days at high and low calcium, effects of DNP (10^{-5}N) on Na^{24} uptake measured at 6 and 12 hours after DNP application are shown in Table 4:4. It will be noted from the table that DNP strongly reduced Na^{24} uptake at high calcium in shoots at both 6 and 12 hours of DNP treatment; root uptake was also reduced at 12 hours though not as strongly as at 6 hours. At low calcium, Na^{24} uptake into shoot and roots was not affected by DNP at either sampling times.

DISCUSSION

Salt uptake in plants is a dynamic system, ions moving both inwards and outwards of the cell. Net uptake of ions occurs when inflow exceeds outflow. With an increase of internal salt concentration of tissues the outflow of ions also increases. Such a loss or efflux has been observed in various plant organs, such as root discs, excised roots, healthy shoots and intact plants. In the present experiments loss of amino acids and potassium from control plants shows that this loss was due to normal physiological conditions. However, superimposed upon this loss are the pathological conditions, particularly those which damage membranes

(Thoday 1918), which enhence the loss of ions from the plants.

In the present experiments the greater loss of ions at low calcium than at high calcium or control was therefore related to membrane permeability indicating that the capacity of ion retention was reduced at low calcium.

Generally, the loss of ions to the medium as observed above is greater in the high salt plants than in the low salt plants (Long et al. 1956). In the present experiments the initial ion concentration i.e. concentration before measuring the loss, was higher in high calcium than in low calcium plants. Yet the plants at high calcium lost less amounts of these salts compared to low calcium plants. This observation supports the assumption that permeability in low calcium plants has been increased.

Other conditions, interfering in the fixation of ions into metabolic intermediates i.e. in the formation of complexes between ions and organic protoplasmic constituents, also result in the release of these ions to the medium. Thus Long (1956) reported greater loss of potassium in the dark-grown seedlings than those grown in the light. In the present experiments loss of potassium at low calcium level might have also been due to a lack of its metabolic fixation in addition to increased permeability.

The disturbed metabolic conditions at low calcium are shown

by greater loss of amino acids (Expt. 1). It may be noted that in this treatment substantial amounts of amino acids were lost even after 1 day of NaCl application and the presence of high calcium had a protective effect against these losses. This first day observation suggests that reduced cell synthesis, preventing the utilization of amino acids, contributed to this loss. On the subsequent days, however, when roots had emerged, both reduced synthesis and increased permeability of root cells would have been responsible for the loss of amino acids.

Loss of potassium and calcium from the roots noted in Experiment 9 (Fig. 4:5D and F) also shows that 1 day NaCl treatment resulted in the loss of inorganic ions as well. In excised roots also, potassium loss at low calcium was greater after 1 and subsequent days of NaCl application than at high calcium. At both levels of calcium the potassium loss did not apparently increase with time (Fig. 4:4A). In fact, the absolute amount of potassium loss from low calcium roots (Table 4:3) increased with time after NaCl application but this was greatly masked in the Fig. 4:4B on day 4 of NaCl application by higher potassium intake (probably passive) due to increased permeability. It has already been stated that the results of loss experiments are expressed as percentages of total content. Calcium concentrations in roots at low calcium treatment decreased progressively as the length of NaCl treatment increased (Fig. 4:5F). Calcium uptake into shoots at low calcium did not take place during

the whole experiment whereas at high calcium and at control, calcium uptake into shoots increased with time and there was no loss of calcium from the roots. This suggests that increased permeability at low calcium might have been due to lower calcium concentration of the plant tissue and Marschner (1964) has also shown that calcium deficiency in barley roots results in potassium loss. Loss of inorganic ions at low calcium did not occur from shoot, as retranslocation of ions from shoots is very insignificant (Greenway and Pitman 1965). In Experiment 6 the plants were first treated with NaCl for 5 days before Rb^{86} application for potassium uptake and loss measurements. This long pretreatment with NaCl had greatly reduced potassium concentration of roots at low calcium treatment (Table 4:2), presumably both by lower uptake and greater loss at low calcium than at the high calcium treatment. Yet the results showed that loss of tracer was greater at low than at high calcium, indicating that 5 days pretreatment with NaCl had considerably damaged the root cells so that tracer absorbed in the 24 hours after pretreatment was lost more rapidly from low than high calcium plants (Fig. 4:3B). Possibly this greater loss of potassium at low calcium than at high calcium could have been detected in a shorter pretreatment with NaCl as suggested by the results in Experiment 9 on potassium loss.

In the Cl^{36}-loss experiments, plants were growing in the

tracer solutions for 3 days. Chloride concentrations in plants at low and high calcium were 68 and 100 micro equivalents respectively after 3 days of tracer application and the amount of chloride lost to the medium in the 8 hour period was 19 and 14.5 micro equivalents respectively (Table 4:1). Pitman (1965) stated that roots in a steady state system come into flux equilibrium with the surrounding medium. This means that in order to compensate for the loss of chloride, the roots must have had a considerable uptake. Chloride loss from the plant as reported in the present chapter may also be considered in terms of chloride regulation during NaCl treatment as investigated by Greenway and Thomas (1965). These authors could not detect chloride loss to the solution but showed that chloride was regulated during transfer from root to the shoot. In the present experiments, as already stated, chloride loss to the solution was detected. Chloride concentrations of roots which reach equilibrium with the surrounding medium may then be regulated by export to shoots at high calcium and loss to the solutions at low calcium. This may be the reason for lower chloride concentrations at low calcium as far as the roots are concerned. Alternatively, the chloride normally leaking out may be reaccumulated more efficiently at high than at low calcium.

The results of sodium loss at low calcium are in agreement with those reported by Handley et al. (1965) in that they also found that loss of previously absorbed sodium was less in the presence

of calcium and strontium than in its absence.

The chief membranes responsible for retaining ions in the vacuole and cytoplasm are the tonoplast and plasmalemma respectively. In the present experiments no potential measurements were made between various cell phases and it is difficult to estimate permeability changes in these membranes. However some deductions can be made from the present observations. Chloride and sodium being non-essential ions are not metabilically utilized and tend to accumulate in the vacuole. Potassium also accumulates in the vacuole to some extent. Losses of these ions indicated that the permeability of either tonoplast or plasmalemma or of both was affected. It is not very likely that the permeability of the tonoplast was involved. On the one hand it is well protected by the surrounding solution and on the other it was shown that roots at low calcium treatment which were severely affected in growth, contained at times, higher chloride and sodium concentrations than the medium (Chapter 3, Table 3:3). Further, the loss of amino acids which are normally utilized in the cytoplasm showed that the permeability of the plasmalemma was increased. This assumption, however, should be viewed with caution as greater losses of amino acids in low calcium treatments could also be due to reduced cell synthesis. Increased permeability in this treatment could act via a decreased organization of the protoplasm, and hence a decreased

localization of the intermediates required for synthesis. Alternatively, permeability changes might have resulted in a more adverse ion unbalance of the protoplasm i.e. penetration of chloride and sodium would have increased and loss of potassium and calcium enhanced.

ION UPTAKE

In the general introduction it was stated that interionic influences on the uptake process were studied in the past at low electrolyte concentrations of the medium. The present results provide information on mutual effects of NaCl, calcium and potassium on their uptake behaviour in a saline environment. In the main, observations using radioactive tracers have confirmed the results of ion uptake reported in the previous chapters which were based on chemical analysis. They have also given additional information in the instances when ion uptake was measured over a short interval of time. Higher sodium, chloride and potassium concentrations at high than at low calcium concentration reported in Chapters 2 and 3, both at seedling and at germination stages, have also been found in the present series of experiments (Expt. 3 and 9). Increased permeability may partly be responsible for lower ionic concentrations at low calcium than at high calcium as has been said in the previous section of this chapter, but the following discussion will show that uptake may have also been reduced at low calcium treatment. In most of the

experiments reported in the previous chapters, plant tissues were analysed for sodium, chloride and potassium 5 days after NaCl application when plant growth was also reduced. But ion uptake studies in plants treated with NaCl for a short period are more important in relating its effects on growth. Results of Experiment 3 show that, at low calcium, Cl^{36} uptake was higher than at high calcium when NaCl treatment was imposed for 2 days, but when NaCl treatment was prolonged to 5 days, Cl^{36} concentration was more reduced at low than at high calcium treatment (Fig. 4:2A). Similar results for sodium concentrations in plants were noted in Experiment 9 (Figs. 4:5A and B). Possible causes for higher sodium and chloride concentrations at low than at high calcium in the initial period of NaCl application have been mentioned in Chapter 2. It was deduced that these higher sodium and chloride concentrations at low calcium change the ion balance of cells and bring about growth reduction and losses of other ions due to increased permeability in the latter period of NaCl treatment. But the results of Experiment 5 where Na^{24} uptake was measured at low and high calcium from 1 to 8 hours, in plants previously treated with NaCl for 4 days, show that Na^{24} uptake at low calcium was lower than at high calcium during the whole period of uptake measurements (Fig. 4:3A). Lower sodium uptake at low than at high calcium, particularly in 1 hour, is an important indication that real sodium uptake at low calcium was reduced because loss due to permeability

would have been negligible in 1 hour period. These results show that the presence of calcium reduced sodium and chloride in plants so long as the health of the plant was not affected by a long NaCl treatment.

In Experiment 9, uptake of calcium and potassium as affected by NaCl at low and high calcium were studied. After 1 day of NaCl application both calcium and potassium concentrations in the roots were reduced at low calcium treatment (Fig. 4:5 D and F). This must be the real loss of calcium and potassium from the roots of low calcium plants since these roots had higher potassium and calcium concentrations before NaCl application. In the excised roots experiment, the first 3 days of NaCl treatment showed that uptake of potassium was greater at high than at low calcium (Fig. 4:4A). The sudden increase in potassium uptake after 4 days of NaCl treatment was probably due to increased permeability similar to that noted in excised leaf tissues and may not be related to uptake process. These results indicate that, in a short term NaCl treatment, potassium concentration at low calcium would have been reduced by lower uptake as well as increased loss. The same applies when the NaCl treatment is prolonged to 5 days; this is shown by the time course absorption curve of potassium uptake by intact plants treated with NaCl at low and high calcium for 5 days (Expt. 5, Fig. 4:3C). Similar to the observations on Na^{24} uptake, potassium

uptake at low calcium was more reduced than at high calcium in the first 2 hours, and also, in the subsequent periods, indicating again that potassium uptake was reduced rather than potassium loss increased.

The need for calcium in maintaining potassium uptake of roots, as noted in the present experiments, has long been known. Jacobson et al. (1950) presumed that the same metabolically-produced binding ions served for the absorption of sodium and potassium and that potassium uptake was markedly decreased in the presence of increasing amounts of NaCl. On the other hand, Epstein et al. (1952) pointed out that sodium was not competitive with regard to K-Rb-Cs sites where the reaction was analagous to the combination of a substrate with an enzyme. Either of these two possibilities might explain the results of the present experiments. Epstein (1961) who has shown that calcium is essential for maintaining the integrity of the selective ion transport believes it is necessary to maintain the cell membrane (see Chapter 6).

Ion transport across the membrane depends upon passive and active uptake. DNP, an uncoupler of oxidative phosphorylation and also of active ion uptake, was used to establish whether active sodium uptake was reduced at low calcium treatment when plants were treated for 4 days with NaCl (Table 4:4). Plants not treated with DNP had a greater sodium concentration in the roots at low than at high calcium. The results of DNP treatment showed

that higher sodium uptake in roots at low calcium was probably passive, because roots of DNP-treated plants at low calcium had as high sodium concentration as roots with no DNP treatment. This higher passive uptake at low calcium can also be seen by comparing root uptake at low and high calcium in the DNP treatment, where the uptake at low calcium over 6 hours is less sensitive to DNP than at high calcium.

In the shoot, on the other hand, sodium uptake at low calcium is totally insensitive to DNP, showing that only passive uptake remains, whereas at high calcium a significant portion of uptake was active. This observation established that when subjected to high NaCl and low calcium for 4 days, there is some formidable barrier to ion transport to shoots, since the shoot concentration remains low though roots in low calcium contain as much sodium as roots in high calcium. This could be more conclusively demonstrated by showing that the sodium concentration in the transpiration stream of low calcium plants is not higher than the concentration of sodium in the culture solution.

CHAPTER 5

PHOSPHORUS UPTAKE AND METABOLISM AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}

Introduction

The previous chapter showed that low calcium, high NaCl, increased the permeability of root cells suggesting among other things that the reduced growth also observed at low calcium, high NaCl may be due partly to loss of some important metabolic intermediates e.g. amino acids. An increased permeability of the cell may also be an indication that the permeability of other cellular components (such as mitochondria) has been increased. If so metabolism could be impaired by reduction of such reactions as oxidative phosphorylation. Phosphorus is an important metabolite as synthesis depends on energy transfer by phosphorylated compounds. Its retention in the cell could be expected to be reduced if permeability increased.

In a saline environment competition from chloride ions might also reduced phosphorus levels by lowering phosphorus uptake. The aim of the present series of experiments was to determine whether the growth differences in plants grown in NaCl at low and high calcium were associated with the differences in phosphorus uptake and metabolism. To investigate this problem, uptakes of P^{32} at special times after treatment application, and after transference to high

calcium were determined (Expt. 1); uptake of P^{32} to different aged segments of roots was established (Expt. 2). Finally the incorporation of phosphorus compounds into metabolites was studied by using P^{32}.

METHODS

(a) General

These experiments were done with Hordeum vulgare at the 1st leaf stage. Seeds were sown in river sand in the glasshouse and transplanted 5 days after sowing to 3 litre culture dishes containing $^{1}/_{40}$ nutrient. Each dish had 18 plants. The composition of the nutrient solution was the same as stated in Chapter 1. Treatments were imposed when the 1st leaf had fully developed. The basic culture solution was $^{1}/_{40}$ strength of nutrient and NaCl was at 0 and 50 me/l and calcium at 0.2 and 0.8 me/l; these calcium levels with NaCl will be designated as low and high respectively. Solutions were aerated continuously and replaced daily. In some cases additional solution changes were given to ensure that phosphorus did not become limiting for growth and alter the specific activity of phosphate compounds. At harvest, roots were rinsed thrice in cold distilled water or in treatment solutions to get rid of P^{32} in the free spaces, and then dried between filter papers. The plants were separated into root and shoot. Further details of the experiments are given in the appropriate sections.

Fractionation of Phosphorus Compounds

The procedure consisted of extracting the phosphorus compounds with acid, then determining these compounds by quantitative methods. The acid extraction method was that used by Loughman and Martin (1957) who showed that grinding barley roots at 3 to 4°C in 0.2N HCl extracted inorganic phosphorus, sugar-phosphates and nucleotides. The quantitative determination of these phosphorus compounds was carried out in the following way:-

Orthophosphate: The two colorimetric methods which are commonly used for determining phosphate content are those developed by Allen (1940) and Fiske and Subbarow (1925) as modified by Bartlett (1959). These methods are not satisfactory for determining inorganic phosphorus in the presence of organic phosphorus as errors may be introduced due to hydrolysis of phosphate esters and poly-phosphate.

Marsh's (1959) method of inorganic phosphorus determination prevents phosphate ester hydrolysis and is more accurate and sensitive. In this method molybdate-catalysed hydrolysis of ATP is prevented by butanol extraction of phosphomolybdate and removal of excess molybdate as a citrate complex. Since, in the present experiments, the acid root extract contained both inorganic and organic phosphorus the method of Marsh was used. A brief description of the procedure is as follows: From 10 day old barley

plants root samples weighing 0.3 to 0.7 gms were taken. The roots were washed thrice with distilled water and blotted between tissue paper. To stop the metabolic activity, roots were then immediately transferred to a deep freeze unit. Subsequently they were ground for 5 minutes with 2 lots of 10 ml of 0.2N HCl. The total volume was made to 30 ml in plastic centrifuge tubes. The whole operation was carried out in a cold room. The cell debris was removed by centrifuging at 3000g for 15 minutes. The supernatant was used for measuring inorganic phosphorus, sugar phosphate and nucleotides. For inorganic phosphorus determination, 1 to 2 ml was transferred to a test tube and the following were added: 5 ml of deionized water, 6 ml of butanol, and 0.6 ml of molybdate. The contents were mixed, then 1.2 ml of sodium citrate, pH7, was added and the contents were mixed again. An aliquot of 3 ml of upper phase was transferred to a glass centrifuge tube and centrifuged for 15 minutes to remove the last droplets of water. Part of the upper phase was then decanted into a spectrophotometer cell and the absorbance measured at 310 mu.

