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EFFECT OF CALCIUM ON PERMEABILITY OF ROOTS OF PLANTS GROWN AT HIGH SODIUM CHLORIDE CONCENTRATION

S. Z. HYDER

Botany Department, Sind University, Jamshoro

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Effect of calcium on loss of inorganic and organic ions from the *Hordeum vulgare* was studied at germination and at the first leaf stage when these plants were grown in low nutrient solution containing high sodium chloride. The saline treatments resulted in greater loss of amino acids, Na^{24}, Cl^{36} and K labelled with Rb from plants receiving low Ca^{++} than from plants receiving high calcium in their growth medium. At low calcium, the greater loss of ions was due to increase in permeability of plant cells in this treatment.

The concentration of some inorganic ions in the growth medium is an important factor in counteracting the adverse effects of salinity on plant growth. Heimann[4] emphasised the importance of potassium in counteracting the deleterious effects of high sodium. Greenway[3] tested the effects of high NaCl on the growth of barley in water culture at two levels of nutrient concentration. He noted that at 50 m-equiv/l NaCl growth of barley was reduced more at low nutrient concentrations i.e. 1/40 strength Hoagland solution than at full strength Hoagland solution. This adverse effect at low nutrient where calcium was at 0.2 m-equiv/l was much less prominent when extra calcium was added to the medium.[3,7]

In the present experiment the calcium–sodium interaction was studied by measuring permeability of root treated with high doses of NaCl at low and high calcium level in their nutrient solution.

Methods and Materials

General

Experiments were done with *Hordeum vulgare* both during germination and at the first leaf stage. In germination experiments the seeds were germinated in the dark on nylon mesh suspended over the medium. The growth room temperature was at 22°C and the atmosphere around the seeds was kept humid by placing petri dishes over the culture dishes. These petri dishes were removed when root development had satisfactorily advanced. In experiments at the first leaf stage, seeds were sown in river sand in the glass house and transplanted five days after sowing to 3 l culture dishes containing 1/40 strength nutrient solution. Each dish had 15 plants. The nutrient solution contained in m-equiv/l Ca^{++} 8; $Mg^{++}4$, $K^{+}5$: NH_4^{+} 2; NO_3^{-} 13; $SO_4^{-}4$: $H_2PO_4^{-}2$. Micro-elements were added as described by Arnon and Hoagland. Saline treatments were imposed when the first leaf had fully developed. These consisted of addition of 50 m-equiv/l NaCl to the plants under following conditions:

(a) Nutrient solution at 1/40 strength with Ca^{++} at 0.2 m-equiv/l, (b) as under (a) but receiving an additional 0.6 m-equiv/l Ca^{++} thus increas ing Ca^{++} to 0.8 m-equvi/l. These two levels of calcium in nutrient solution containing NaCl will be designated in the text as low Ca^{++} and high Ca^{++} respectively. Control consisted of 1/40 nutrient solution with no NaCl. Usually the treatments lasted for 4 to 5 days.

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. Potassium was labelled with Rb^{86}. 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 124/01 liquid Geiger Muller tube. The plant tissues were treated with boiling dil HNO_3 to extract Rb^{86} and Na^{24}. Total counts for each sample were minimally 1000 disintegrations and at high counting rates counting was continued for at least 5 min. The counts were converted to micro-equivalents per gram of fresh or dry weight.

Sampling of Plant Material

After the period of tracer absorption plants were rinsed for 3 min in cold (nonlabelled) 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.

Measurement of Permeability

Permeability in plant roots was measured by:

1. Feeding the labelled ions to the plants and determining their loss to the nonlabelled solution.

2. Determining the loss of naturally occurring metabolites (amino acids) from the seeds to the culture solution.

Individual Experiments

Amino Acid Loss from Germinating Seeds (Expt. 1).—This experiment was done at germination stage. Fifty seeds were placed at nylon mesh suspended over the culture solution in 150 ml dishes. Treatments consisted of 0 and 80 m-equiv/l NaCl in the nutrient solution containing high and low calcium. There were two culture dishes in each treatment. Fresh solution was replaced in each culture dish after every 24 hr.

The loss of amino acids of the seeds was determined by estimation of amino acids in the culture solution in which seeds were grown for 24 hr. The culture solution was reduced to 2 ml and amino acids were quantitatively estimated by the ninhydrin method.[2] The experiment was continued for 5 days after commencement of treatment.

Na^{24} *Loss at Germination* (Expt. 2).—Treatments were the same as for the amino acids (Expt. 1.) except control treatment. 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 hr and then transferred to nonlabelled solution. The loss of tracer was determined by counting the culture solutions as well as by tissue analysis at 2, 3, 5, 8 hr after tracer removal.

Cl^{36} *Loss at First Leaf Stage* (Expt. 3).—This experiment was done during the first leaf stage. NaCl, 50 m-equiv/l, at low and high calcium was labelled with Cl^{36} and treatments were continued for 80 hr. There were 3 replicates of 4 plants in each treatment. Seventy two hours after tracer application one replicate was harvested. The remaining two replicates were transferred to nonlabelled solution and Cl^{36} loss from these plants was measured at intervals of 1, 3, 5, and 8 hr. Eighty hours after tracer application these plants were also harvested. These harvests were made for determining Cl^{36} content in the tissues.

K^+ *Loss at First Leaf Stage* (Expt. 4).—This experiment was done at the first leaf stage. NaCl was at 0 and 50 m-equiv/l at low and high calcium. There were eight replicates of 4 plants for each treatment. Plants were treated with NaCl for 5 days before transferring them to the tracer solution. Two replicates of each treatment were harvested prior to tracer application. The rest of the plants were transferred to the respective treatment solution in which potassium was labelled with Rb^{36}. The uptake was measured by harvesting 2 replicates of each treatment at intervals of 4, 16 and 24 hr. After the absorption period, the loss of tracer was determined by transferring 3 replicates from each treatment to nonlabelled solutions. These nonlabelled solutions were replaced at 2, 5, 16 and 24 hr.

