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## The effect of low concentrations of sodium on potassium uptake and growth of wheat

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**Abstract.** Sodium is a beneficial mineral for some plant species when external concentrations are low. The role of Na<sup>+</sup> in energising K<sup>+</sup> acquisition in terrestrial plants has recently been suggested because of evidence demonstrating that wheat root cells express a high-affinity Na<sup>+</sup>-energised K<sup>+</sup> symporter. To determine whether low concentrations of Na<sup>+</sup> improve the K<sup>+</sup> nutrition and growth of wheat, long-term growth and short-term tracer flux experiments were conducted. Long-term growth experiments were conducted over a range of K<sup>+</sup> concentrations, at acidic and alkaline pH, with and without 500 μM NaCl. Plant biomass and tissue Na<sup>+</sup> and K<sup>+</sup> content was measured. Short-term experiments were conducted using tracers to determine whether low concentrations of Na<sup>+</sup> or K<sup>+</sup> stimulate Rb<sup>+</sup> or Na<sup>+</sup> uptake, respectively. Sodium stimulated the growth of wheat only at low (20 μM) external K<sup>+</sup> in one of the long-term experiments, but not in two other experiments. Na<sup>+</sup> did not stimulate Rb<sup>+</sup> uptake, but K<sup>+</sup> stimulated Na<sup>+</sup> uptake in short-term tracer flux experiments. The results suggest that low concentrations of Na<sup>+</sup> do not increase K<sup>+</sup> uptake to a large extent, and only when light levels are low does Na<sup>+</sup> have a beneficial effect on the growth of wheat.

**Keywords:** high-affinity, potassium, sodium, uptake, wheat.

### Introduction

Even though the amount of K<sup>+</sup> required for optimal growth is very low (Asher and Ozanne 1967), plants must be able to adapt to soil conditions where potassium concentrations may limit growth. Plants may adapt to changes in soil solution ion concentrations by the deployment of additional K<sup>+</sup> uptake mechanisms (Glass and Siddiqi 1984; Fernando *et al.* 1992; Wang *et al.* 1998) or may take up other more readily available minerals such as Na<sup>+</sup>. The use of Na<sup>+</sup> by plants may be particularly important in saline soils where K<sup>+</sup> and Na<sup>+</sup> often compete for uptake (Schachtman and Liu 1999).

Sodium is a mineral that may be essential (Brownell 1979) or toxic for plant growth (Munns 1993). In some C<sub>4</sub> plant species, Na<sup>+</sup> is an essential micronutrient (Brownell and Crossland 1972) whereas in other plant species it is not required for plant growth, but may be beneficial (Montasir *et al.* 1966; Leigh *et al.* 1986). The beneficial role of Na<sup>+</sup> is thought to be in replacing potassium (Flowers and Läuchli 1983) in non-specific physiological and biochemical processes.

Plant roots have a number of independent K<sup>+</sup> uptake mechanisms that operate in parallel to ensure plants have an adequate supply of this essential macronutrient. At high external concentrations, K<sup>+</sup> channels (Kochian and Lucas 1982; Schroeder and Fang 1991) are important for uptake and, at low external K<sup>+</sup> concentrations, energised high-affinity K<sup>+</sup> uptake systems are required to accumulate K<sup>+</sup> against the electrochemical gradient. To maintain a steady state flux

of K<sup>+</sup> (Glass and Siddiqi 1984) plant roots may alter the expression of various K<sup>+</sup> uptake mechanisms. At least two different high-affinity K<sup>+</sup> uptake mechanisms have now been shown to be induced by K<sup>+</sup> starvation (Fernando *et al.* 1990; Santa-Maria *et al.* 1998; Wang *et al.* 1998). One of the high-affinity K<sup>+</sup> uptake mechanisms (Schachtman and Schroeder 1994) that is derepressed when external K<sup>+</sup> concentrations are low is a Na<sup>+</sup>-coupled high-affinity K<sup>+</sup> symport HKT1 (Rubio *et al.* 1995). The K<sub>m</sub> for sodium of this transporter is below 500 μM (Gassmann *et al.* 1996; Diatloff *et al.* 1998) and therefore a Na<sup>+</sup>-coupled K<sup>+</sup> uptake mechanism may play an important role in K<sup>+</sup> acquisition when external Na<sup>+</sup> concentrations are low.

The presence of Na<sup>+</sup> as the energising ion for high-affinity K<sup>+</sup> acquisition may provide several advantages for plants growing in K<sup>+</sup>-depleted conditions. Sodium can partially replace K<sup>+</sup> in its contribution to the osmotic potential of the cell and in the generation of turgor (Flowers and Läuchli 1983). Therefore, the activity of a Na<sup>+</sup>-coupled high-affinity K<sup>+</sup> transporter, may provide plant cells with the essential macronutrient K<sup>+</sup>, and the beneficial osmoticum, Na<sup>+</sup>. In addition, Na<sup>+</sup>-coupled K<sup>+</sup> uptake systems may have physiological significance under alkaline conditions (Walker and Sanders 1991; Maathuis *et al.* 1996) when the flux through a H<sup>+</sup>-energised transporter would be significantly reduced, such that a Na<sup>+</sup>-coupled transporter may become essential for K<sup>+</sup> acquisition.

While there is evidence for Na<sup>+</sup>-coupled uptake systems

in aquatic macrophytes (Smith and Walker 1989; Walker *et al.* 1993), the search for Na<sup>+</sup>-energised K<sup>+</sup> transport activity in higher plants, using a limited range of radioactive tracer flux analyses and electrophysiological transport assays, has been unsuccessful (Maathuis and Sanders 1994; Maathuis *et al.* 1996). Therefore this study focused on determining whether low concentrations of Na<sup>+</sup> play a role at the whole plant level, over a long time period. We also extended previous research that has used tracer flux techniques, by examining Rb<sup>+</sup> influx at different pH levels, the uptake of Rb<sup>+</sup> along the root profile and the effect of K<sup>+</sup> on Na<sup>+</sup> uptake.