Sugar phosphate and nucleotides: These compounds were determined by hydrolysing them into inorganic form. To do this 5 ml of supernatant solution was transferred to a 25 ml Kjeldahl flask and 10 mls of conc. HNO_3 and 1 ml of $HClO_4$ (70%) were added and the mixture gently heated until a vigorous reaction took place. The residue was transferred with successive washing into the measuring

flask and the volume made to 25 ml. An aliquot of 5 ml of this solution was used to determine inorganic phosphorus as described above.

Residual Phosphorus: The residue of the initial extract was transferred to Kjeldahl flask by successive washing with water. The water was evaporated at 110°C as the addition of acid in the presence of water accelerated bumping. Digestion was carried out as described for sugar phosphates. The residue was made up to 100 ml; an aliquot of 2 ml of this solution was taken for inorganic phosphorus determination.

Total Phosphorus: Oven dried roots weighing 0.03 to 0.05 gm were transferred to Kjeldahl flask and total phosphorus determined as described for residual phosphorus.

Chromatorgraphy: Paper partition chromatography was used. The procedure described by Bailerki and Young (1963) was followed. Various solvent systems were tried. These included -

1. Solvent tertiary butanol/water/picric acid.
2. Solvent isopropyl ether/formic acid.
3. Methanol/formic acid.
4. Tertiary amyl alcohol/water/formic acid.

Volume ratio and R.f. values are given in Appendix II. The last solvent (Walker 1957) was used in the present experiment as it gave

better separation of phosphorus compounds. Acid and alcoholic extracts were reduced to suitable volume by evaporation under vacuum at temperatures below 30°C. 100 microlitre of these extracts was applied to acid washed Whatman No.1 paper and chromatogrammed in the descending system for 24 hours at room temperature. A mixture of marker compounds was run beside the unknown. The spots were sprayed and developed under ultra violet light. Radioautographs of the chromatograms were developed by placing them in contact with X-ray film for 5 days and the activity in the spot was counted with an end window counter.

EXPERIMENTAL DETAIL

P^{32} uptake at high NaCl as affected by high and low Ca^{++}. Expt. 1.

In this experiment, plants first grown in NaCl solution at low and high calcium for 1, 2, 3, 5 and 6 days were transferred to the respective treatment solution which was labelled with P^{32}. The radioactivity in the plants was counted after a period of 6 hours. Table 5:1 gives relative counts of P^{32} i.e. counts per minute per 100 mg dry weight of samples. There were two replicates of three plants each in each treatment. Since this preliminary experiment showed that P^{32} uptake was higher in the shoot of high calcium plants, the next experiments were designed

to establish the speed of recovery in P^{32} uptake upon calcium addition to plants previously treated at low calcium level. The recovery was studied both in the short term and long term experiments.

In the short term recovery experiment there were two treatments as follows:

	Low Ca^{++}	High Ca^{++} following low Ca^{++}
Expt. (a)	48 hours	8 hours
Expt. (b)	24 "	24 "

In the long term experiment measurements of P^{32} uptake were at the following time:

Treatments	3 days	5 days	6 days	8 days	11 days
Low Ca^{++}	+	+	+	+	-
High Ca^{++}	+	+	+	+	+
First low then high Ca^{++} on day 6			+	+	+

To measure P^{32} uptake plants were transferred to the treatment solution containing P^{32} at 1.66 microcurie/l for 6 hours, and the results are expressed as counts per minute per 100 mg dry weight.

P^{32} uptake at high NaCl in different aged root segments as affected by low and high Ca^{++}. Expt. 2.

P^{32} uptake was measured in different root segments of plants grown in NaCl at low and high calcium. In addition to other regions the segments included meristematic zone (2 to 3 mm from the tip). Since, as Kramer and Wiebe (1952) for example, have shown this meristematic zone is a region of higher P^{32} uptake, it was thought that treatment differences might become particularly apparent in this zone. Plants were treated with NaCl at low and high calcium for 36 hours. Solutions in the respective treatments were then replaced with fresh solution containing P^{32} at 50 microcurie/l. Two plants per treatment comprised a replicate and there were 2 replicates in each treatment. At harvest shoots were separated and roots were cut into following segments:

1. Tips 2 to 3 mm in length.
2. Elongation zone, 2 cm behind the tip.
3. Rest of roots

Harvests were taken at the following times after P^{32} application - 5 minutes, 30 minutes, 1.5 hours, 3 hours and 6 hours. Results are expressed as total counts for each zone.

Different Phosphorus Compounds as affected by NaCl at low and high Ca^{++}. Expt. 3.

Concentration of different phosphorus compounds formed in the roots of plants grown in NaCl treatment at high and low calcium were examined. Plants were treated with NaCl at low and high calcium for a period of 1 to 3 days. There were 3 replicates of 4 plants each. Amounts of phosphorus compounds have been expressed as content per plant.

Incorporation of P^{32} in different phosphorus compounds in NaCl treatment at low and high Ca^{++}. Expt. 4.

As no significant differences between treatments were apparent in Experiment 3, a more sensitive method to detect any differences between the above treatments was tried in Experiment 4. This was done by using P^{32} and measuring the specific activity of the phosphorylated compounds in the above treatments. These compounds were also separated by chromatography and percentage distribution of P^{32} was determined. Length of time of NaCl treatment was the same as in Experiment 2. Dishes (250 ml) each containing 4 plants were used. There were 4 dishes under each treatment. Thirty-six hours after the commencement of NaCl treatment, fresh solutions containing P^{32} at 50 microcuries/l were replaced and P^{32} uptake was measured after 1 hour. Six plants in duplicate

were used for each harvest in each treatment. Roots were extracted in 10 ml of 0.2N formic acid at 4°C and volume made to 30 ml. Formic acid was preferred to HCl as it is easy to evaporate under reduced pressure for chromatographic separation. The other 4 plants were used for alcoholic extraction. To do this roots and shoots were extracted with boiling 80% ethyl alcohol and then with 50% ethyl alcohol and finally with distilled water. The combined extracts were evaporated to a small volume under low pressure. This was done as a check on the acid extraction process. The acid extracts were fractionated for phosphorus compounds. Total counts and specific activity were measured in these compounds and results are expressed as content per plant. An additional set of 4 plants in each treatment was used for total phosphorus determination. Extracts of the different fractionations were saved for chromatography.

RESULTS

P^{32} uptake at high NaCl as affected by high and low Ca^{++} over short time intervals. Expt. 1.

Results are presented in Table 5:1. P^{32} uptake by the shoot was lower at low calcium than at high calcium. This difference was already pronounced 1 day after NaCl application and became very large after the treatment had been imposed for longer

TABLE 5:1

P^{32*} UPTAKE AT HIGH AND LOW Ca^{++} EXPRESSED AS COUNTS PER 100 mg DRY WEIGHT AS AFFECTED BY NaCl AFTER ITS APPLICATION FROM 1 TO 5 DAYS. P^{32} UPTAKE WAS MEASURED OVER A 6 HOUR PERIOD

	Low Ca		High Ca	
Days after NaCl Application	Shoot	Roots	Shoot	Roots
1	190	1817	320	1650
2	218	2200	570	1550
3	180	3440	725	4113
5	450	2550	1150	4200
6	329	2600	1120	3650

* These experiments were done at different times. Therefore the values of counts at low and high Ca^{++} are comparable only on the days indicated and cannot be used in a time sequence.

FIGURE 5:1

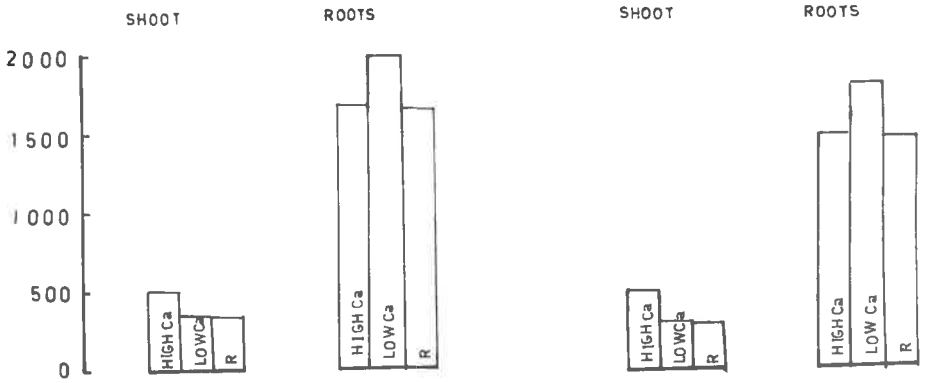
Effect of NaCl (50 me/l) in Hordeum vulgare at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrient (high Ca^{++}) on P^{32} uptake.

Recovery in P^{32} uptake upon extra Calcium addition to plants previously treated at low Ca^{++} was measured in both short (A) and long (B) term experiments - P^{32} uptake was measured over 6 hour period.
(Expt. 1 of Chapter 5).

FIG. 5.1 (A) SHORT TERM RECOVERY IN P UPTAKE

(a) LOW Ca 48 HOURS, THEN HIGH Ca 8 ,,

(b) LOW Ca 24 HOURS, THEN HIGH Ca 24 ,,



(B) LONG TERM RECOVERY IN P UPTAKE

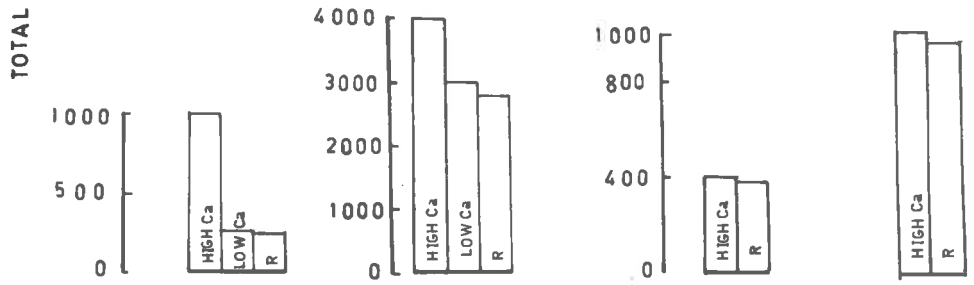
(a) LOW Ca 6 DAYS, THEN HIGH Ca 48 HOURS

(b) LOW Ca 6 DAYS, THEN HIGH Ca 5 ,,

SHOOT ROOTS

SHOOT ROOTS

plants at low Ca died therefore comparasion not possible



R= FIRST LOW THEN HIGH Ca

periods. During the first 2 days of treatment, phosphorus uptake by the root was higher at low than at high calcium. However, after longer treatment the deleterious effects of low calcium also became apparent in the roots. In both short term experiments, shoot uptake did not rise upon calcium addition to the low calcium medium, though root uptake dropped to the level of high calcium (Figs. 5:1A (a) and (b)). When plants were treated for 6 days at low calcium and then transferred to high calcium, the P^{32} uptake did not recover after 2 days (Fig. 5:1B (a)) but was restored to that of high calcium after 5 days (Fig. 5:1B (b)).

P^{32} uptake at high NaCl in different aged root segments as affected by low and high Ca^{++}. Expt. 2.

Results are presented in Fig. 5:2 for both calcium levels as well as for the control. P^{32} uptake to the shoot was lower than that to the root. In roots P^{32} uptake decreased in the following order: rest of the roots > expanding zone > tips. Tips comprised a very small portion of total roots but nevertheless showed a large absorption of P^{32} from the medium, and relative to its size the root tip was presumably the most efficient absorption region. In the initial period of 30 minutes there was a sharp rise in P^{32} uptake in the elongation zone in all 3 treatments; this rise was particularly pronounced in the rest of the roots and was followed by a stationary phase and second rise again after 3 hours of

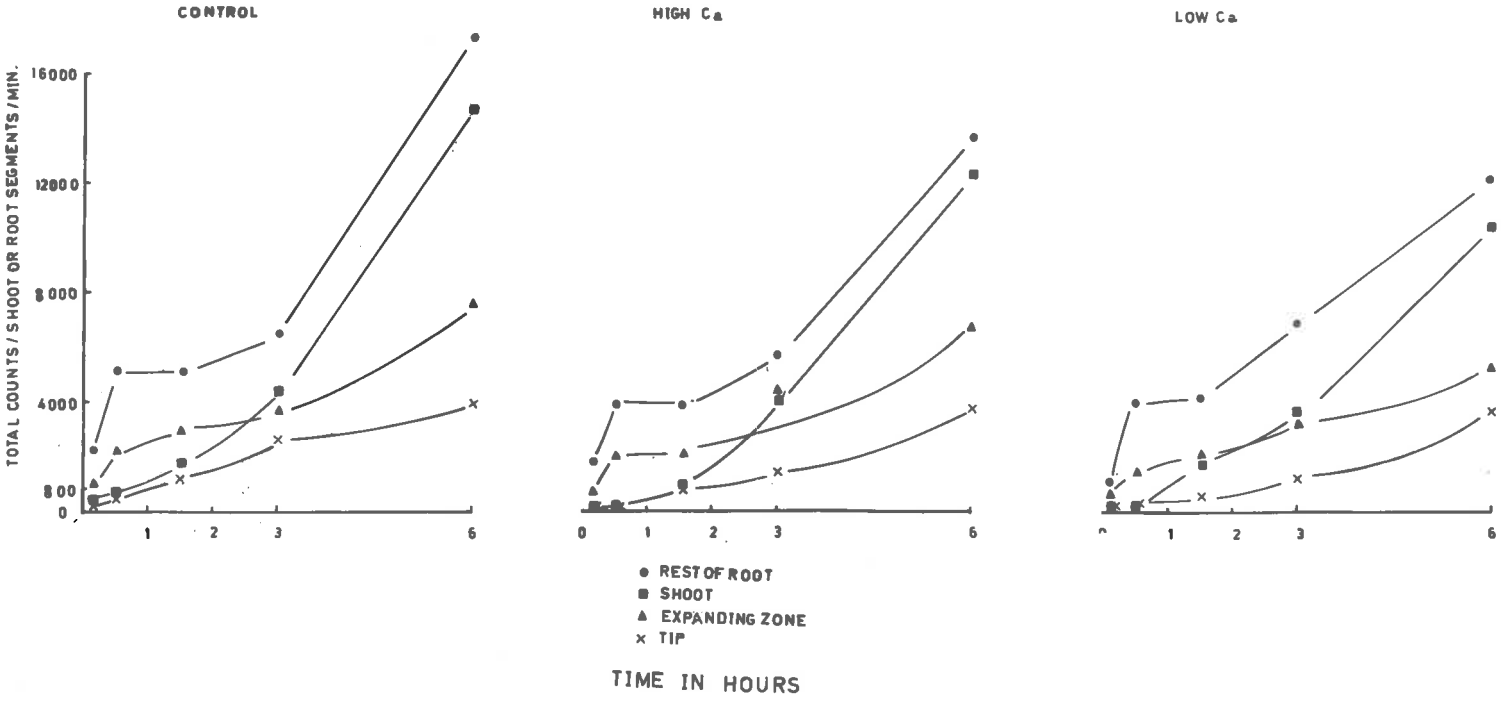
FIGURE 5:2

Effect of NaCl (50 me/l) in *Hordeum vulgare* at $^{1}/_{40}$ nutrient (low Ca^{++}) and $^{1}/_{40}$ nutrient + Calcium of $^{3}/_{40}$ nutrients (high Ca^{++}) on P^{32} uptake in different aged root segments and roots.

(Expt. 2 of Chapter 5).

FIG. 5.2

P UPTAKE IN SHOOT AND ROOT SEGMENTS



P^{32} application. Uptake in tips showed a gradual increase with time. P^{32} uptake in control and high calcium plants was higher than in plants at low calcium yet root tip uptake was similar in these plants.

Different Phosphorus Compounds as affected by NaCl at low and high Ca^{++}. Expt. 3.