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

Results

Amino Acids Loss (Expt. 1).—In all treatments amino acid losses were high at first but decreased with time after the first day. Losses of amino acids were much higher from low than from either control or high calcium (Fig. 1).

Na^{24} *Loss* (Expt. 2).—Sodium loss to the solution after 4 days of NaCl treatment was greater at low than at high calcium at all sampling occasions (Fig. 2). This loss at low calcium was more prominent in the first 4 hr of loss measurements. At high calicum Na^{24} loss to thes olution which increased slowly during the first 4 hr did not increase further in the later period. The losses measured in solutions were in good agreement with those measured by counting of the tissues (Fig. 3).

Cl^{36} *Loss* (Expt. 3).—At both levels of calcium, the rate of Cl^{36} loss to the solution after 3 days of labelling was greater in the first hour than during the subsequent hours (Fig. 4). Low calcium plants lost more Cl^{36} than plants high in calcium. Table 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 transfer to non-radioactive solutions and 8 hr later were plotted (Fig. 5). This figure shows 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. The rise of Cl^{36} in shoot of only high calcium plants may be due to the fact that Cl^{36} absorbed by the roots was translocated up in the shoot while it was lost to external medium in low calcium plants.

TABLE 1.—Cl^{36} LOSS IN M-EQUIV/G DRY WEIGHT MEASURED OVER 1 TO 8 HR AFTER Cl^{36} REMOVAL.

Treatments	1 hr	3 hr	5 hr	8 hr
High Ca++	3	5.9	6.0	14.5
Low Ca++	2.7	6.6	12.7	19.1

Ca++ 0.8 m-equiv/l NaCl 50 m-equvi/l (High Ca)
Ca++ 0.2 m-equiv/l NaCl 50 m-equvi/l (Low Ca)

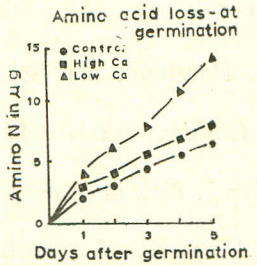

Fig. 1

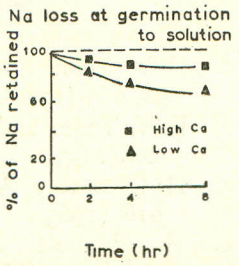

Fig. 2

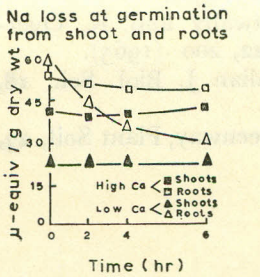

Fig. 3

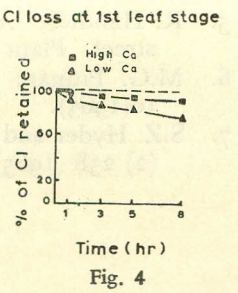

Fig. 4

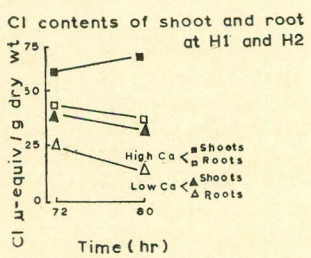

Fig. 5

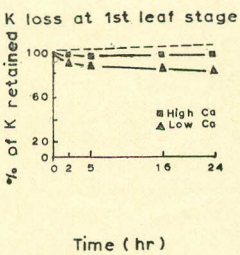

Fig. 6

K^+ *Loss* (Expt. 4).—The rate of loss of rubidium from plants, after treating plants with NaCl for 5 days, was high initially at both levels of calcium, but slowed later and became linear with time (Fig. 6). 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.

Discussion

The results presented in this paper show clearly that when treated with high NaCl there was a greater loss of ions from low calcium than from high calcium or control plants. One effect of increased permeability of root cells might be growth reduction due to loss of organic and inorganic ions. The reduced growth observed in low calcium plants[7] could have been due to such loss of metabolites. The loss of amino acids is an indication of disturbed metabolic conditions at low calcium. As shown in Fig. 1 substantial amount of amino acids were lost from low Ca^{++} plants even after one day of NaCl application and presence of high calcium in the growth medium had a protective effect against these losses. This observation suggests that in low calcium plants impaired cell synthesis prevented the utilization of amino acids and contributed to their 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.

The loss of inorganic nutrient from plants due to increased permeability has been demonstrated by greater loss of potassium, chloride and sodium ions (Fig. 6, 4, and 2). In the experiment on potassium loss, plants were first treated with NaCl for 5 days, both at high and at low calcium level, before Rb^{86} application for potassium loss measurement. The 5 days pretreatment with NaCl had considerably damaged the roots cell, so that the tracer absorbed in 24 hr after pretreatment was lost more rapidly from low than from high calcium plants.

In the Cl^{36} loss experiments, plants were grown 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 hr period was 19 and 14.5 micro-equivalents respectively (Table 1). Pitman[6] 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 low Ca^{++} high NaCl roots must have had a considerable uptake. 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 Calcium than at low calcium.

The results of sodium loss at low calcium are in agreement with those reported by Handley *et al.*[5] They found that loss of perviously 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 electrical 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 are non essential ions for nonhalophytes and tend to accumulate in the vacuole. Losses of these ions indicate 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 because it is well-protected by the surrounding solution.

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 high NaCl treatments could also be due to the reduced protein synthesis.

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