## Materials and methods

### Plant growth conditions

Seeds of *Triticum aestivum* (cv. Spear) were selected within a 10-mg weight range (30–40 mg). The seeds were surface sterilised in a 1% sodium hypochlorite and dilute detergent solution for 5 min and rinsed thoroughly with deionised water. Four d prior to transfer to the light, the seeds were germinated on filter paper with 0.6 mM CaCl<sub>2</sub> in the dark at room temperature. Plants were grown in 50-L hydroponics tanks in a glasshouse. Seedlings were held in 12-mm closed cell foam disks which were inserted into holes in the lids of the hydroponics tanks. Each tank supported a maximum of 40 wheat seedlings.

For both the growth and uptake experiments, plants were grown on a complete nutrient medium (with the exception of the specified Na<sup>+</sup> and K<sup>+</sup> concentrations) equivalent to 1/4 strength modified Hoagland's solution: 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>·H<sub>2</sub>O, 0.25 mM NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, plus micronutrients [Fe-citrate (FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·5H<sub>2</sub>O) (100 μM), H<sub>3</sub>BO<sub>3</sub> (4.6 μM), MnCl<sub>2</sub>·4H<sub>2</sub>O (0.5 μM), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.2 μM), (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O (0.1 μM) and CuSO<sub>4</sub>·5H<sub>2</sub>O (0.2 μM)]. The pH of the unbuffered nutrient solution varied between pH 5 and 5.5. The solutions were continuously aerated and the air lines were fitted with a 5 μm pre-filter (AF 3000-03) and a 0.1 micro-mist separator (AFD 3000; SMC Pneumatics) to eliminate oil contamination.

### Growth experiments

Two growth experiments were performed in the unbuffered 1/4 strength Hoagland's solution. In the first experiment, plants were grown over 32 d (March/April 1997; 499 MJ m<sup>-2</sup>, 9.5–11 h d<sup>-1</sup>) in 2000, 100 or 20 μM KCl in the presence or absence of 500 μM NaCl. In the second experiment, plants were grown in 20 μM KCl in the presence or absence of 500 μM NaCl over a longer (42 d) growth period (May/June 1997; 326 MJ m<sup>-2</sup>, 8–8.5 h d<sup>-1</sup>).

In the third growth experiment (March/April 1998, 9.5–11 h d<sup>-1</sup>), plants were grown over 40 d in 2000, 100 or 20 μM KCl in the presence or absence of 500 μM NaCl in solutions that varied between pH 8 and 9. Ca(OH)<sub>2</sub> was added daily to increase the pH of the solutions to pH 9. Over a 24-h period the plants were capable of changing the pH of the nutrient solutions to 8. The pH of the nutrient solutions was regularly monitored and the solutions were supplemented daily with Ca(OH)<sub>2</sub>. The maximum Ca<sup>2+</sup> concentration, resulting from the addition of Ca(OH)<sub>2</sub>, was 1.2 mM.

In all growth experiments, the nutrient solutions were changed once each week. Glasshouse temperatures were maintained at 23°C during the day and 18°C at night. The K<sup>+</sup> concentration was regularly monitored using flame photometry and the solutions were supplemented to the designated K<sup>+</sup> concentration every 3 d. Although plant biomass increased over time, seedlings were continuously harvested such that the rate of K<sup>+</sup> depletion did not significantly change over the period of growth. The Na<sup>+</sup> concentration of the 'Na<sup>+</sup>-free' treatments was 5–10 μM Na<sup>+</sup>, well below the K<sub>m</sub> of Na<sup>+</sup> in the wheat root high-affinity K<sup>+</sup> transporter, HKT1 (K<sub>m</sub> = 175–450 μM).

In the first growth experiment (32 d), plants from each of the six treatments were harvested at 6, 13, 20 and 32 d; in the second growth experiment (42 d), plants were only harvested twice, at 20 and 42 d; and in the third growth experiment (40 days), plants were harvested at 6, 13, 20 and 40 d. The roots and shoots were separated and the fresh weights measured. Roots were desorbed in 10 mM CaCl<sub>2</sub> for 10 min and the shoots were rinsed in deionised water to remove surface salts. Samples were oven-dried at 70°C, transferred to a desiccator for cooling and the dry weights measured.

Ions were extracted from dried root and shoot samples with 0.5 M hydrochloric acid (Hunt 1982). Finely-chopped samples were transferred to acid-washed plastic beakers at a maximum ratio of 0.1 g per 50 mL of 0.5 M acid and heated for 2 h in a 50°C oven. The Na<sup>+</sup> and K<sup>+</sup> concentrations of the sample solutions were determined using flame photometry (Corning, Model 410). Tissue ion concentrations, in mM, were determined by dividing the total tissue K<sup>+</sup> or Na<sup>+</sup> content by the plant water content, calculated from the difference in the total fresh weight and dry weight.

### Tracer flux analysis

Wheat seedlings were grown in the 1/4 strength modified Hoagland's solution in the glasshouse and intact seedlings 6–8-d-old, which had two leaves, were used for all tracer flux experiments. Uptake experiments were performed in aerated 125-mL solution vials and, with the exception of the specified K<sup>+</sup>, Na<sup>+</sup> or Rb<sup>+</sup> concentrations, all the solutions were equivalent to the 1/4 strength modified Hoagland's growth solution.

For the Rb<sup>+</sup> uptake experiments wheat seedlings were grown in solutions with and without 500 μM Na<sup>+</sup> and 2000, 100 or 20 μM K<sup>+</sup>. Plants were first transferred to a complete nutrient solution of the same K<sup>+</sup> and Na<sup>+</sup> composition as the growth solution, buffered to pH 6 (0.5 mM Ca(OH)<sub>2</sub>, 1.5 mM MES) or pH 9 (0.5 mM Ca(OH)<sub>2</sub>, 1.5 mM CHES) for 1 h. Following the 1-h adaptation, plants were desorbed for 10 min in a buffered solution without K<sup>+</sup> or Na<sup>+</sup> to remove the cell wall contents. Plants were then transferred to a buffered radioactive solution for 20 min which contained Rb<sup>+</sup> (as RbCl) in concentrations equivalent to the K<sup>+</sup> concentration of the growth media. Approximately 8 μCi of <sup>86</sup>Rb<sup>+</sup> was present in each 125-mL vial. Uptake experiments were performed with or without 500 μM Na<sup>+</sup>. The roots were subjected to two 1-min rinses in a buffered nutrient solution without K<sup>+</sup>, Na<sup>+</sup> or Rb<sup>+</sup>, and then excised from the shoots, blotted and weighed. Root samples were counted in a liquid scintillation counter (Beckman LS 3801) in 4 mL of scintillation fluid. The uptake of Rb<sup>+</sup> was linear over the 20-min period and there was no detectable translocation of Rb<sup>+</sup> to the shoot or significant depletion of Rb<sup>+</sup> from the growth medium over this period.