For the first two days after treatment was started there was no difference in the concentrations of inorganic phosphorus, sugar phosphorus and nucleotides in the roots of low and high calcium plants, though residual phosphorus was higher in the high calcium treatment (Table 5:2). Three days after NaCl application, the root fresh weight at low calcium was reduced and so were the amounts of different phosphorus compounds compared with the plants at high calcium. Reduction in amounts of the various compounds would be expected because of the reduced growth and no inference can be drawn about the effects of treatment on phosphorus incorporation in particular compounds. Because of the variability the relations between the amounts of the different compounds is not clear.

TABLE 5:2

FRESH WEIGHT AND AMOUNTS OF PHOSPHORUS COMPOUNDS* OF ROOTS EXPRESSED AS u gm/PLANT AT LOW AND HIGH Ca^{++} AFTER THE PLANTS WERE TREATED FOR 1 TO 3 DAYS WITH NaCl. (MEAN STANDARD ERROR INDICATED)

1 Day Treatment

Treatments	Fresh weight in Mg	Inorganic Phosphorus	Sugar Phosphorus and Nucleotides	Residual Phosphorus
High Ca^{++}	13 $\pm$ 0.75	12.6 $\pm$ .5	5.7 $\pm$ 0.9	13.25 $\pm$ 2.3
Low Ca^{++}	11.7 $\pm$ 0.5	10.6 $\pm$ 0.35	5.7 $\pm$ 2.5	14.0 $\pm$ 3.4

2 Days Treatment

High Ca^{++}	11.25 $\pm$ 0.75	10.9 $\pm$ 1.2	7.7 $\pm$ 1.9	17 $\pm$ 3.5
Low Ca^{++}	9.7 $\pm$ 0.5	10 $\pm$ 1.6	6.5 $\pm$ 1.1	10.7 $\pm$ 3.2

3 Days Treatment

High Ca^{++}	10.7 $\pm$ 1	12.5 $\pm$ 1.5	12 $\pm$ 1.7	20 $\pm$ 2.4
Low Ca^{++}	7.5 $\pm$ 0.4	8.7 $\pm$ .75	7.5 $\pm$ 1.0	13 $\pm$ 1.5

* These experiments were done at different times. Therefore values of phosphorus compounds at high and low Ca^{++} are comparable only in the individual days of treatments.

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Incorporation of P^{32} in different phosphorus compounds in NaCl treatment at low and high Ca^{++}. Expt. 4.

Total Phosphorus: The total phosphorus and specific activities were similar in the shoots of control and of high calcium treatment plants and were greater than in low calcium plants. Similar trends were noted in the roots (Table 5:3).

Formic acid fractionation: In roots, the total counts for inorganic phosphorus were greater in the control and high calcium plants than in plants at low calcium, but there was no difference in the specific activity of inorganic phosphorus between the treatments. Other phosphorus fractions showed similar trends (Table 5:4 (a)), except that counts and specific activity in sugar phosphorus were higher in the plants of high calcium treatment than in those of either control or low calcium. Distribution of the P^{32} label in different phosphorus compounds was not different in control, high calcium and low calcium plants nor was the ratio $\frac{\text{Pi + Sugar P}}{\text{Residue}}$ different (Table 5:4 (b)).

Alcoholic extraction: Alcoholic extraction showed that total counts both in roots and shoots were greater in control and in high calcium plants than in low calcium plants (Table 5:5).

Chromatographic separations of phosphorylated compounds

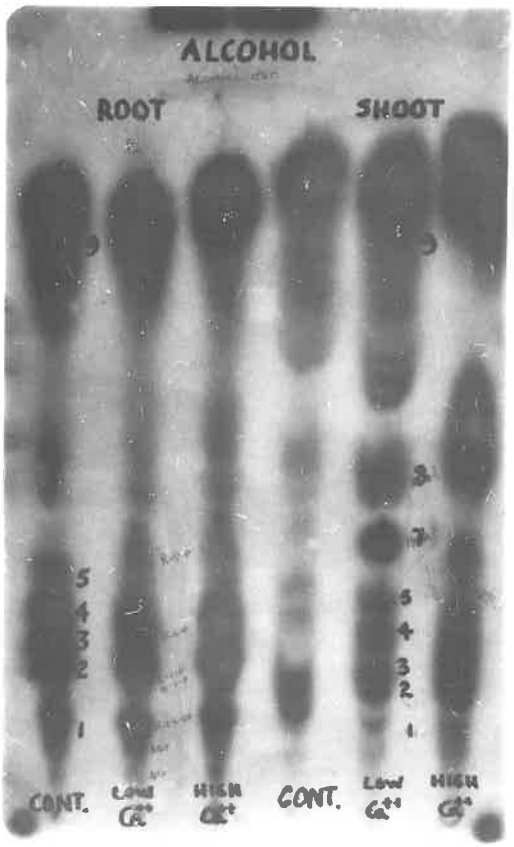
PLATE 5:1

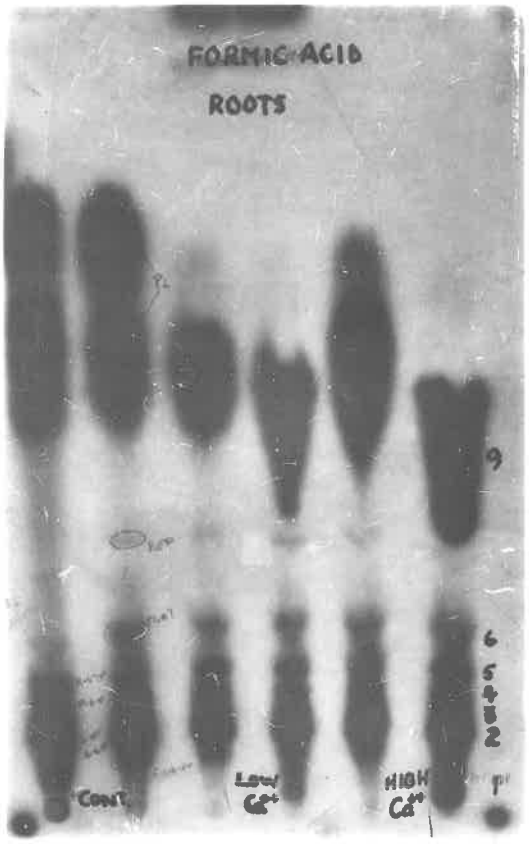
Autoradiograph of labelled phosphorus compounds in alcohol and formic acid extract separated on acid washed Whatman No. 1 paper in NaCl (50 me/l) at low (0.2 me/l), high (0.8 me/l) and control (0.2 me/l) Calcium treatments.

Solvent: Tertiary amyl alcohol/water/formic acid
3 : 1 : 3

The numerals in the figures on the plate in respective extracts represent the following compounds.

Alcohol extract		Formic acid extract	
1.	ATP/ADP	1.	ATP/ADP
2.	F-1-6DP	2.	F-1-6DP
3.	G.1.P.	3.	G.I.P.
4.	G.6-P	4.	G.6-P
5.	F.6.P.	5.	F.6.P.
6.	R.5.P.	6.	R.5.P.
7.	P.G.A.	7.	Unknown
8.	P.E.P.	8.	P.G.A.
9.	Pi	9.	Pi


ALCOHOL
ROOT
SHOOT
5
4
3
2
1
CONT.
HIGH
CONT.
HIGH


FORMIC ACID
ROOTS
9
6
5
4
3
2
1
Cont.
HIGH

of both alcoholic and acid extracts are shown in Plate 5:1 and the percentage distribution of P^{32} in these compounds in Tables 5:7 and 5:8. It will be noted in Plate 5:1 that no qualitative differences appeared among control, high and low calcium plants. Percentage distribution of P^{32} also showed no differences except that the alcohol extract of shoots of low calcium treatments had a greater activity than of other treatments, possibly due to phosphoglyceric acid. It must be stated here that the exact identity of these compounds is not certain as the spots were not co-chromatogrammed but were identified by R.f. values.

DISCUSSION

Most of the studies on phosphorus uptake by plants suggest that phosphorus is rapidly incorporated into organic form, but that its transport to the shoots is mainly in the inorganic form (Loughman and Russell 1957). The present experiments are consistent with this view since most P^{32} in the shoot was in inorganic form (Table 5:8). Van Andel, Arisz and Helder (1950) have proposed that three metabolic processes are involved in phosphate uptake (i) accumulation in the cell sap; (ii) metabolic incorporation into the zone of growth; (iii) radial transport and secretion to the xylem. Results of Experiment 1 suggest that process (iii) was affected in plants at low calcium, high NaCl, because shoot uptake over short periods was much reduced

TABLE 5:3

TOTAL COUNTS, P CONTENT AND SPECIFIC ACTIVITY OF P^{32} PER SHOOT AND PER ROOTS AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}

SHOOT

Treatments	Phosphorus in µg	Total counts /min.	Specific activity
Control	170	4.6×10^3	27
High Ca	183	5.6×10^3	31
Low Ca	110	1.8×10^3	16

ROOTS

Control	74	15.3×10^3	196
High Ca	70	17.4×10^3	248
Low Ca	55	9.3×10^3	167

TABLE 5:4

TOTAL COUNTS, P CONTENT AND SPECIFIC ACTIVITY IN DIFFERENT PHOSPHORUS COMPOUNDS PER ROOT AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}; FORMIC ACID EXTRACTION

a)

Treatments	Inorganic P			Inorganic P & S P and nucleotide		Sugar P			Residual P		
	c.p.m.	P in ug	s.p.a.	c.p.m.	P in ug	c.p.m.	P in ug	s.p.a.	c.p.m.	P in ug	s.p.a.
Control	16.4×10^3	34.3	480	24.1×10^3	40	7.6×10^3	6	1301	7.1×10^3	45	156
High Ca^{++}	14.5×10^3	30	490	24.9×10^3	35.6	10.4×10^3	5.6	2000	5.1×10^3	36	141
Low Ca^{++}	13.3×10^3	27	479	20.6×10^3	33.3	6.9×10^3	4.8	1494	4×10^3	30	129

b) Distribution of label from P^{32} in inorganic P, Sugar P and residue.

Treatments	c.p.m.	Inorganic P percentage	Sugar P percentage	Residue percentage
Control	31.25×10^3	52	24	23.5
High Ca	30×10^3	47	35	17
Low Ca	24×10^3	55	27.5	17

$\frac{\text{Inorganic P + Sugar P}}{\text{residue}}$	Control	High Ca	Low Ca
	3.3	4.3	5.1

P = Phosphorus

S.P. = Sugar phosphate

c.p.m. = Total counts per minute/ plant roots.

s.p.a. = Specific activity i.e.

$\frac{\text{c.p.m.}}{\text{ug P}}$ / plant

TABLE 5:5

TOTAL COUNTS AND COUNTS IN THE RESIDUAL PHOSPHORUS, PER SHOOT OR PER ROOT AND THE RATIO OF TOTAL COUNTS TO COUNTS IN THE RESIDUAL PHOSPHORUS AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}; ALCOHOL EXTRACTION

SHOOTS

Treatments	Total Counts/min.	Residual Phosphorus	Total counts/min. / Residual Phosphorus
Control	4.3×10^3	3.6×10^3	1.2
High Ca	5×10^3	2.6×10^3	1.18
Low Ca	2.6×10^3	2.2×10^3	1.92

ROOTS

Control	22.4×10^3	5.8×10^3	3.86
High Ca	20.3×10^3	6.7×10^3	2.98
Low Ca	19.5×10^3	4.9×10^3	3.97

TABLE 5:7

PERCENTAGE DISTRIBUTION OF P^{32} ACTIVITY IN THE FORMIC ACID EXTRACTION OF ROOTS AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}.

Solvent - Tertiary amyl alcohol/water/formic acid.

		Treatments		
Compound	Rf.(PGA)	Control	High Ca	Low Ca
1. ATP/ADP	4-8	3	2	trace
2. F-1-6 DP	15-17	2.3	2.5	2.0
3. G.I.P.	22-26	14.4	19	14.5
4. G-6-P.	2-.34	7.0	5.0	6.2
5. F-6.P.	39-45	5.1	2.9	3.0
6. R-5.P.	55-56	6.5	4	5.1
7. Unknown	66	trace	trace	trace
8. P.G.A.	100	trace	trace	trace
9. Pi	134-200	55.5	65	63

TABLE 5:8

PERCENTAGE DISTRIBUTION OF P^{32} ACTIVITY IN THE ALCOHOL EXTRACTION OF SHOOT AND ROOTS AS AFFECTED BY NaCl AT LOW AND HIGH Ca^{++}

Solvent - Tertiary amyl alcohol/water/formic acid.

		Treatments					
		Control		High Ca^{++}		Low Ca^{++}	
Compounds	Rf. (PGA)	Shoot	Roots	Shoot	Roots	Shoot	Roots
ATP/ADP	9	-	1.28		2.01	-	1.72
F-1-6 DP	28	5.07	5.1	0.58	4.3	0.39	5.4
G.I.P.	34	9.9	16.9	1.64	15.5	6.2	12.66
G-6.P.	37	-	1.23	-	-	-	-
F-6.P.	53	-	6.65	4.8	13.4	16.5	13.04
R-5.P	71	-	2.31	2.5	3.62	2.83	2.14
P.G.A.	100	-	0.6	1.5	1.01	7.2	1.75
P.E.P.	48	-	-	3.9	-	7.3	-
Pi	81	78	.64	82	57	66.16	63

compared with plants at high calcium.

The higher root uptake in Experiment 1 (Table 5:1) for the first 2 days in low calcium does not seem to be due to a concurrent reduced shoot uptake because the addition of calcium showed that root uptake was reduced to that of the high calcium level, and there was still a persistent decreased shoot uptake (Fig. 5:1A). There is no ready explanation for the increased P^{32} uptake by the roots in low calcium. Perhaps permeability of some cytoplasmic compartments was increased, leading to increased leakage of internal phosphate, thus providing the opportunity for increased exchange of labelled phosphorus from the external solution.

Both control plants and high calcium, NaCl treated plants had higher total phosphorus than NaCl treated plants at low calcium (Table 5:3). This indicates that phosphorus uptake was not reduced by high chloride concentration, though such reduction has been noted by several other workers (Gausman and Awan 1956; Ferguson and Hedlin 1963). Calcium, at least at high NaCl, increased phosphorus uptake. Such a stimulating effect of calcium on phosphorus uptake has been found by Tanda (1955), Palfi (1965) and Legget et al. (1965). In the NaCl treatment the higher uptake of phosphorus at high calcium than at low calcium could have been due to the following possibilities:

1. Increased turnover of a rate limiting intermediate in phosphorus absorption (as proposed by Legget et al.)

2. Metabolic absorption of cytoplasmic sites (as suggested by Loughman and Russell 1957).

3. Restoration of normal membrane permeability, thereby decreasing leakage as shown in the previous chapter.

P^{32} uptake in different root segments and by the shoot (Expt. 2, Fig. 5:2) showed that maximum P^{32} absorption per unit amount of tissue occurred in apical meristematic region. This is in agreement with the results reported by other authors. However P^{32} uptakes by tips at low and high calcium and by tips in controls were similar, showing that meristematic regions at low calcium, high NaCl did not lose their ability for incorporating phosphorus. The patterns of P^{32} uptake in other segments and in the shoots were similar in all three treatments.

The general trends will now be discussed. Wiebe et al. (1954) found little translocation of P^{32} from tips. In the present experiment a slow but gradual rise of P^{32} uptake in tips for a period of 6 hours suggests that if translocation was occurring from this region the input was greater than output. The steep rise in P^{32} uptake of the rest of roots and of the elongation zone (Fig. 5:2) in the initial period of 30 minutes and then after

about 1.5 hours of tracer application, could have been due to mature cells with larger vacuoles in these regions. In such cells salt uptake would be both by absorption by the cytoplasm and absorption into vacuoles. Perhaps both of these processes occurred simultaneously so that the effect is a marked increase in P^{32} uptake. The stationary phase for 40 minutes in P^{32} uptake following the initial rise in these regions is difficult to explain; one possibility is that after the initial higher uptake the saturation point was reached or the absorbed P^{32} was translocated to the shoot. The second alternative is not very likely because at the stationary phase the shoot uptake rate was not as pronounced as it was later. Furthermore, after the stationary phase there was a second rise in P^{32} uptake when concurrently shoot uptake was also high.

The differences in phosphorus uptake between high and low calcium, stimulated interest in a possible differential treatment effect on phosphorus incorporation into organic compounds. The results in Table 5:2 show that even after 1 day of NaCl application the residual phosphorus was somewhat higher at high calcium than at low calcium. The residue mostly consisted of nucleic acids, phosphoprotein and phospholipids. Amounts of other phosphorus compounds, however, were similar. After 1 and 2 days of NaCl application, the fresh weights of roots at low calcium were 90% and 86% respectively of those at high calcium (Table 5:2).

On the 3rd day of treatment when root weight at low calcium was reduced to 70% of that at high calcium (Table 5:2), the amounts of all individual phosphorus compounds at low calcium was also reduced. But this reduction has little relevance since the development between plants had become too diverse.

Incorporation of P^{32} into organic compounds was also studied. Specific activity measurements of compounds gives a better idea of turnover of these compounds under different treatments. Results present in Table 5:4 show that although amounts of inorganic phosphate, sugar phosphate and nucleotides were higher in high calcium and control than in low calcium, there was no difference in the specific activity of these compounds in the three treatments. This means that rate of turnover of these compounds in control, high calcium and low calcium was similar. The specific activity in the residue, on the other hand, was somewhat higher in high calcium and control than in low calcium. Chromatographic separation involved the measurement of radioactivity in the phosphorylated sugar compounds and no difference was found among all three treatments (Tables 5:7 and 5:8). This suggested that the metabolic pathway of phosphorus incorporation into phosphorylated sugars at low calcium, high NaCl is similar to that in control and high calcium. The results presented above show that adverse effects of NaCl at low calcium observed previously could have been due to reduced uptake of phosphorus and also due to reduced rate

of nucleic acid, phosphoprotein and phospholipid formation because of somewhat reduced specific activity in the residue at low calcium treatment. There was no evidence in the present experiment that phosphorylated sugars were concerned in growth reduction.

Apart from high NaCl effects at low calcium these results may be considered in terms of general saline conditions on plant growth. It has been stated that salinity reduced growth by

1. Increased diffusion pressure deficit of the medium. (Chapter 1).
2. Ion unbalance. (Chapter 2).

As regards the DPD effect, Wilson and Huffaker (1964) could not demonstrate conclusively the change in the concentration of phosphorylated intermediates in Trifolium subterraneum by increasing moisture stress. Such changes in their experiment were only apparent when severe wilting stage was reached. At wilting stage, however, any difference in concentration has little relevance because that could be easily ascribed to the difference in the total phosphorus pool between severely wilted and healthy plants. The ion unbalance condition due to salinity in the present experiment also had no effects on the concentration of phosphorylated sugars (Expt. 4).

These two observations partly support the statement of Nieman (1965) that salt affected plants are small but have the same metabolic capacity as control plants.

CHAPTER 6

Ca^{45} MOVEMENTS IN PLANTS AND ANATOMICAL CHANGES IN ROOT TIPS AT SUBMICROSCOPIC LEVEL AS AFFECTED BY NaCl AT LOW AND HIGH CALCIUM

Introduction

In Chapter 3 it was shown that addition of 0.6 me/l calcium at $^{1}/_{40}$ strength of nutrient greatly alleviated the adverse effect of NaCl on the growth of Hordeum vulgare. Calcium concentration of tissues in low calcium, high NaCl treatment was more reduced than in controls and in high calcium, high NaCl treatment (Chapter 4). These observations suggest that ion unbalance condition at low nutrition, due to high NaCl might have depleted calcium from the plant to the critical limit, and the growth reduction observed under this condition could have been as a direct or indirect effect of limited amount of calcium. Growth reductions associated with low calcium, high NaCl were pronounced mainly in roots and younger leaves (Chapter 2, Chapter 3). Roots and younger organs are important indicators of growth and any condition bringing about the deficiency of a certain element will be first visible in these organs.

The present experiments investigated whether calcium movement, in plants grown in low calcium, high NaCl was reduced more than in plants in high calcium, high NaCl or than in controls and also how quickly calcium moved to younger organs when low calcium plants were transferred to high calcium, high NaCl solution (Expt. 1.)

An attempt was also made to study the anatomical changes in the root tips at sub-microscopic level in plants treated with and without NaCl at low and high calcium for a period of 7 days (Expt. 2).

Ca^{45} movement at low and high calcium. (Expt. 1)

Methods: Hordeum vulgare at 1st leaf stage was used. Culture methods were the same as described previously.

Treatment: NaCl was at 0 and 50 me/l and calcium at 0.2 and 0.8 me/l. As in other chapters these two levels of calcium with NaCl will be called low and high respectively. Since calcium movement using Ca^{45} was determined in both short and long term experiments, first harvest (H_1) was taken 1 day after NaCl application and the second harvest (H_2) 5 days later. To note the recovery in calcium movement following calcium addition, one group

of plants previously treated with low calcium for 4 days, was transferred to high calcium solution prior to second harvest. At each harvest, plants were transferred from green house to constant temperature growth cabinet. In order to have the same specific activity in all three treatments, Ca^{45} was added to the culture solution at 80 microcurie/l in high calcium and 20 microcurie/l in low calcium and control treatments. Plants were allowed to absorb tracer for a period of 1 hour and then returned to non-labelled solution. At the time of tracer removal the first sample was taken. This is designated as 1 hour (sample). Subsequent samplings were at 5 and 24 hours after tracer removal. At each sampling occasion 2 plants in duplicate under each treatment were removed and radioautographed.

For radioautography plant samples were dried and placed on a sheet of blotting paper. These samples were covered with another sheet of blotting paper, and pressed gently for a few seconds and then transferred to fresh sheet of blotting paper. A sheet of aluminium foil 0.001 ins thick was placed on the top of the plant specimens as well as underneath the blotting paper. In the darkroom a sheet of Kodak X-ray film was placed on the upper sheet of aluminium foil and the whole lot was put between

two sheets of ½" thick polythene foam. The various layers were kept in position by placing two heavy glass sheets on both sides of polythene foam and fitting them closely inside cardboard cases. The cardboard cases were wrapped in black paper to exclude light and placed in refrigeration at $0^{o}C$ for 30 days. After this period the cases were reopened in the dark and the X-ray film developed.

Anatomical changes in the root tips of plants treated with NaCl at low and high calcium. Expt. 2.

Method: Hordeum vulgare at 1st leaf stage was used. NaCl and calcium levels in culture solutions were the same as in Experiment 1. Plants at low and high calcium were treated with and without NaCl for 7 days and then harvested. At harvest several root tips (2mm long) under each treatment were sampled for electron microscopy.

Fixation of the material for electron microscopy

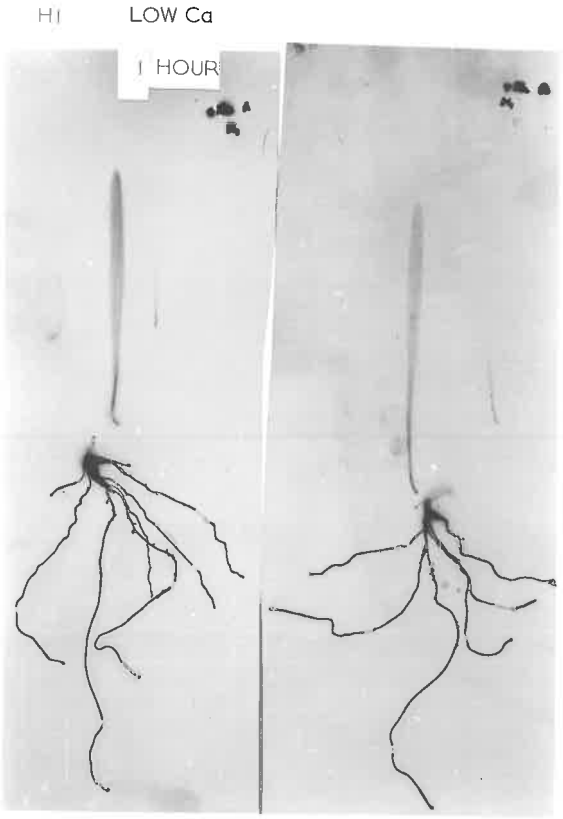
A 2% buffered solution of $KMNO_4$ (veronal acetate pH 7.2) was the most satisfactory fixative. Root tips 1 mm long were fixed for 2 hours at room temperature, dehydrated in a graded acetone series of the following strength:-

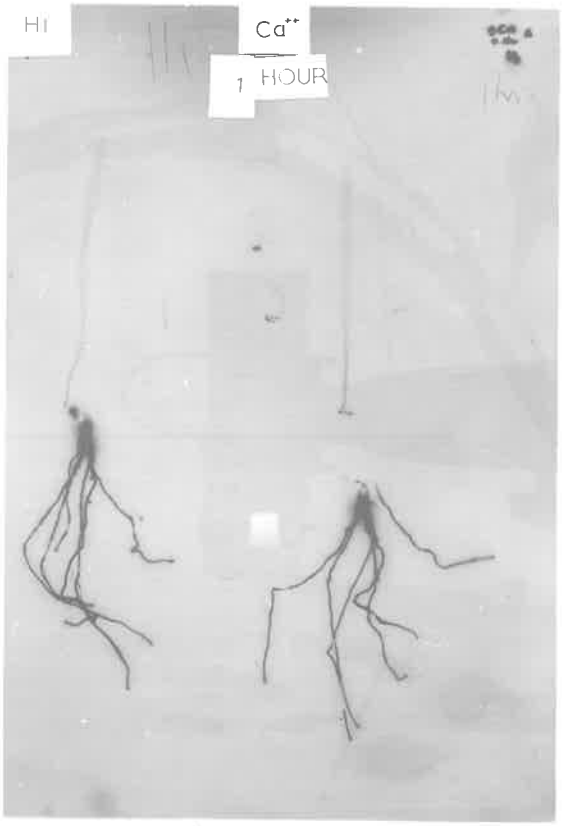
(25% - 30 mins; 50% - 30 mins; 75% plus 1% phosphotungstic acid and 1% uranyl acetate overnight; 95%, 100% 1 hr each).

PLATE 6:1

Ca^{45} radioautograms showing the distribution of labelled calcium, 1 hour after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at low (0.2 me/l) and high (0.8 me/l) calcium for 1 day before tracer application.

(In the figure Ca^{++} means high calcium).


HI
LOW Ca
1 HOUR


HI
Ca++
1 HOUR

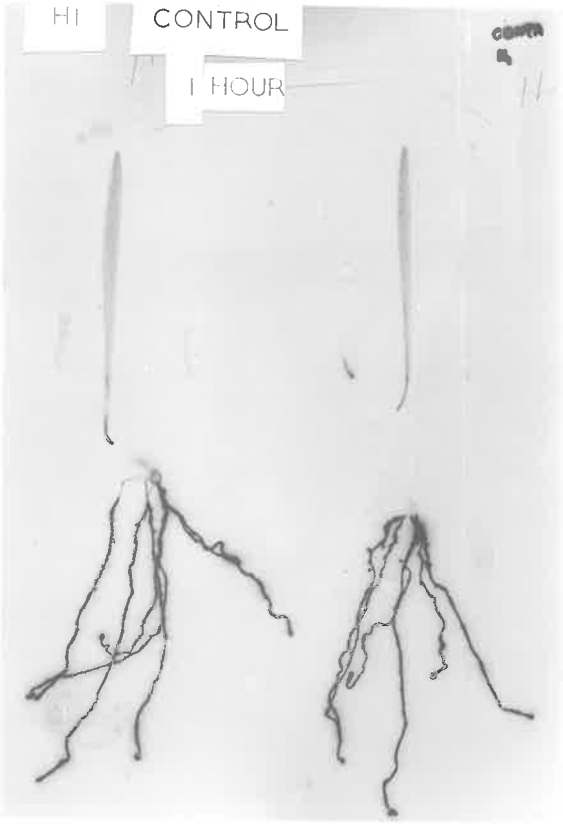

HI
CONTROL
1 HOUR

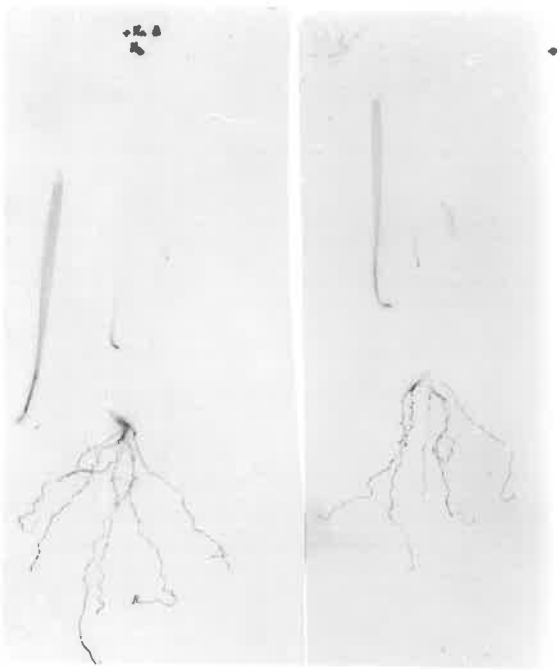
PLATE 6:2

Ca^{45} radioautograms showing the distribution of labelled calcium, 5 hours after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at low (0.2 me/l) and high (0.8 me/l) calcium for 1 day before tracer application.

(In the figure Ca^{++} means high calcium).

HI LOW Ca

5 HOURS



HI Ca++

5 HOURS

HI CONTROL

5 HOURS

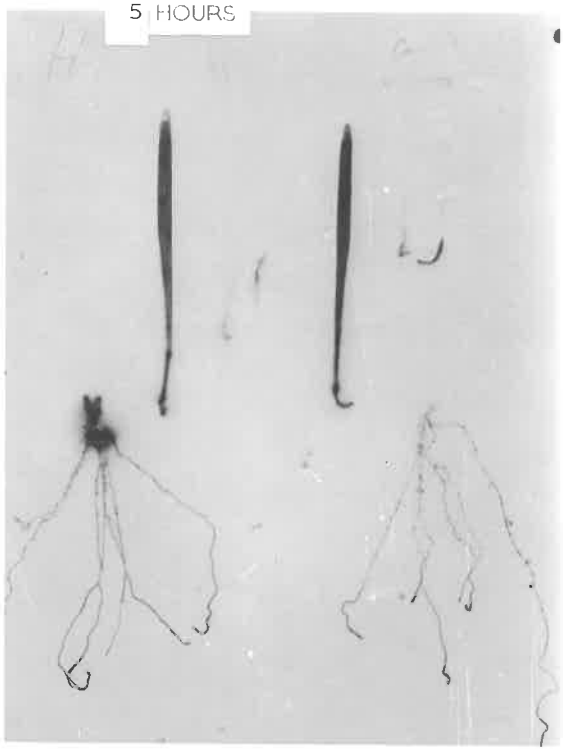
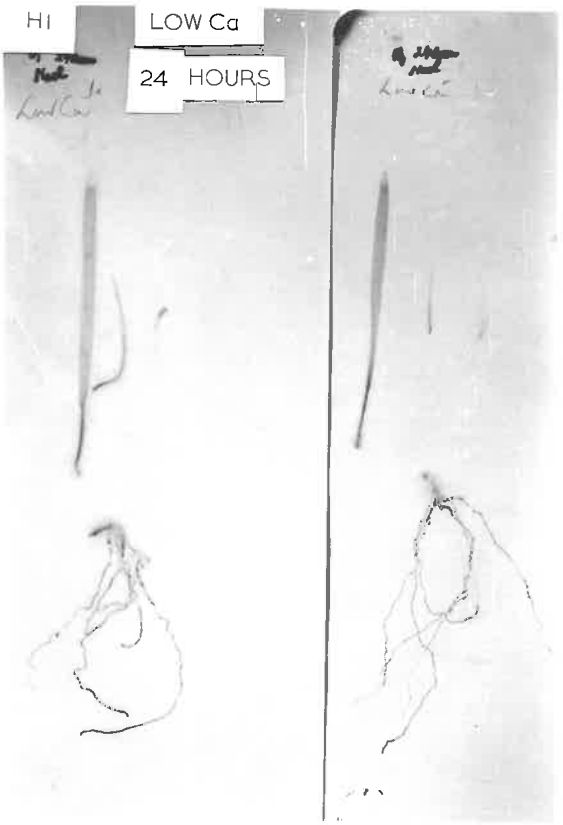
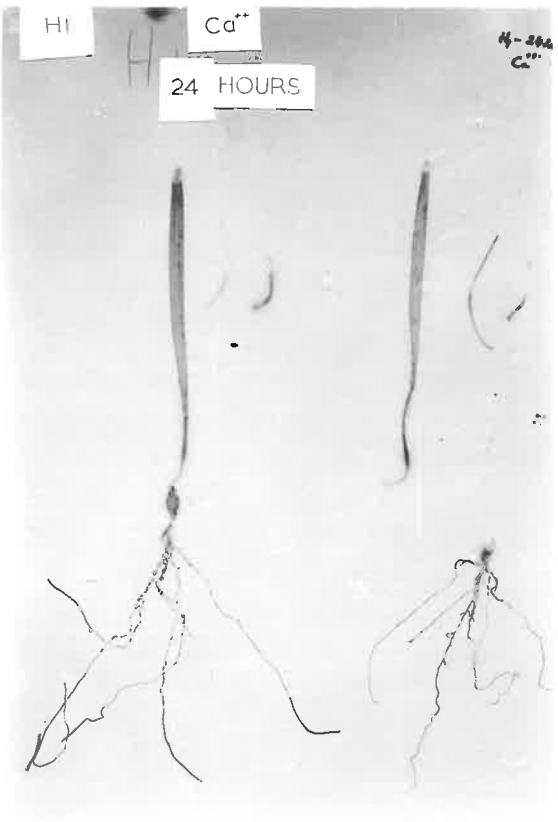