In addition to the whole-root <sup>86</sup>Rb<sup>+</sup> uptake experiments, the effect of Na<sup>+</sup> on the uptake of Rb<sup>+</sup> along the root was also determined. Wheat plants were grown in 5 or 20 μM K<sup>+</sup> in the presence of 500 μM Na<sup>+</sup> and uptake experiments were performed in pH 6 solutions containing 5 or 20 μM Rb<sup>+</sup>, with or without 500 μM Na<sup>+</sup>. The general method for desorption, uptake and rinsing was as described for the whole-root uptake experiments. Following the rinses, the two longest seminal roots were selected from the root system of each plant and the roots were cut into 1-cm segments from the root tip up to the point where the roots began branching. Each 1-cm segment was paired with the other root and the two segments were counted in a scintillation vial, as described above.

For the Na<sup>+</sup> uptake experiments wheat seedlings were grown in the 1/4 strength modified Hoagland's solution with 5 μM K<sup>+</sup> and 500 μM Na<sup>+</sup>. Plants were first transferred to a Na<sup>+</sup>- and K<sup>+</sup>-free unbuffered desorption solution for 10 min followed by a 20-min uptake period. Uptake solutions were buffered to pH 6 and contained 200 μM Na<sup>+</sup> and 0, 5 or 20 μM K<sup>+</sup>. Each 125-mL uptake vial contained approximately 1 μCi of <sup>22</sup>NaCl and the uptake of Na<sup>+</sup> was linear over the 20-min period. After the uptake period, the seedlings were rinsed, weighed and

counted in a liquid scintillation counter, as described for the whole-root <sup>86</sup>Rb<sup>+</sup> uptake experiments.

#### Statistical analysis

Analysis of variance was performed using JMP statistical software for Apple Macintosh from the SAS Institute Inc. When more than one factor was tested, a Tukey Kramer HSD Test was used to compare the differences between means.

## Results

### Wheat growth

#### Experiment 1

In the first experiment there were no differences in the dry weights of the wheat seedlings up to 20 d across the three external K<sup>+</sup> concentrations (Fig. 1a). After 20 d, the growth rate of the plants in all treatments increased. By 32 d, the total dry weight of the wheat grown in 20 μM K<sup>+</sup> was significantly lower than the wheat grown in 100 or 2000 μM K<sup>+</sup> ( $P < 0.001$ ), but there was no difference in the total biomass of plants grown in 100 and 2000 μM K<sup>+</sup>. In this experiment the external K<sup>+</sup> concentration had the same effect on root and shoot growth, when analysed separately (data not shown). The addition of 500 μM external Na<sup>+</sup> did not lead to a significant enhancement of root, shoot (data not shown) or total biomass (Fig. 1a) at any of the external K<sup>+</sup> concentrations that were used.

#### Experiment 2

In a second experiment at low pH, wheat plants were grown in 20 μM K<sup>+</sup> (with and without 500 μM Na<sup>+</sup>) for 42 d, to determine if external Na<sup>+</sup> influenced plant growth over a longer time period. At 20 d there was no difference in the total dry weight of wheat grown in the presence or absence of Na<sup>+</sup> (Fig. 1b). At 42 d plants grown with 500 μM Na<sup>+</sup> accumulated significantly more dry weight ( $P < 0.004$ ) than wheat grown without Na<sup>+</sup> (Fig. 1b).

#### Experiment 3

In a third growth experiment, wheat plants were grown at high pH over a 40-d period, to determine if external Na<sup>+</sup> enhanced wheat growth when H<sup>+</sup> concentrations were reduced. The effect of the external K<sup>+</sup> concentration on wheat growth was similar to the trend observed in experiment 1, with no differences in dry weight until the final harvest (Fig. 1c). At 40 d, the total dry weight of wheat grown in 20 μM K<sup>+</sup> was significantly lower than in the other K<sup>+</sup> concentrations ( $P < 0.034$ ). As in experiment 1 there was no significant difference between the biomass of plants grown in the presence or absence of external Na<sup>+</sup> at any external K<sup>+</sup> concentration.

### Ion accumulation

The effect of the external K<sup>+</sup> concentration on root and shoot K<sup>+</sup> accumulation over the 32-d growth period of experiment 1 is illustrated in Fig. 2. The concentration of K<sup>+</sup> in the wheat

roots was dependent on the K<sup>+</sup> concentration of the growth solution, and in all treatments the root K<sup>+</sup> concentration declined over time (Fig. 2). Initially (d 6), root K<sup>+</sup> concentrations at the three external K<sup>+</sup> concentrations were significantly different ( $P < 0.001$ ) whereas by d 32, root K<sup>+</sup> concentrations of plants grown at 20 and 100 μM were similar, but lower than in plants grown at 2000 μM K<sup>+</sup>.

The K<sup>+</sup> concentration in the shoots was higher than in the roots of the wheat seedlings for each of the three K<sup>+</sup> treatments (Fig. 2). The shoot K<sup>+</sup> concentration of the wheat grown in 20 μM K<sup>+</sup> was lower than shoot samples from other treatments and decreased at 20 and 32 d. At 20 d, when the external K<sup>+</sup> concentration had no effect on the total dry weight, the K<sup>+</sup> concentration in the roots and shoots of the 20 μM K<sup>+</sup> grown wheat was 15 ± 4 mM and 80 ± 13 mM, respectively. Chlorosis on the tips and margins of the older leaves of the 20 μM K<sup>+</sup>-grown wheat was first observed on d 26 and the severity of the K<sup>+</sup> deficiency symptoms increased to d 32. At the final harvest, when the dry weight of the wheat grown at 20 μM K<sup>+</sup> was significantly lower, the K<sup>+</sup> concentration had been reduced to 6 ± 0.4 mM in the roots and 42 ± 3 mM in the shoots (Fig. 2).