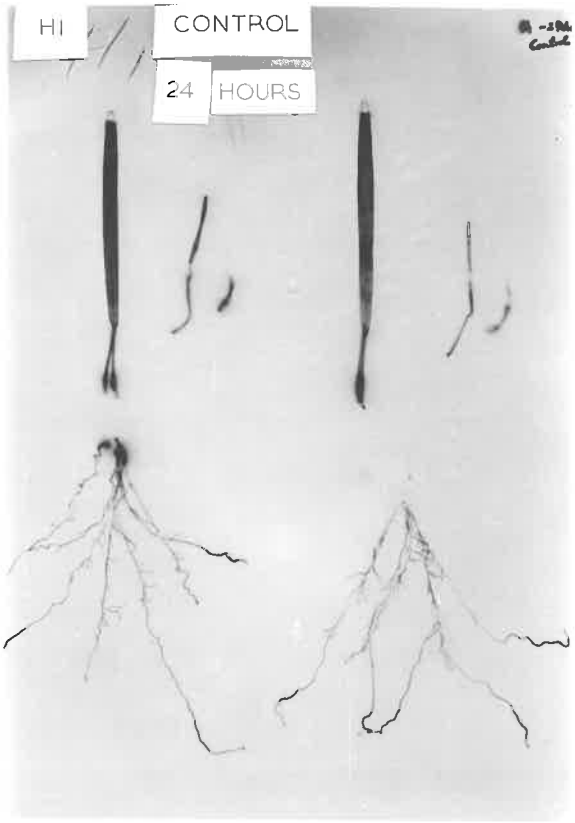
PLATE 6:3

Ca^{45} radioautograms showing the distribution of labelled calcium, 24 hours after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at low (0.2 me/l) and high (0.8 me/l) calcium for 1 day before tracer application.

(In the figures Ca^{++} means high calcium).


HI
LOW Ca
24 HOURS


HI
Ca++
24 HOURS


HI
CONTROL
24 HOURS

The tips were then embedded in "Araldite" and sections (500 - 1000A° thick) were cut using a "Si-RO-Flex" ultramicrotome and mounted on collodion grids.

RESULTS

Ca^{45} movement at low and high calcium as affected by NaCl.

Expt. 1.

The results of Experiment 1 are presented as a series of radioautograms representing the distribution of Ca^{45} at 1 to 5 and 24 hours, after tracer application. An examination of radioautograms shows that both at one day from NaCl treatment (H_1) and five days from NaCl treatment and in controls (H_2) most of the tracer absorbed during the 1 hour period stays in the root under all the three treatments (Plate 6:1,4).

In control, most of the absorbed calcium passes from roots to shoot in the 5 hour period and this relative distribution of tracer between roots and shoot remains unchanged for rest of the 24 hours at both harvests (Plates 6:2, 3, 5, 6). But calcium movement in NaCl treatment is greatly restricted particularly when plants were treated only for 1 day with NaCl. In this short term treatment very little calcium moved from root to shoot even after 24 hours of tracer application in low calcium treatment

PLATE 6:4

Ca^{45} radioautograms showing the distribution of labelled caldium 1 hour after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at

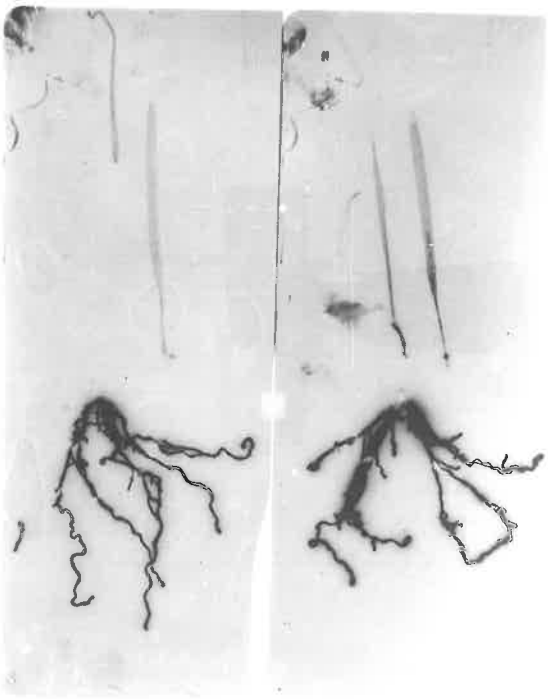
1. Low (0.2 me/l) calcium 5 days
2. High (0.8 me/l) " 5 days
3. First low Ca^{++} 4 days, then high Ca^{++} 1 day.

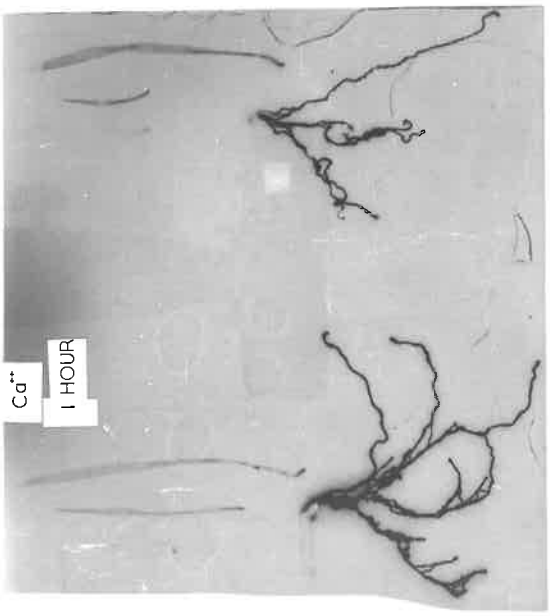
(In the figures Ca^{++} means high calcium).

H2

LOW Ca

1 HOUR





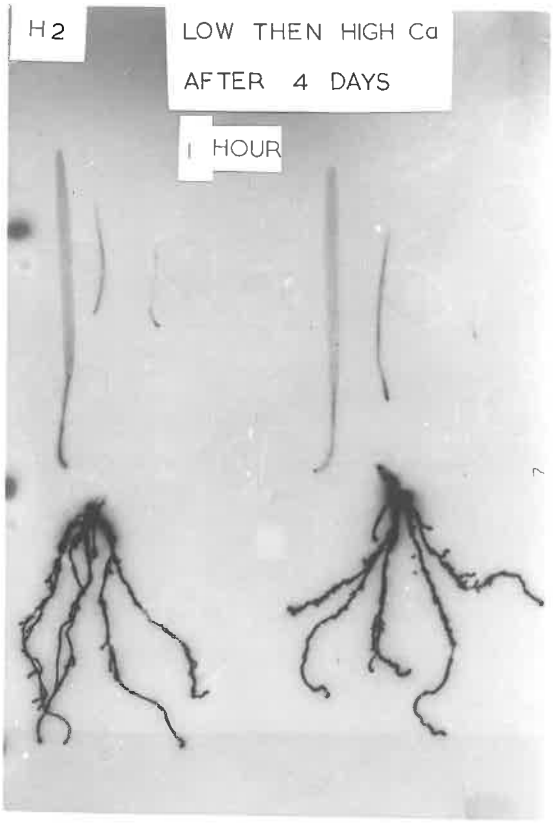
H2

H2

LOW THEN HIGH Ca

AFTER 4 DAYS

1 HOUR



H2

CONTROL

1 HOUR

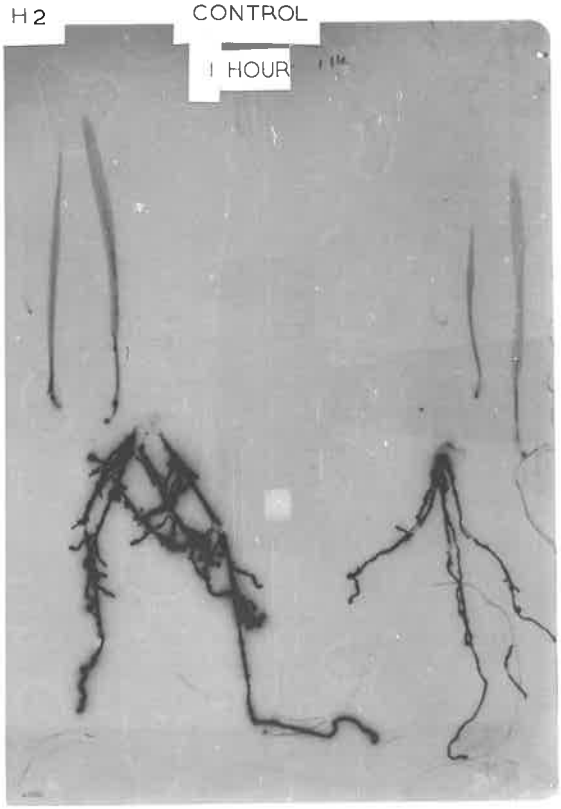


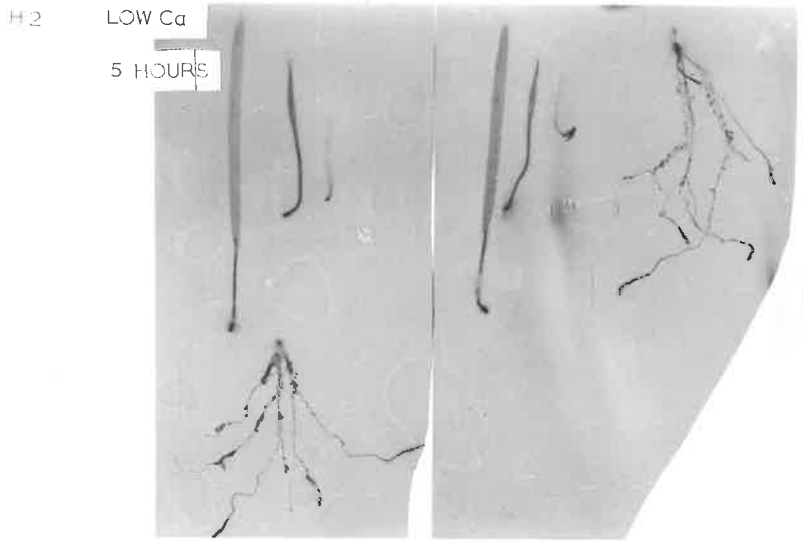
PLATE 6:5

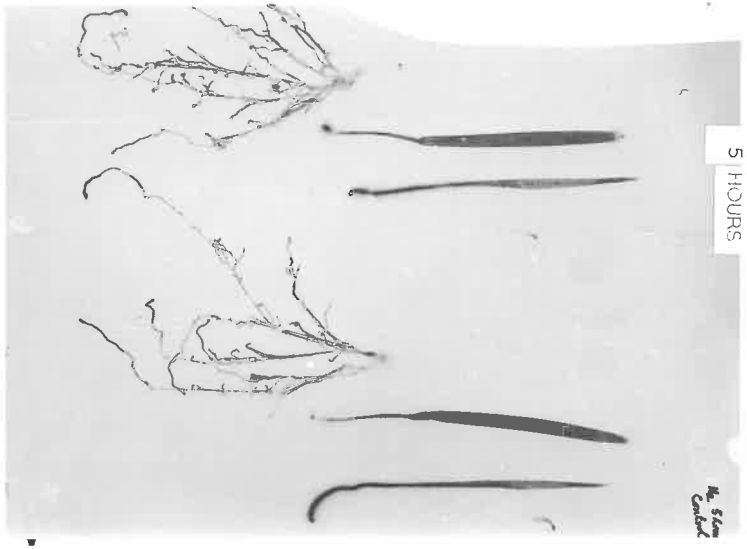
Ca^{45} radioautograms showing the distribution of labelled Calcium, 5 hours after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at:-

1. Low (0.2 me/l) Calcium 5 days.
2. High (0.8 me/l) " 5 days.
3. First low Ca^{++} 4 days, then high Ca^{++} 1 day.

(In the figures Ca^{++} means high Calcium).


H 2
LOW THEN HIGH Ca
AFTER 4 DAYS
5 HOURS


H 2
LOW Ca
5 HOURS


H 2
CONTROL
5 HOURS

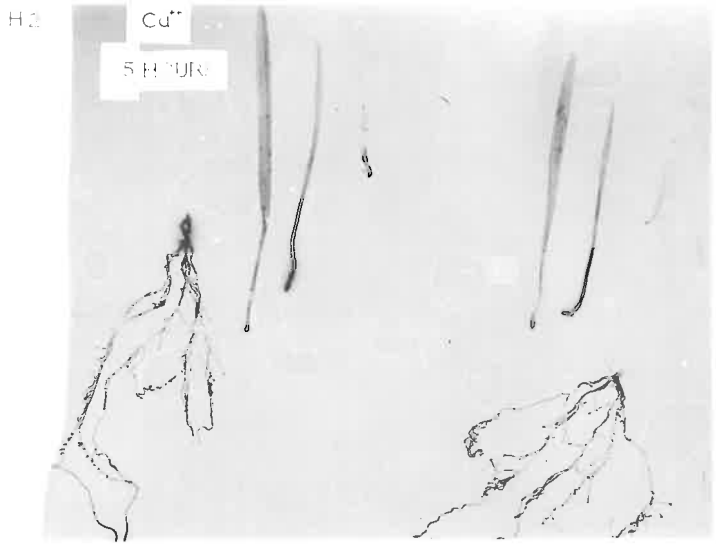

H 2
Cu++
5 HOURS

PLATE 6:6

Ca^{45} radioautograms showing the distribution of labelled calcium, 24 hours after its application to the roots via treatment solution. Plants were treated with and without NaCl (50 me/l) at

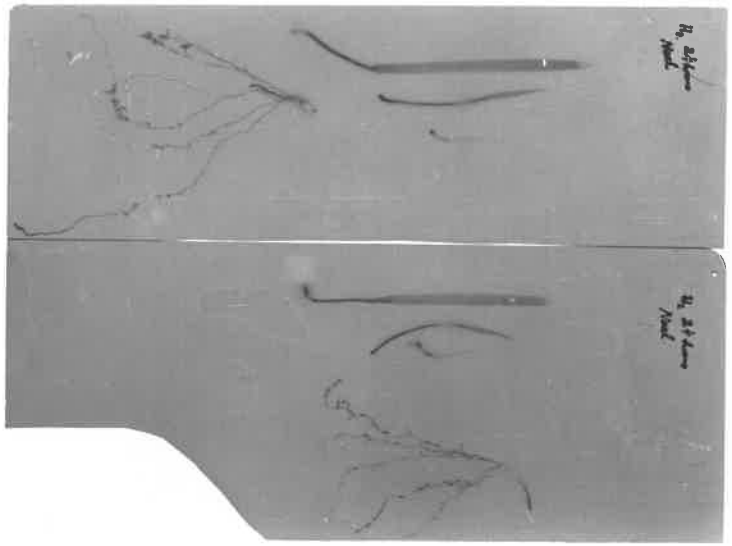
1. Low (0.2 me/l) calcium 5 days
2. High (0.3 me/l) " 5 days
3. First low Ca^{++} 4 days, then high Ca^{++} 1 day.