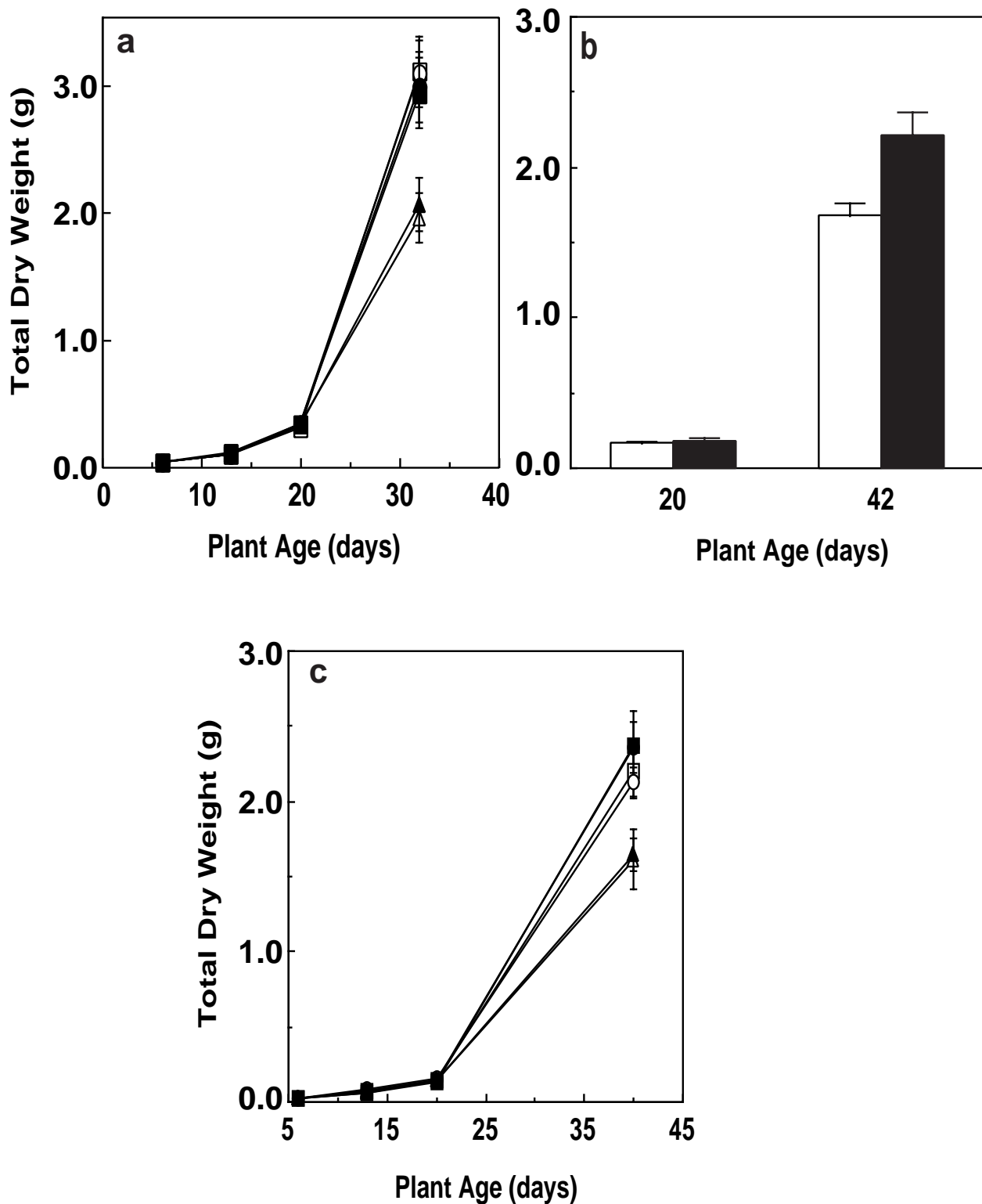
In the first growth experiment sodium accumulated in the roots and to a lesser extent in the shoots of the wheat grown with Na<sup>+</sup> (Fig. 2). The highest Na<sup>+</sup> concentration was observed in the roots and shoots of wheat grown in 20 μM K<sup>+</sup>. By d 32, the internal Na<sup>+</sup> concentration of the wheat grown in 20 μM K<sup>+</sup> had significantly increased, corresponding to the decrease in root and shoot K<sup>+</sup> concentration. The increased internal concentrations of Na<sup>+</sup> had no effect on the plant dry weight, but a significant increase was observed in the fresh weight to dry weight ratios of the wheat shoots grown with 20 and 100 μM K<sup>+</sup> in the presence of 500 μM Na<sup>+</sup> at d 32 [ $P < 0.004$  (20 μM K<sup>+</sup>);  $P < 0.019$  (100 μM K<sup>+</sup>)] (Table 1).

Due to the effect of Na<sup>+</sup> on the fresh weight to dry weight ratio of K<sup>+</sup>-starved wheat, the K<sup>+</sup> concentration of wheat grown in the presence or absence of external Na<sup>+</sup> has also been expressed in mmol per gram of dry weight (Table 2). In experiment 1, the presence of 500 μM Na<sup>+</sup> in the growth

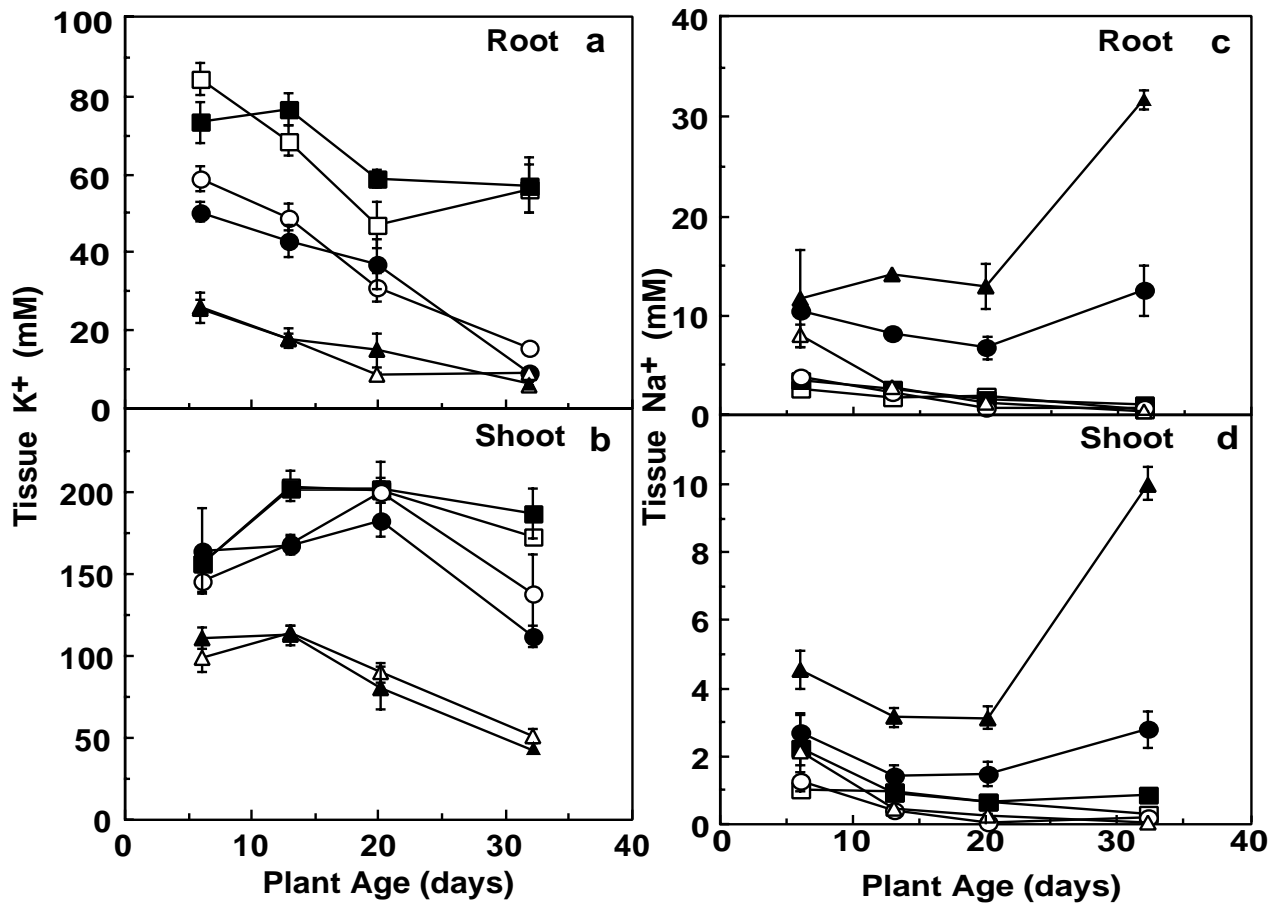
**Table 1. The shoot fresh weight to dry weight ratios of plants grown at different K<sup>+</sup> concentrations in the presence or absence of 500 μM Na<sup>+</sup>**

Plants of *Triticum aestivum* cv. Spear were grown in 20, 100 and 2000 μM K<sup>+</sup> at acidic pH (experiment 1) for 32 d. Means ± SE are shown for  $n = 7$  plants. \* indicates that the shoot FW:DW ratio of the plants grown with Na<sup>+</sup> was significantly different from those grown without Na<sup>+</sup> ( $P < 0.05$ )

K <sup>+</sup> Concentration	Shoot FW:DW (minus Na <sup>+</sup> )	Shoot FW:DW (plus Na <sup>+</sup> )
2000 μM	8.23 ± 0.08	8.32 ± 0.03
100 μM	7.66 ± 0.21	8.40 ± 0.17*
20 μM	7.11 ± 0.12	7.97 ± 0.21*



**Fig. 1.** The effect of external  $[\text{K}^+]$  and the presence or absence of  $\text{Na}^+$  on the total dry weight of *Triticum aestivum* cv. Spear. (a) Plants were grown at acidic pH with 2000  $\mu\text{M}$   $\text{K}^+$  minus  $\text{Na}^+$  ( $\square$ ), 2000  $\mu\text{M}$   $\text{K}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  ( $\blacksquare$ ), 100  $\mu\text{M}$   $\text{K}^+$  minus  $\text{Na}^+$  ( $\circ$ ), 100  $\mu\text{M}$   $\text{K}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  ( $\bullet$ ), 20  $\mu\text{M}$   $\text{K}^+$  minus  $\text{Na}^+$  ( $\triangle$ ) and 20  $\mu\text{M}$   $\text{K}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  ( $\blacktriangle$ ). Symbols represent means  $\pm$  SE,  $n = 7$  plants. (b) Plants were grown at acidic pH with 20  $\mu\text{M}$   $\text{K}^+$  minus  $\text{Na}^+$  (open bars) and 20  $\mu\text{M}$   $\text{K}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  (closed bars). Bars represent means  $\pm$  SE,  $n = 8$  plants. (c) Plants were grown at alkaline pH in three external  $\text{K}^+$  concentrations in the presence or absence of 500  $\mu\text{M}$   $\text{Na}^+$  [symbols as in (a)]. Symbols represent means  $\pm$  SE,  $n = 8$  plants.



**Fig. 2.** The effect of external [K<sup>+</sup>] and the presence or absence of Na<sup>+</sup> on the potassium and sodium concentrations of the roots [(a) and (c)] and shoots [(b) and (d)] of *Triticum aestivum* cv. Spear over a 32-d growth period. Plants (from experiment 1) were grown at acidic pH in three external K<sup>+</sup> concentrations in the presence or absence of 500 μM Na<sup>+</sup> (symbols as in Fig. 1).

medium did not increase K<sup>+</sup> concentrations in the shoots or roots of 32-d-old wheat grown in 20 μM K<sup>+</sup> (Table 2). In fact, the K<sup>+</sup> content per gram of dry weight in the roots of these wheat seedlings was slightly, but significantly, lower when grown in the presence of Na<sup>+</sup> ( $P < 0.001$ ). Even in the second growth experiment, where the presence of external Na<sup>+</sup> enhanced wheat growth, there was no evidence for higher K<sup>+</sup> concentrations in the roots or shoots of the 42-d-old wheat plants (Table 2). Similarly in the third experiment at high pH, ion analysis revealed that the presence of external Na<sup>+</sup> did not lead to increased tissue K<sup>+</sup> content (Table 2).