(In the figures Ca^{++} means high calcium).

H 2 LOW THEN HIGH Ca

AFTER 4 DAYS

24 HOURS

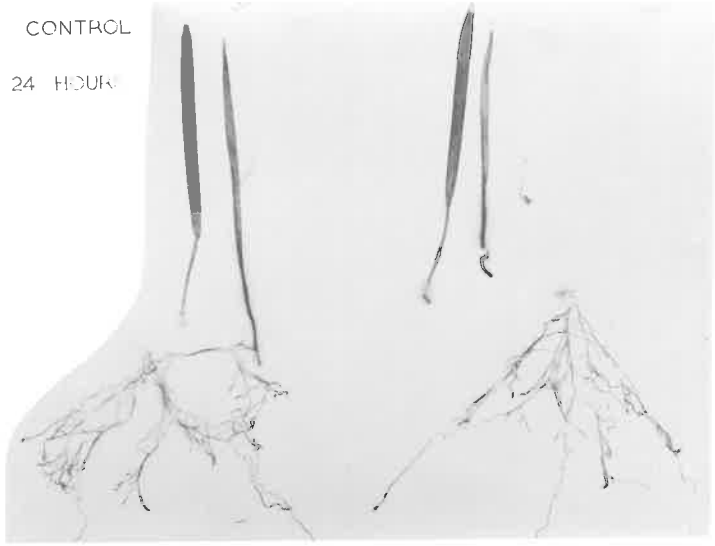


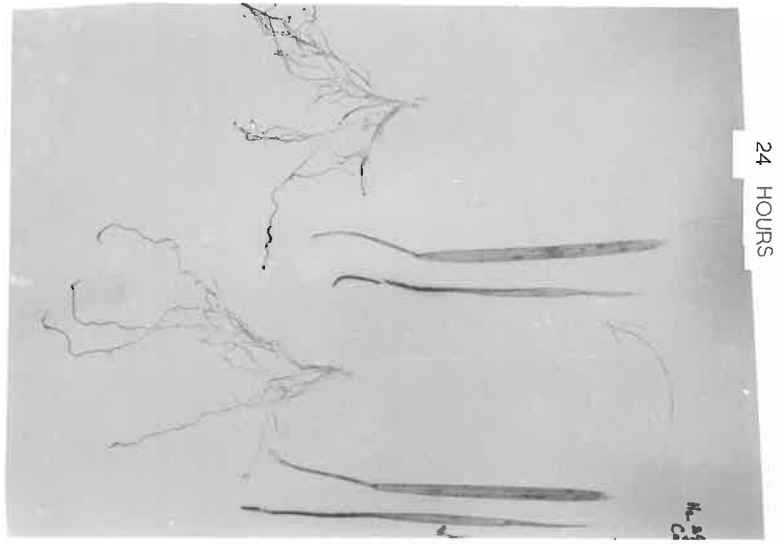
H 2 LOW Ca

24 HOURS

H 2 CONTROL

24 HOURS





H 2 Ca^{++}

24 HOURS

(Plate 6:3) though some more movement of tracer in high calcium treatment was noted at this period (Plate 6:3).

In the long term treatment i.e. when plants were first treated with NaCl for 5 days prior to tracer application some Ca^{45} in high calcium treatment moved from root to shoot in 5 hour period (Plate 6:5) and after 24 hours of tracer application this movement was also shown (Plate 6:6). Similar trends in calcium movement were noted in plants which were transferred from low to high calcium on day 4 of the experiment (Plate 6:6). At low calcium on the other hand there was little indication of calcium movement from root to shoot even after 24 hours of tracer application (Plate 6:6). In this treatment calcium absorbed in roots in the 1 hour period was lost after 24 hours; the tracer lost from the root did not apparently move to the shoot. At both harvests young leaves of the plant contained more radioactivity in the control, high calcium and recovery treatments than low calcium treatment (Plates 6:1 to 6).

Anatomical changes in root tips as affected by NaCl at low and high calcium. Expt. 2.

Satisfactory comparison of morphological changes at the sub-microscopic level can only be made by examining the cells at similar position in the tissue in different treatments.

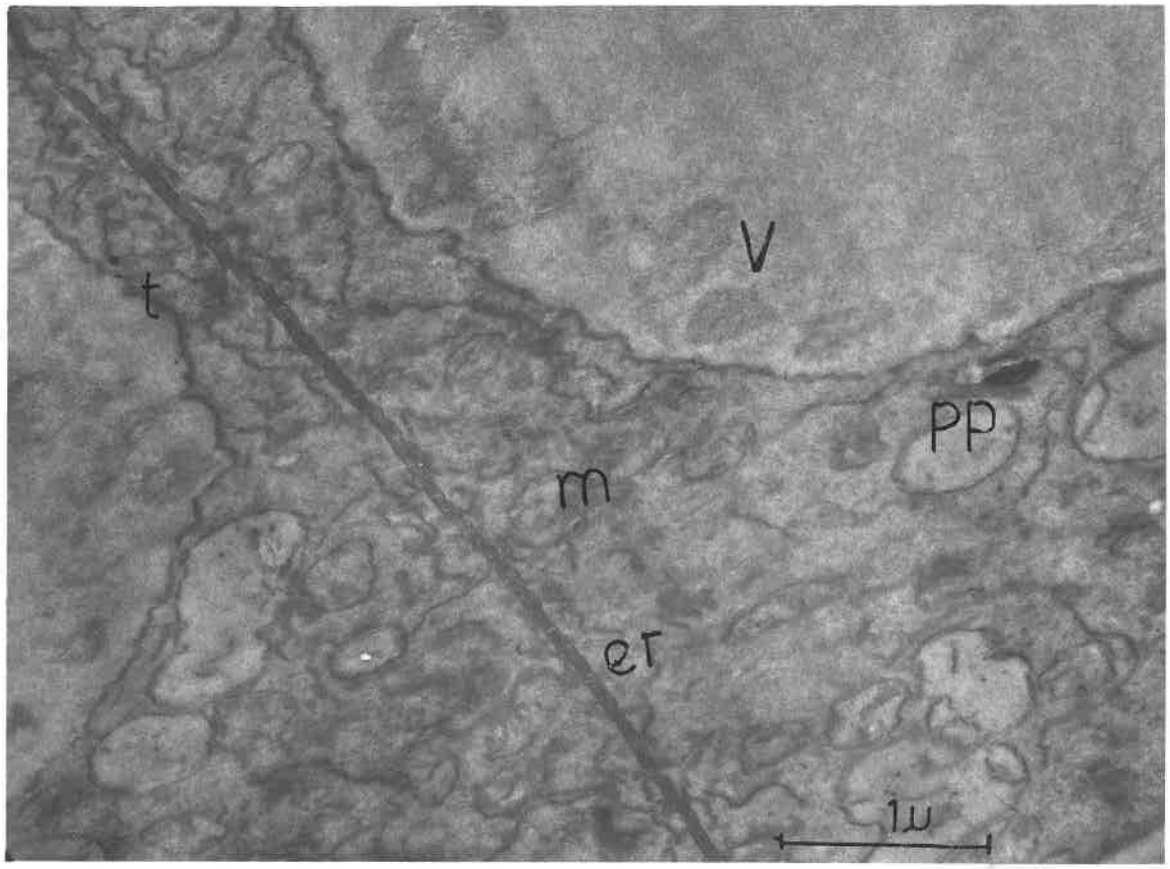
PLATE 6:7

Electron micrographs of cells approximately 1 mm from the root tip of Hordeum vulgare growing in $^{1}/_{40}$ nutrient for a period of 7 days.

Fig. 1. Micrographs showing vacuoles (v) and cytoplasm. The tonoplast membrane is clearly defined (t). Numerous organelles appear in the cytoplasm: mitochondria (m), endoplasmic reticulum (e.r.), proplastids (p.p.).

Fig. 2. Similar cells with a darkly stained small vacuole (s.v.) and golgi bodies (g).

Both micrographs (x 27,600).


V
t
PP
m
er
1μ

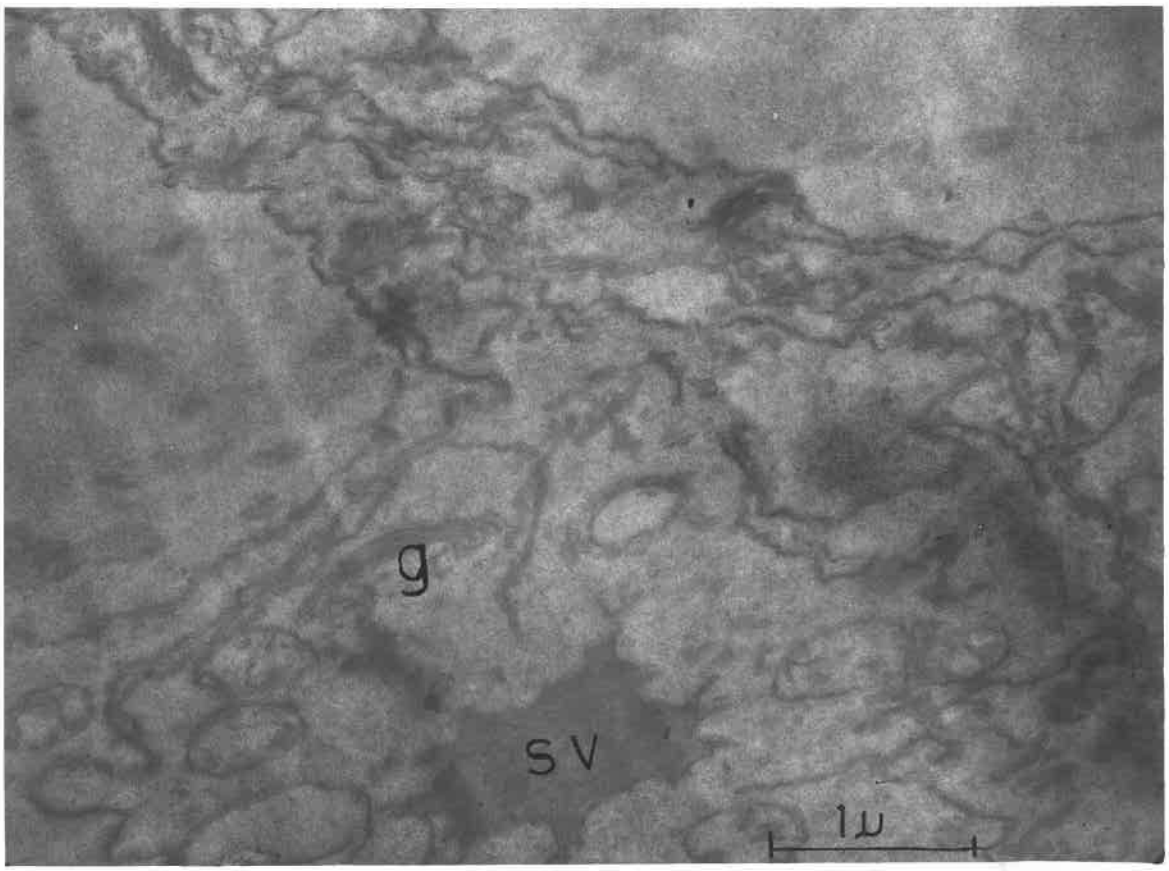

g
SV
1μ

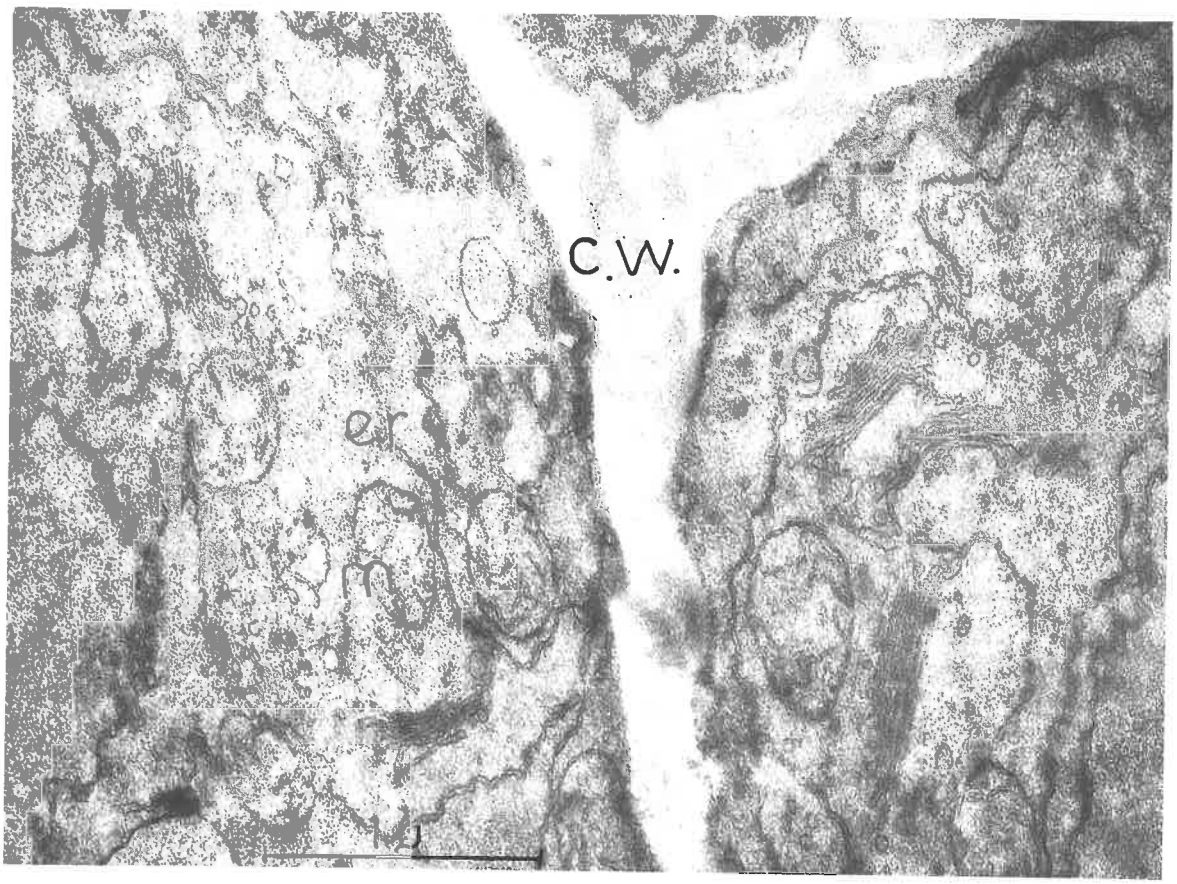
PLATE 6:8

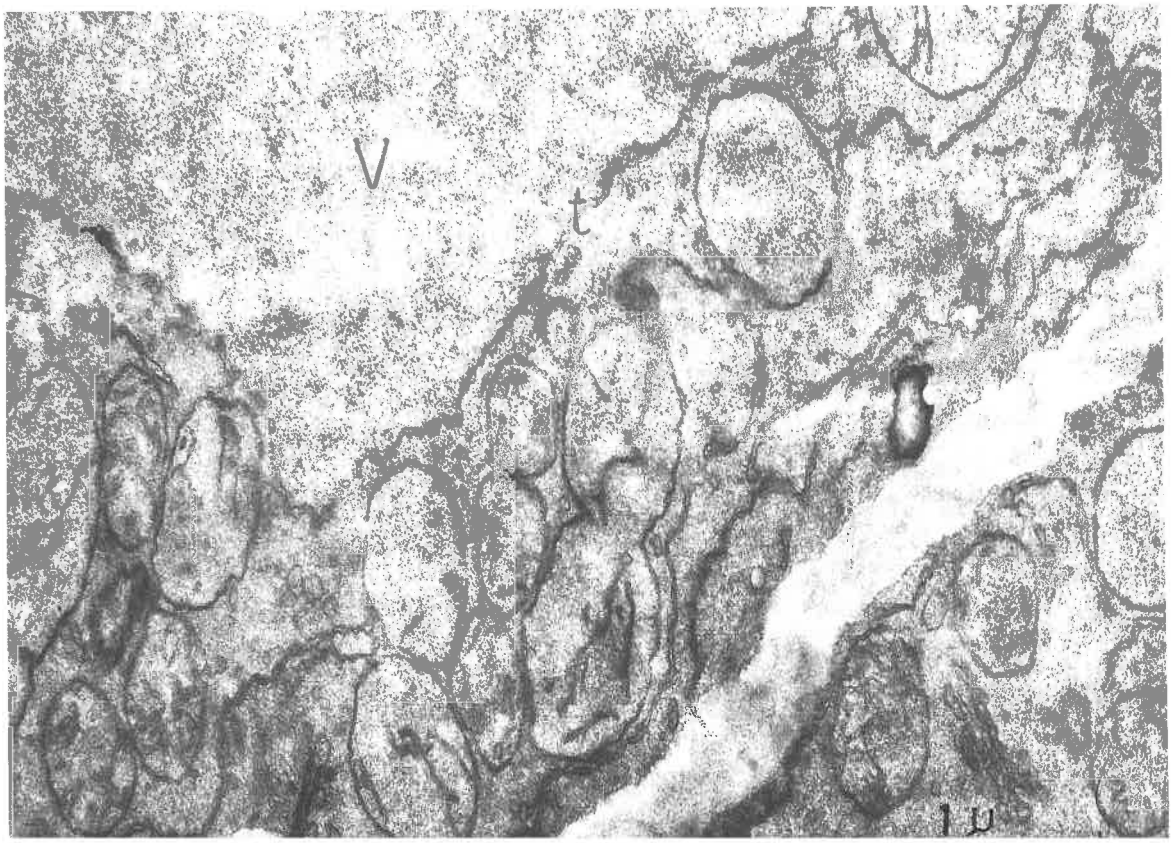
Electron micrographs of cells approximately 1mm from the root tip of Hordeum vulgare growing in $^{1}/_{40}$ nutrient + NaCl (50 me/l) for a period of 7 days.