#### Tracer flux analysis

##### The effect of Na<sup>+</sup> on Rb<sup>+</sup> uptake

Wheat plants were grown in 20, 100 or 2000 μM K<sup>+</sup> (plus 500 μM Na<sup>+</sup>) and exposed to an uptake solution of equivalent Rb<sup>+</sup> concentration. Rubidium was used as a tracer because it has been shown to be a suitable tracer for K<sup>+</sup> uptake in some plant species (Epstein *et al.* 1963). In uptake solutions buffered to pH 6, the mean Rb<sup>+</sup> influx rates were between 3.5

and 4 μmol (g root FW)<sup>-1</sup> h<sup>-1</sup> in all three external Rb<sup>+</sup> concentrations (Fig. 3a). The Rb<sup>+</sup> uptake rates remained constant, independent of the K<sup>+</sup> concentration in the growth solution and the Rb<sup>+</sup> or Na<sup>+</sup> concentration in the uptake solution. At pH 9, the average Rb<sup>+</sup> influx into the intact wheat roots from all three K<sup>+</sup>/Rb<sup>+</sup> treatments was less than 0.75 μmol (g root FW)<sup>-1</sup> h<sup>-1</sup> (Fig. 3b). Although the Rb<sup>+</sup> influx rates were significantly reduced in alkaline uptake conditions, there was no measurable stimulation of Rb<sup>+</sup> uptake by the presence of 500 μM Na<sup>+</sup>. The reduction in Rb<sup>+</sup> influx measured during short-term tracer flux experiments (Fig. 3b) was much greater than the growth reduction observed in the whole plant growth experiments at high pH (Fig. 1c). In addition, Rb<sup>+</sup> uptake rates were constant from 20–2000 μM, but tissue K<sup>+</sup> concentrations of 6-d-old wheat (Figs 2b, c) were different between K<sup>+</sup> treatments. This highlights the potential problems in using short-term fluxes to analyse long-term processes. In the low K<sup>+</sup> treatments, there was no evidence for Na<sup>+</sup>-coupled Rb<sup>+</sup> influx into the roots of 6-d-old intact seedlings of wheat.

The results presented in Figs 3a and b are for wheat grown in the presence of 500  $\mu\text{M}$   $\text{Na}^+$ .  $\text{Rb}^+$  uptake experiments were also performed on wheat grown in the absence of added  $\text{Na}^+$  and, while the average influx rates were lower [ $< 2.5 \mu\text{mol} (\text{g root FW})^{-1} \text{h}^{-1}$ ], there was no measurable stimulation of  $\text{Rb}^+$  uptake in the presence of  $\text{Na}^+$ .

As the rate of  $\text{K}^+$  influx has been shown to vary along the longitudinal profile of barley and maize roots (Hanson and Kahn 1957; Eshel and Waisel 1973), so may the activity of  $\text{Na}^+$ - $\text{K}^+$  co-transport mechanisms. The effect of  $\text{Na}^+$  on the uptake of  $\text{Rb}^+$  along the apical 6 cm of wheat grown in 5 and 20  $\mu\text{M}$   $\text{K}^+$  (without  $\text{Na}^+$ ) was examined (Fig. 3c). Although  $\text{Rb}^+$  influx varied along the root length, the presence of 500  $\mu\text{M}$  external  $\text{Na}^+$  had no stimulatory effect on the rate of  $\text{Rb}^+$  uptake along the root profile.

#### The effect of $\text{K}^+$ on $\text{Na}^+$ uptake

$\text{Na}^+$  uptake rates were measured in the presence of 0, 5 or 20  $\mu\text{M}$   $\text{K}^+$  on wheat roots from plants grown in a solution of 5  $\mu\text{M}$   $\text{K}^+$  and 500  $\mu\text{M}$   $\text{Na}^+$  (Fig. 4). The mean  $\text{Na}^+$  influx rate significantly increased from 0.1  $\mu\text{mol} (\text{g root FW})^{-1} \text{h}^{-1}$  to 0.15  $\mu\text{mol} (\text{g root FW})^{-1} \text{h}^{-1}$  upon addition of 5  $\mu\text{M}$   $\text{K}^+$  to the uptake medium ( $P < 0.001$ ). However, there was a distinct reduction in the stimulatory effect of  $\text{K}^+$  on  $\text{Na}^+$  influx in the presence of 20  $\mu\text{M}$   $\text{K}^+$ , such that there was no significant difference in  $\text{Na}^+$  uptake between the control and the 20  $\mu\text{M}$   $\text{K}^+$  treatment.

This experiment was repeated several times and, although  $\text{Na}^+$  uptake was consistently stimulated in the presence of micromolar  $\text{K}^+$ , the stimulation varied in magnitude. Experiments were also conducted with wheat grown in the absence of  $\text{K}^+$  or in the absence of  $\text{Na}^+$ . In wheat grown under both these conditions, the  $\text{Na}^+$  uptake rates were higher and there was no measurable stimulation of  $\text{Na}^+$  uptake by the presence of external  $\text{K}^+$ .

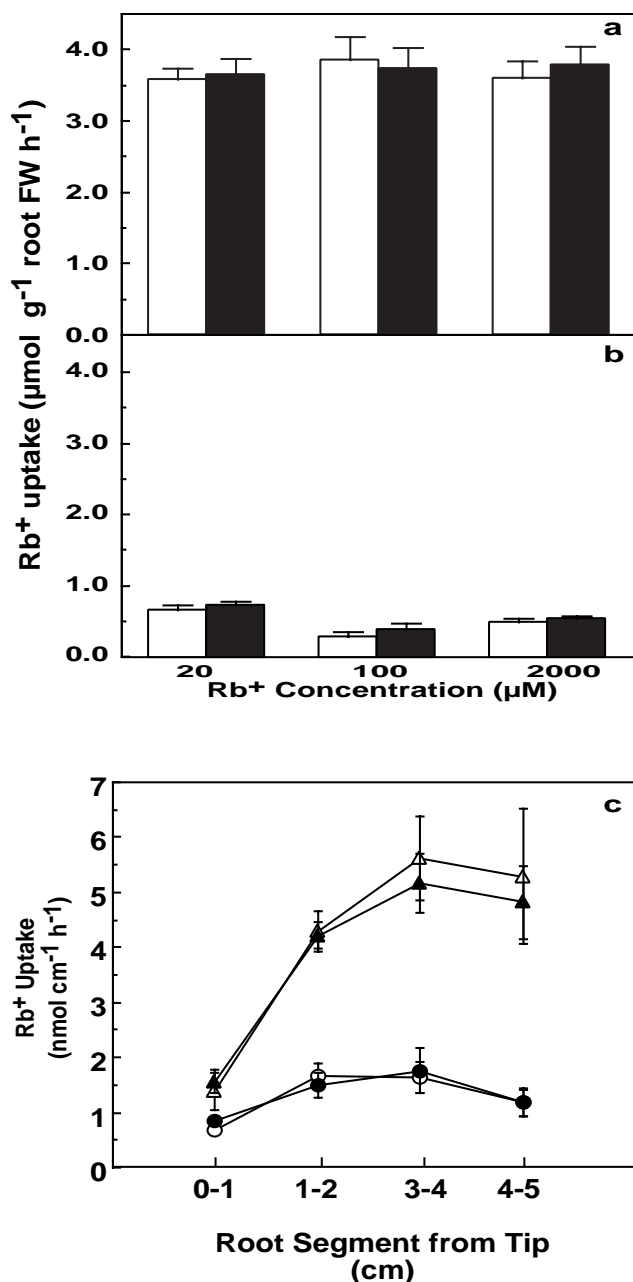
**Table 2.** The  $\text{K}^+$  content of the roots and shoots of wheat grown in 20  $\mu\text{M}$   $\text{K}^+$  with or without 500  $\mu\text{M}$  external  $\text{Na}^+$

In experiment 1, plants were grown at acidic pH and harvested at 32 d. In experiment 2, plants were grown at acidic pH and harvested at 42 d. In experiment 3, plants were grown at pH 8 to 9 and harvested at 40 d. Values represent means  $\pm$  SE,  $n = 7$ –8 plants. \* indicates that the  $\text{K}^+$  concentration of the root or shoot tissue of the plants grown with  $\text{Na}^+$  was significantly different from those grown without  $\text{Na}^+$  ( $P < 0.05$ )

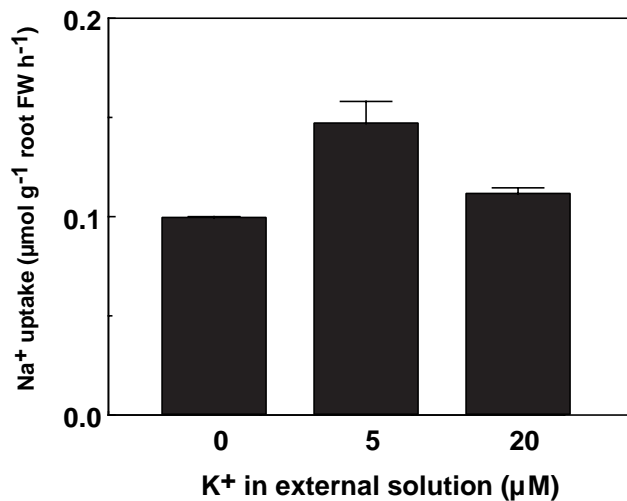
Experiment and treatment		K <sup>+</sup> Content [mmol (g DW) <sup>-1</sup> ]	
		Root	Shoot
Experiment 1	minus Na <sup>+</sup>	0.21 $\pm$ 0.01	0.30 $\pm$ 0.02
	plus Na <sup>+</sup>	0.15 $\pm$ 0.01*	0.28 $\pm$ 0.02
Experiment 2	minus Na <sup>+</sup>	0.16 $\pm$ 0.02	0.36 $\pm$ 0.01
	plus Na <sup>+</sup>	0.12 $\pm$ 0.02	0.37 $\pm$ 0.02
Experiment 3	minus Na <sup>+</sup>	0.11 $\pm$ 0.02	0.43 $\pm$ 0.08
	plus Na <sup>+</sup>	0.09 $\pm$ 0.02	0.40 $\pm$ 0.04

## Discussion

The response of wheat seedlings to the range of external  $\text{K}^+$  concentrations provided insight into the capacity of the root  $\text{K}^+$  uptake mechanisms to supply this essential nutrient. Growth experiments showed that 100  $\mu\text{M}$   $\text{K}^+$  was sufficient for optimum rates of growth, but at 20  $\mu\text{M}$   $\text{K}^+$  wheat could not



**Fig. 3.**  $\text{Rb}^+$  uptake into whole roots of 6-d-old intact seedlings of *Triticum aestivum* cv. Spear.  $\text{Rb}^+$  uptake rates in the absence (open bars) and presence (closed bars) of 500  $\mu\text{M}$   $\text{Na}^+$  at (a) pH 6 and (b) pH 9 and (c) along the first 5 cm of roots in solutions containing 5  $\mu\text{M}$   $\text{Rb}^+$  minus  $\text{Na}^+$  ( $\circ$ ); 5  $\mu\text{M}$   $\text{Rb}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  ( $\bullet$ ); 20  $\mu\text{M}$   $\text{Rb}^+$  minus  $\text{Na}^+$  ( $\triangle$ ); or 20  $\mu\text{M}$   $\text{Rb}^+$  plus 500  $\mu\text{M}$   $\text{Na}^+$  ( $\blacktriangle$ ). Means  $\pm$  SE are shown,  $n = 8$  plants.