Fig. 1. The micrograph shows that there is little morphological difference in the tonoplast and organelles of the cytoplasm from Plate 6:7. The cell walls (c.w.), mitochondria (m), endoplasmic reticulum (e.r.), golgi (g) and proplastids (p.p.) are clearly visible.

Fig. 2. The tonoplast (t) is well defined and appears as a double membrane separating the cytoplasm-vacuole phase.

Both micrographs (x 36,300).


C.W.
er
m
1μ


V
t
1μ

In the present experiment, cells at a distance 1 mm away from the tips were studied for morphological changes in all the treatments. Marschner et al. (1964) have shown that deficiency of calcium resulted in the breakdown of the tonoplast in the root tips of barley. It was thought that in low calcium treatment a similar breakdown of tonoplast may occur due to limited uptake or greater loss of calcium from the plants. The low calcium treatment was compared with the control and the electron micrographs are presented in Plates 7 and 8. It appears from the plates that no detectable difference in the structure of tonoplast and organelles occurs between the two treatments.

DISCUSSION

It has been generally agreed that after its initial deposition calcium does not recirculate in plants because of its immobility (Williams 1955; Zimmerman 1960).

Charles (1953) suggested that xylem elements may be negatively charged and Bell and Bidulph (1963) have shown that calcium in the xylem moves by an exchange process where its movements could be arrested by divalent ions but not by monovalent ions. In the present experiment calcium movement at low calcium status of the medium was greatly reduced by NaCl treatment (Plates 6:3, 6) suggesting that monovalent ions may also prevent calcium movement, if $\frac{\text{monovalent ions}}{\text{calcium ion}}$ equivalence ratio greatly increases.

In the present experiment this ratio (NaCl/calcium) was 83, whereas in the experiment of Bell and Bidulph (1963) the ratio (KCl/calcium) was 1. At high monovalent/calcium ratio, exchange sites for calcium may be restricted because of the presence of other monovalent ions competing for these sites.

In the NaCl treatment due to restricted Ca^{45} movement, a distinctly different pattern of distribution of Ca^{45} to that in control plant has been demonstrated. In this connection it may be noted that in the short term experiment when plants were treated with NaCl only for 1 day, calcium movement from roots to shoot was greatly reduced in low calcium treatment and also though to a less extent in high calcium treatment. This restricted movement of calcium in low calcium treatments gives an indication of the cause of adverse growth which occurs in the later stages in these experiments; after 5 days, growth in low calcium, high NaCl treatment is strongly reduced (Fig. 3:6, Chapter 3). Since calcium movement in low calcium treatment is reduced even after 1 day of NaCl application when there are no obvious differences in growth compared to control plants, it is suggested that reduced calcium uptake is the cause rather than effect of reduced growth which occurs when this treatment is prolonged.

Younger parts such as leaf 2 and root tips are the seat of active synthesis. Radioactivity in these parts was greatly

reduced in low calcium treatment compared to high calcium and control treatment (Plates 6:1 to 6). This indicates that high NaCl at low calcium either restricts the calcium movements to growing sites or calcium moving to these sites may be lost again. Which ever may be the possibilities, the net effect will impair the functional or organizational capacity of these regions, thus leading to reduced growth. The alternative that calcium may be lost at least from roots to the solution gets stronger support from the observation that roots in this treatment neither transport calcium to shoot nor retain it. This may well be conceived from earlier observation (Chapter 4) of increased permeability in this treatment.

In Chapter 3 it was shown that plants previously treated with low calcium, high NaCl for 5 days had considerably recovered when transferred to high calcium solution for seven days. In the present experiment it should be noted that plants subjected to low calcium, high NaCl for 4 days then transferred to high calcium for only 1 day showed greater movement of calcium to the shoot and greater retention in the roots than the plant in the continued low calcium treatment, indicating that growth recovery may depend partly upon calcium moving to the shoot and partly on that retained in the root.

The results of these radioautograms are in good agreement

with the results of calcium analysis of plants reported in Chapter 4. Although low calcium and control treatments had the same calcium concentration in the nutrient solution, calcium movement into plants in the control was far greater. Even in the high calcium treatment which had four times more calcium concentration in the nutrient solution than the control, the plants showed less calcium movement than the control plants.

From the results of these radioautograms it looks certain that growth reduction under saline environment, at least at low calcium status of the medium are due to lack of calcium reaching to the growing points, but it is difficult to make definite conclusions whether it is the direct or indirect effect of calcium which reduces growth. It has been claimed that calcium plays an important role in cell physiology by maintaining structure of the protoplasm and semipermeability of the membranes (Fischer 1956). Epstein (1961) has shown that calcium is essential for maintaining the integrity of the selective ion transport mechanism. These observations suggest that the direct effect of calcium deficiency would be the disorganization of protoplasmic structure (Marinos 1962) and loss of important metabolite (Chapter 4). Calcium on the other hand is also essential for the uptake and utilization of other ions like potassium (Kahn and Hanson, 1957; Viet 1944; and Chapter 4), phosphorus (Tanda 1955; and Chapter 5) and nitrate (Burström 1954). Thus the calcium deficiency may include both its primary and secondary effects on growth.

It is therefore reasonable to assume that growth reduction at low calcium high, NaCl may be caused both by a primary and secondary effect of calcium deficiency. The electron micrographs of root tips at low calcium did not show any major characteristic deficiency symptoms for calcium. This observation does not rule out the possibility that direct effect of calcium was involved. It has already been seen that high NaCl at low calcium reduces calcium uptake (Plate 6:3). The electron micrographs show that this reduction did not involve any gross morphological disorganisation of cell structure.

CHAPTER 7

GENERAL CONCLUSIONS

The purpose of the experiments described in this thesis was to investigate effects of high NaCl concentration on plant growth in a low nutrient solution. In recent years, a distinction has been made between osmotic and non-osmotic effects of certain salts by comparing growth in solutions of electrolytes with that in non-ionic osmotic solutions (Lagerwarff and Eagle 1961). Osmotic effects influence plant growth by controlling cell turgor and the non-osmotic effects are physiological and possibly biochemical.

Before the distinction between these two effects was appreciated, an over emphasis was placed on the osmotic interpretation of growth reduction in a solution of high electrolyte concentration. Possibly this was because, in most salinity studies, salts in high concentrations were added to the full strength of nutrient solution. Under such conditions the non-osmotic or the physiological effects due to ion unbalance were counter-balanced to a great extent by the ions of the nutrient solution and the growth reduction was then primarily due to osmotic effects. In the dilute

nutrient solution, on the other hand, non-osmotic effects of salts are very pronounced as the antagonistic power of the nutrient ions is very low and the growth is depressed both by osmotic and non-osmotic effects of high salt concentration in the root medium. In the present thesis this point has been clearly demonstrated by comparing growth in high NaCl and iso-osmotic solutions of mannitol at high and low nutrient level (Chapter 3, Expt. 3). For inducing an ion unbalance condition at low nutrient the particular ionic species is not important so growth was similarly reduced in the solutions of both high KCl and high NaCl (Chapter 3, Expt. 8). Information regarding NaCl effects at lower than full nutrient solution is important in studying plant response to saline conditions because the ionic composition of full nutrient solution represents the condition of a fertile soil whereas some saline soils are not fertile; they lack calcium due to low solubility of its carbonate and sulphate salts (Kelley 1951).

It has also been shown that when plants are exposed to a solution of high concentration of salts, their internal osmotic pressure is adjusted by electrolyte absorption from the external solution (Eaton 1942; Gauch and Wadleigh 1945; Bernstein 1961, 1963). Probably this osmotic pressure adjustment is more efficient at high than at low nutrient, since growth in NaCl treatments (Chapter 3) was better than in iso-osmotic mannitol at high nutrient though the reverse was true at low nutrient (Fig. 3:2). The inadequate osmotic

pressure adjustment at low nutrient could be due to loss of ions in long term NaCl treatment, as shown by the increased permeability of plants under this condition (Chapter 4).

Halophytes occurring in saline habitat show great salt tolerance. The main features of "Regulation types" (Arnold 1955) halophytes are:-

1. Resistance to high internal salt concentration.
2. Rapid salt uptake providing rapid osmotic pressure adjustment (Greenway 1965).

In the non-halophyte, barley, these two factors of salt tolerance were adversely affected by NaCl at low nutrient in the experiments reported in the present thesis. Determination of NaCl effects at low nutrient in salt tolerant halophytes would contribute to the general understanding of ion unbalance under saline conditions. There are many reports that changes in ionic composition of the plant tissues, such as increases in sodium and chloride and decreases in potassium, are responsible for plant injury and growth reduction on saline media. Such changes in ionic balance are more pronounced in sensitive than resistant varieties (Ehlig 1960; U.S. Salinity Lab. Staff 1954). It appears that, at low nutrient, NaCl brings about similar changes in the ionic composition of the plant and decreases the salt tolerance. It may be pointed out here that sodium and chloride

concentration, which reduce growth at low nutrient level, increased rapidly soon after NaCl application (Chapter 4, Fig. 4:2A and Figs. 4:5A, B). In the later period these concentrations decreased (Chapter 3, Fig. 3:7) but the growth of the plant did not improve (Chapter 3, Fig. 3:6). This suggests that metabolic changes which reduce growth under such conditions are brought about immediately upon NaCl application. Perhaps more detailed studies of the enzymes which are involved in various metabolic paths may elucidate the sequence of events bringing about growth reduction. There is some evidence in the present thesis that production of RNA and DNA might be limiting at low nutrient, high NaCl (Chapter 5, page 112) but a direct determination of the content of these nucleic acids as affected by NaCl at low and high nutrient would be more confirmatory. It is also important to get information on the mitochondrial activity of the plants at low and high nutrient because they fulfil a vital link in metabolism, being the seat of oxidative phosphorylation.

In Chapter 4 it was shown that at low nutrient level, NaCl treatment reduced uptake and increased the loss of potassium and calcium (Figs. 4:5C, D, E, F) from the plants. This observation suggests that growth at low nutrient, high NaCl may have been partly reduced due to deficiency of any one of the essential elements. A simple approach which migh help to ascertain which element or elements is responsible for growth reduction would be first to establish

the critical concentration of individual elements in plants below which the growth is reduced at low nutrition. If the concentration of these elements falls below this level in the high NaCl, low nutrient treatment this might give some convincing evidence that high NaCl at low nutrient level brings about the deficiency of the particular element.

The important conclusion from this thesis is that calcium is the principal ion in the nutrient medium overcoming the toxic effects of high NaCl. Calcium has been shown to have important effects on the permeability of the cells and thus probably to affect the structural organization of the cytoplasm (Chapter 6). At present the question of where the excess NaCl at low calcium becomes toxic in the metabolism remains unanswered.

General Abbreviations and Terminology

Abbreviations

D.P.D. = Diffusion pressure deficit

D.N.P. = 2 - 4 dinitrophenol

me/l = milli equivalent per litre

O.P. = osmotic pressure

Terminology

Halophytes: Salt tolerant species of saline habitat.

Active ion uptake: The process whereby ions move against the electrochemical potential gradient and is therefore dependent on metabolic energy.

Passive ion uptake: Ion uptake due to diffusion into the free spaces and absorption to electrically charged points with no metabolic process involved.

Membranes: Lipoprotein membranes.

(a) plasmalemma : outer surface of cytoplasm

(b) tonoplast : boundary between cytoplasm and vacuole.

Free space: The part of the cell into which solute and solvent from external solution move readily.

APPENDIX I

Abbreviations of phosphorylated compounds

A.D.P.	:	Adenosine diphosphate
A.T.P.	:	Adenosine triphosphate
F.I.6.D.P.	:	Fructose 1, 6-diphosphate
F.6.P.	:	Fructose-6-phosphate
G.I.P.	:	Glucose-1-phosphate
G.6.P.	:	Glucose-6-phosphate
P.E.P.	:	Phosphoenolpyruvic acid
P.G.A.	:	Phosphoglyceric acid
Pi	:	Inorganic phosphate
R.5.P.	:	Ribose-5-phosphate

APPENDIX II

Rf values of phosphorylated compounds on acid washed Whatman No. 1 Chromatography paper in different solvents.

(1) Solvent tert - butanol/water/picric acid 80:20:3 (v.v.w.)

Compounds	Rf (G.I.P.)
G.I.P.	13
R.5.P.	22.5
G.6.P.	14.0
F.1.6.D.P.	13.2
P.G.A.	31
A.T.P.	6
A.D.P.	9.2
Pi	21.8

(2) Solvent isopropyl ether/(90 ml) 90% formic acid (60 ml)

Compounds	Rf (A.T.P.)
A.T.P.	28
A.D.P.	30.5
F.6.P.	32.5
R.5.P.	30.4
G.I.P.	26.3
G.6.P.	24.2
P.G.A.	30.5
F.1.6.D.P.	27
Pi	34

APPENDIX II (Continued)

(3) Solvent Methanol/formic acid

Compounds	Rf (R.S.P)
R.S.P.	34
F.1.6.D.P.	33.7
P.G.A.	36.5
F.6.P.	34.5
G.I.P.	30
P.E.P.	39
R.5.P.	35.3
F.1.D.D.P.	33
P1	39.4

REFERENCES

Allen, R.J.L. (1940). - The estimation of phosphorus. Biochem. J. 34 : 858-865.

Andel, O.M. van, Arisz, W.H., and Helder, R.J. (1950). - Influence of light and sugar on growth and salt intake by maize. Proc. Kon. Ned. Akad. Wetensch Amsterdam 53.

Arisz, W.H., Camphuis, I.J., Heikens, H. and van Tooren, A.J. (1955). - The secretion of the salt glands of Limonium latifolium. Ktze. Acta Bot. Neerl. 4 : 322-338.

Arnold, A. (1955). - "Die Bedeutung der Chlorionen für die pflanze". (Fischer : Jena)

Arnon, D.I. and Hoagland, D.R. (1940). - Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. 50 : 663-684.

Ayers, A.D. (1953). - Germination and emergence of several varieties of barley in salinized soil cultures. Agron Jour. 45 : 68-71.

Ayers, A.D., Brown, J.W. and Wadleigh, C.H. (1952). - Salt tolerance of barley and wheat in soil plots receiving several salinisation regimes. Agron. J. 44 : 307-310.

Bartlett, G.R. (1959). - Phosphorus assay in Column Chromatography. J. Biol. Chem. 234 : 466-468.