**Fig. 4.** The effect of external [K<sup>+</sup>] on Na<sup>+</sup> uptake into whole roots of 6-d-old intact seedlings of *Triticum aestivum* cv. Spear. Uptake solutions contained 200 μM Na<sup>+</sup> and were buffered to pH 6. Plants used for these experiments were grown in solutions containing 5 μM K<sup>+</sup> and 500 μM Na<sup>+</sup>. Bars represent means ± SE, *n* = 16 plants.

acquire sufficient amounts of K<sup>+</sup> to maintain high growth rates. Therefore it appears that certain K<sup>+</sup> uptake mechanisms may operate at 100 μM but not as efficiently at 20 μM K<sup>+</sup>. This is consistent with what has been shown using an *Arabidopsis* mutant. That study showed there was a significant component of channel-mediated K<sup>+</sup> uptake when 100 μM K<sup>+</sup> was supplied in the presence of NH<sub>4</sub><sup>+</sup> (Hirsch *et al.* 1998). However, when the external K<sup>+</sup> concentration was lowered to 10 μM, the *Arabidopsis* mutant, deficient in channel-mediated K<sup>+</sup> uptake, did not grow much slower than the wild type. This suggests that at very low external concentrations of K<sup>+</sup>, somewhere below 100 μM, channel-mediated uptake does not make a large contribution to the K<sup>+</sup> nutrition of plants. The inhibition of wheat growth in the presence of only 20 μM external K<sup>+</sup> suggests that the activity of the plant K<sup>+</sup> transport mechanisms under those conditions cannot efficiently supply the K<sup>+</sup> required for optimum long-term growth. These long-term growth experiments on wheat, at 20, 100 and 2000 μM K<sup>+</sup>, suggest that K<sup>+</sup> uptake mechanisms can operate efficiently at 100 μM, but do not operate efficiently enough at 20 μM to sustain maximal growth rates.

In one out of three growth experiments we observed a beneficial effect of low Na<sup>+</sup> on wheat grown in low external K<sup>+</sup> (20 μM). Since there was no difference in the internal K<sup>+</sup> concentration between wheat grown with or without added Na<sup>+</sup> it is possible that the cause of the increased biomass was due to a direct effect of Na<sup>+</sup> on growth, rather than an enhancement of K<sup>+</sup> uptake. Two other lines of evidence also suggest that Na<sup>+</sup> had a direct effect on growth.

First, the uptake experiments showed only a small component of K<sup>+</sup>-stimulated Na<sup>+</sup> uptake, which was observed at

a maximum rate of 0.05 μmol Na<sup>+</sup> (g root FW)<sup>-1</sup> h<sup>-1</sup>. Assuming a transport stoichiometry of 1 Na<sup>+</sup> : 2 K<sup>+</sup> by the Na<sup>+</sup>-coupled K<sup>+</sup> transporter in wheat roots (Gassmann *et al.* 1996), the maximum rate of K<sup>+</sup> uptake through the Na<sup>+</sup>-coupled system would be less than 3% [0.1 vs 3.5 μmol (g root FW)<sup>-1</sup> h<sup>-1</sup>] of the measured Rb<sup>+</sup> influx. Hence, it is unlikely that a Na<sup>+</sup>-coupled flux, which is less than 3% of the total high-affinity K<sup>+</sup> uptake (as measured with Rb<sup>+</sup>), would contribute to the 23% increase in total dry weight observed in the 42-d-old wheat grown with Na<sup>+</sup>.

Second, the Na<sup>+</sup>-stimulated growth was observed only in one whole-plant growth experiment, and only when plants were grown under shorter day lengths and lower total irradiance. So we hypothesise that, when carbon assimilation and growth are reduced by lower light levels, the contribution of inorganic solutes to plant growth may be of greater significance. Apart from inorganic K<sup>+</sup> and Na<sup>+</sup>, organic solutes such as sugars and amino acids contribute significantly to the osmotic potential of the cell (Greenway and Munns 1980; Munns and Weir 1981). Therefore, when the synthesis of organic solutes is limited by light, the uptake of Na<sup>+</sup> may have contributed significantly to plant growth. Further experiments will be needed to test this hypothesis.

The finding that short-term flux rates were constant from 20 to 2000 μM K<sup>+</sup> was not expected. Whereas most of the flux studies in the past 35 years have used roots grown in low or high salt conditions and have measured the flux rates in a 'perturbed system' (Glass and Siddiqi 1984), we chose to measure ion uptake in the modified Hoagland's solution in which the plants were grown. At least one report also shows that barley roots maintain a constant rate of K<sup>+</sup> uptake between 5 and 100 μM external K<sup>+</sup> (Siddiqi and Glass 1983) in short-term experiments using similar methods to those used in our experiments. Our results confirm that K<sup>+</sup> uptake in wheat is tightly regulated to maintain a constant supply in the short term over at least a 100-fold difference in external K<sup>+</sup> concentration. Whereas root K<sup>+</sup> channels are constitutively expressed (Lagarde *et al.* 1996), others are induced when external K<sup>+</sup> concentrations decline (Santa-Maria *et al.* 1998; Wang *et al.* 1998) which allows plants to maintain a steady supply of K<sup>+</sup> for nutrition and growth.

Our results suggest that Na<sup>+</sup>-energised K<sup>+</sup> uptake is not a major mechanism for high-affinity K<sup>+</sup> acquisition in wheat grown under acidic or alkaline pH conditions. The large reduction in the rate of Rb<sup>+</sup> influx into wheat roots at high external pH suggests that the major component of K<sup>+</sup> uptake is proton-dependent. Even at alkaline pH the rate of high-affinity Rb<sup>+</sup> uptake was not stimulated by the presence of external Na<sup>+</sup>. However we cannot eliminate the fact that Rb<sup>+</sup> may not be a reliable tracer for all K<sup>+</sup> uptake mechanisms (Gassmann *et al.* 1996). Nevertheless, the growth experiment at high pH supports the results of the short-term tracer flux studies, as the presence of external Na<sup>+</sup> had no effect on the biomass or K<sup>+</sup> content of wheat grown in low external K<sup>+</sup>

concentrations. We conclude that in wheat, low concentrations of Na<sup>+</sup> do not enhance the acquisition of K<sup>+</sup> or growth in alkaline environments, except perhaps when light levels limit the production of organic solutes.

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