Bell, C.W. and Bidulph, O. (1963). - Translocation of Calcium. Exchange versus mass flow. Plant Physiol. 38 : 610-614.

Berg, C. Van Den (1952). - The influence of absorbed salts on growth and yield of agricultural crops on salty soils. Versl. Landb. 's Grav. No.58.5.

Bernstein, L. (1958). - Salt tolerance of grasses and forage legumes. U.S. Dept. Agric. Information Bull. No.194.

Bernstein, L. (1961). - Osmotic adjustment of plants to saline media, I. Steady state. Am. J. Bot. 48 : 909-918.

Bernstein, L. (1963). - Osmotic adjustment of plants to saline media. II. Dynamic phase. Am. J. Bot. 50 : 360-370.

Bernstein, L. and Pearson, G.A. (1956). - Influence of exchangeable sodium on the yield and chemical composition of plants. I. Green beans, garden beets, clover and alfalfa. Soil Sci. 82 : 247-258.

Bernstein, L. and Hayward, H.E. (1958). - Physiology of salt tolerance. Ann. Rev. Pl. Physiol. 9 : 25-46.

Best, R.J. (1950). - A rapid and accurate electrotitrimetric method for determining the chloride content of soils, water, and plant materials. Trans. IVth Int. Cong. Soil Sci. Amsterdam 3 : 162-164.

Bieleski, R.L. and Young, R.E. (1963). - Extraction and separation of phosphate esters from plant tissues. Anal. Biochem. 6 : 54-68.

Bisalputra, T. (1960). - Anatomical and morphological studies in the Chenopodiaceae. Aust. J. Bot. 8 : 226-242.

Braverman, I. and Morgulis, S. (1943). - The inhibition of the adenosine triphosphatase activity of actomyosin by magnesium ion. Jour. Gen. Physiology 31 : 411-415.

Briggs, G.E., Hope, A.B. and Robertson, R.N. (1961). - "Electrolytes and Plant Cells." (Blackwell: Oxford).

Bonner, J. and Koepfli, J.B. (1939). - The inhibition of root growth by Auxins. Amer. J. Bot. 26 : 557-565.

Bower and Turk (1946). - Calcium and magnesium deficiencies in alkali soils. J. Am. Soc. Agron. 38 : 723-27.

Boynton, D. and Burrel, A.B. (1944). - Potassium induced magnesium deficiency in McIntosh apple trees. Soil Sci. 58 : 441-454.

Boynton, D. and Compton, O.C. (1944). - The influence of differential fertilization with $(NH_4)_2SO_4$ on the chemical composition of McIntosh apple trees. Proc. Am. Soc. Hort. Sci. 44 :9-17.

Burström, H. (1941). - On formative effects of carbohydrates on root growth. Bot. Notiser 1941 : 310.

Burström, H. (1954). - Studies on the growth and metabolism of roots x investigations on the calcium effect. Physiol. Plant. 7 : 332-342.

Carolus, R.L. (1933). - Some factors affecting the absorption of Mg. in the potato plant. Proc. Am. Soc. Hort. Sci. 30 : 480-484.

Charles, A. (1953). - Uptake of dyes in cut leaves. Nature 171 : 435-436.

Collander, Runar. (1959). - Cell membranes: their resistance to penetration and their capacity for transport. Plant Physiology. A treatise Ed. F.C. Steward vol.II, page 3-93. Academic Press, New York.

Curtis, O.F. and Clarke, D.G. (1950). - An introduction to Plant Physiology. McGraw-Hill Book Co. Inc. New York, Toronto, London.

David, D.J. (1959). - The determination of calcium in plant material by atomic-absorption spectrophotometry. The Analyst 84 : 536-545.

Eaton, F.M. (1942). - Toxicity and accumulation of chloride and sulphate salts in plants. J. Agric. Res. 64 : 357-399.

Ehlig, C.F. (1960). - Effect of salinity on four varieties of table grapes grown in sand culture. Proc. Amer. Soc. Hort. Sci. 76 : 323-335.

Emmert, F.H. (1961). - The bearing of ion interactions in tissue analysis results. Plant analysis and Fertilizer Problem. Wallace T. (Ed.) 231-243.

Epstein, E. (1961). - The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36 : 437-444.

Epstein, E. (1962). - Mutual effects of ions in their absorption by plants. Agrochimica 6 : 293-322.

Epstein, E. and Hagen, C.E. (1952). - A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. 27 : 457.

Fawzy, H.R., Overstreet, R. and Jacobson, L. (1954). - The influence of H ion concentration on cation absorption by roots. Plant Physiol. 29 : 234-237.

Ferguson, W.S. and Hedlin, R.A. (1963). - Effect of soluble salts on plant response to and absorption of phosphorus. Can. J. Soil Sci. 43 : 210-218

Fischer, H. (1956). - Ionenwirkung. Encyclopaedia of Plant Physiology II 706-746.

Fiske, C.H. and Subbarow, Y. (1925). - The colorimetric determination of phosphorus. J. Biol. Chem. 66 : 375.

Gausman, H.W. and Awan, A.B. (1956). - Effect of chloride from calcium chloride on P^{32} accumulation in potatoes. Agron. J. 48 : 431.

Gauch, H.G. and Wadleigh, C.H. (1945). - Effect of high concentration of sodium, calcium, chloride and sulphate on ionic absorption by bean plants. Soil Sci. 59 : 139-153.

Greenway, H. (1962a). - Plant response to saline substrates. I. Growth and ion uptake of several varieties of Hordeum during and after sodium chloride treatment. Aust. J. Biol. Sci. 15 : 16-38.

Greenway, H. (1962b). - Plant response to saline substrates II. Chloride, sodium and potassium uptake and translocation in young plants of Hordeum vulgare during and after a short sodium chloride treatment. Aust. J. Biol. Sci. 15 : 39-57.

Greenway, H. (1963). - Plant response to saline substrate. III. Effect of nutrient concentration on the growth and ion uptake of Hordeum vulgare during a sodium chloride stress. Aust. J. Biol. Sci. 16 : 3, 616-28.

Greenway, H. (1965). - "Plant response to saline media". Ph.D. thesis University of Adelaide, South Australia.

Greenway, H. and Thomas, D.A. (1965). - Plant response to saline substrate v. chloride regulation in the individual organs of Hordeum vulgare during treatment with sodium chloride. Aust. J. Biol. Sci. 18 : 505-524.

Greenway, H. and Pitman, M.G. (1965). - Potassium retranslocation in seedlings of H. vulgare. Aust. J. Biol. Sci. 18 : 235-247.

Groenewegen, H. and Mills, J.A. (1960). - Uptake of mannitol into the shoots of intact barley plants. Aust. J. Biol. Sci. 13 : 1-4.

Handley, Raymond; Metwally, A. and Overstreet, Roy (1965) - Divalent cations and the permeability to Na of the root meristem of Zea mays. Plant and Soil 22 : 200-206.

Hayward, H.E. and Bernstein, L. (1958). - Plant growth relationship on salt affected soils. Bot. Rev. 24 : 584-635.

Hayward, H.E. and Wadleigh, C.H. (1949). - Plant growth on saline and alkali soils. Advanc. Agron. 1 : 1-38.

Heilbrunn, L.V. (1952). -"An outline of general physiology" 3rd ed. Saunders, Philadelphia.

Heimann, H. (1958). - Irrigation with saline water and the ionic environment. In "Potassium symposium". Annu. Mtg. Bdt. Tech. Advisor, Madrid. pp. 173-220 (Int. Potash Inst. Berne).

Heimann, H. and Ratner, R (1961). - The influence of potassium on the uptake of sodium by plants under saline conditions. Bull. Res. Coun. Israel A 10 : 55-62.

Höber, R. (1945). -"Physical chemistry of cells and tissues". Blakistan, Philadelphia.

Hoblyn, T.N. (1940). - Manural trials with apple trees at East Malling, 1920-1939. J. Pomol. and Hort. Sci. 18 : 325-343.

Holmes, M.R.J. (1962). - Field experiments on the Mg requirement of potatoes in Great Britain. J. Agr. Sci. 58 : 281-285.

Hyder, S.Z. and Greenway, H. (1965). - Effect of Ca^{++} on plant sensitivity to high NaCl concentrations. Plant and Soil 23 : 258-260.

Itallie, Th. B. Van (1937). - Mg mangel und Ionenverhält-nisse in Gettreidepflanzen Bodenkunde und Pflanzenernährung 5 : 303-333.

Jacobson, L., Overstreet, R., King, H.M. and Handley R. (1950). - A study of K absorption by barley roots. Plant Physiol. 25 - 639-647.

Kahn, J.S. and Hanson, J.B. (1957). - The effect of calcium on potassium accumulation in corn and soya bean roots. Plant Physiol. 32 : 312-316.

Kelley, W.P. (1951). - "Alkali soils, their formation, properties and reclamation". (Reinhold Publ. Co.: New York).

Kramer, P.J. and Wiebe, H.H. (1952). - Longitudinal gradient of P^{32} absorption in roots. Plant Physiol. 27 : 661-674.

Lagerwerff, J.V. and Gen. Ogata (1960). - Plant growth as function of interacting activities of water and ions under saline conditions. 7th Intern. Congress of Soil Science, Madison, Wisc., U.S.A. Transc. vol.III pp. 475-479.

Lagerwerff, J.V. and Eagle, H.E. (1961). - Osmotic and specific effects of excess salts on beans. Plant Physiol. 36 : 472-477.

Leggett, J.E., Galloway, R.A. and Gauch, H.G. (1965). - Calcium activation of orthophosphate absorption by barley roots. Plant Physiol. 40 : 897-902.

Long, W.G., Sweet, D.V. and Tukey, H.B. (1956). - Loss of nutrient from plant foliage by leaching as indicated by radio-isotopes. Science 123 : 1039-1040.

Loughman, B.C. and Martin, R.P. (1957). - Methods and equipment for the study of the incorporation of phosphorus by intact barley plants in the experiment of short duration. J. Expt. Bot. 8 : 272-279.

Loughman, B.C. and Russell, R.S. (1957). - The absorption and utilization of phosphate by young barley plants. IV. The initial stages of phosphate metabolism in roots. J. Expt. Bot. 8 : 280-293.

Magistad, O.C. (1945). - Plant growth relations on saline and alkali soils. Bot. Rev. 11 : 181-230.

Marschner, H.and Günter, I. (1964). - Ion uptake in relation to cell structure of barley roots as affected by calcium supply. Z. Pflanzenernähr Düng, Bodenkunde 107 : 118-136.

Marinos, N.G. (1962). - Studies on sub-microscopic aspects of mineral deficiencies I. Calcium deficiency in the shoot apex of barley. Amer. Jour. Bot. 49 : 834-841.

Marsh, B.B. (1959). - The estimation of inorganic phosphate in the presence of adenosine triphosphate. Biochem. Biophys. Acta 32 : 357-361.

Matukhin, G.R. and Boiko, L.A. (1957). - Chloride content of plants in the processes of adoption to soil salinity. Uchen. Zap. Rostov-na Donu Univ. 28 : 79-84.

May, L.H., Chapman, H. and Aspinall, D. (1965). - Quantitative studies of root development. I. The influence of nutrient concentration. Aust. J. Biol. Sci. 18 : 25-35.

Mazia, D. (1940). - Binding of ions by cell surface. Cold Spring Harbor Symp. Quant. Biol. 8 : 195-203.

Middleton, L.F. and Russell, R.S. (1958). - The interaction of cations in absorption by plant tissues. J. Expt. Bot. 9 : 115-127.

Millington, A.J., Buroill, G.H. and Marsh, B. (1951). - Salt tolerance, germination and growth tests under controlled salinity conditions. Jour. Agric. W. Aust. 23 : 198-210.

Moulder, A.G. (1956). - N-Mg relationships in crop plants. Plant and Soil 7 : 341-376

Nieman, R.H. (1965). - Expansion of bean leaves and its suppression by salinity. Plant Physiol. 40 : 156-161.

Overstreet, R., Jacobson, L. and Handley, R. (1952). - The effect of Ca on the absorption of K by barley roots. Plant Physiol. 27 : 583-590.

Palfi, G. (1965). - The effect of sodium salts on the nitrogen, phosphorus, potassium, sodium and amino acid contents of rice shoots. Plant and Soil 22 : 127-135.

Pearson and Bernstein (1958). - Influence of exchangeable sodium on yield and chemical composition of plants. II. Wheat, barley, oats, rice tall fescue and tall wheat grass. Soil Sci. 86 : 254-261.

Pitman, M.G. (1965). - Sodium and potassium uptake by seedlings of Hordeum vulgare. Aust. J. Biol. Sci. 18 : 10-24.

Reifenberg, A. and Rosovsky, R. (1947). - Saline irrigation water and its effect on the intake of ions by barley seedlings. Palest. J. Bot. Jerusalem 4 : 1-13.

Seifriz, W. (1949). - Toxicity and chemical properties of ions. Science 110 : 193-196.

Shakhov, A.A. (1956). - Salt resistance of plants
Moskva Akad. Nank. SSSR 551

Slatyer, R.O. (1961). - Effect of several osmotic substrates on water relationship of tomato.
Aust. J. Biol. Sci. 14 : 519-540.

Smit, J. and Mulder, E.G. (1942). - Mg deficiency as the cause of injury in cereals. Meded. Landbouwhogeschool, Wageningen 46, No.3 : 3-43

Smith, F.C. and Epstein, E. (1964). - Ion absorption by shoot tissue: Technique and first findings with excise leaf tissue of corn. Plant Physiol. 39 : 338-341.

Sutcliffe, J.F. (1957). - The selective absorption of alkali cations by storage tissues and intact barley plants. In "Potassium Symposium, 1956". Ann. Mtg. Bd. Tech. Advisors London. pp. 1-11 (Int. Potash Inst.: Berne)

Tanda, T. (1955). - Effect of ultraviolet radiation and Ca on their interaction on salt absorption by excised mung bean roots. Plant Physiol. 30 : 221-225.

Thoday, D. (1918). - Some observations on the behaviour of turgescent tissue in solution of cane sugar and certain toxic substances. New Phytologist 17 : 57-68.

Toniolo, L., de Poli, E. (1958). - Contribution to the study of the behaviour of various crops on saline soils.
Agr. delle Venezie 12 : 260-283.

Uhvits, Rachell (1946). - Effect of osmotic pressure on water absorption and germination of alfalfa seeds.
Amer. J. Bot. 33 : 278-285.

U.S. Salinity Laboratory Staff (1954). - Diagnosis and improvement of saline and alkali soils. U.S. Dept. Agr. Handb. No.60

Viets, F.G. Jr. (1944). - Ca and other polyvalent cations as accelerators of ion accumulation by excised barley roots.
Plant Physiol. 19 : 466-480.

Wadleigh, C.H. and Ayers, A.D. (1945). - Growth and biochemical composition of bean plants as conditioned by soil moisture tension and salt concentration.
Plant Physiol. 20 : 106-32.

Wadleigh, C.H. and Bower, C.A. (1950). - The influence of Ca ions activity in water culture on the intake of cations by bean plants. Plant Physiol. 25 : 1-12.

Walker, A.D. (1957). - Biochem. J. 67 : 73-79.

Walsh, T. (1945). - The effect on plant growth of substituting strontium for calcium in acid soils. Proc. Roy. Irish Acad. B50 : 287-294.

Wiebe, H.H. and Kramer, P.J. (1954). - Translocation of radioactive isotopes from various regions of roots of barley seedlings. Plant Physiol. 29 : 342-348.

Williams, R.F. (1955). - Redistribution of mineral elements during development. Annu. Rev. Pl. Physiol. 6 : 25-42.

Wilson, A.M. and Huffaker, R.C. (1964). - Effect of moisture stress on acid soluble phosphorus compounds in Trifolium subterraneum. Plant Physiol. 39 : 555-560.

Yemm, E.W. and Cocking, E.C. (1955). - The determination of amino acids with ninhydrin. Analyst 80 : 209-212.

Zimmerman, M.H. (1960). - Transport in the phloem. Annu. Rev. Pl. Physiol. 11 : 167-90.

Hyder, S. Z., & Greenway, H. (1965). Effects of Ca^{++} on plant sensitivity to high NaCl concentrations. *Plant and Soil, 23*(2), 258-260.

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