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## ABSTRACT

**To facilitate the development of forages with mineral composition optimal for ruminant production, knowledge of the biochemical and physiological processes determining genotypic differences in nutrient uptake is needed. Experiments were conducted to identify the mechanism(s) that regulated the difference in leaf blade K concentration between two selections of *Lolium* × *Festuca* hybrid derivatives. Selection differences in rates of K uptake were estimated by measuring Rb uptake by excised roots, and the selectivity of cation uptake for K over Na was determined using intact tillers grown in modified Hoagland's solutions containing varying ratios of K:Na. Differences in Rb uptake by excised roots were of sufficient magnitude to explain differences in leaf blade K concentration. Results indicated differences in K and Na uptake between these two selections reflected an alteration in cation uptake selectivity by roots. Perturbing the plasma membrane integrity by Ca depletion treatments eliminated the difference between selections in K uptake but not in Na uptake. Differences in K uptake were associated with differences in the stimulation of the plasma membrane ATPase by K. These results were interpreted in terms of at least two transport processes contributing to the differences in leaf blade cation concentrations, one process on the plasma membrane affecting K accumulation and another affecting Na accumulation, possibly on the tonoplast membrane.**

GRASS TETANY, or hypomagnesemia, is an economically important metabolic disorder of ruminants characterized by low serum Mg (Littledike et al., 1983). Dietary levels of K have been reported to reduce serum Mg levels by several mechanisms (Newton et al., 1972; Tomas and Potter, 1976). Thus, ruminants grazing herbage that is low in K and high in Mg should have less tendency to develop grass tetany than animals grazing forages high in K and low in Mg. Accordingly, plant breeders have been attempting to develop cultivars with more desirable levels of these two cations.

Genetic differences in mineral nutrition have been intensively studied and reviewed numerous times (Epstein, 1972; Clark, 1983; Memon et al., 1985 and references cited within). In many instances, genetic differences in mineral levels can be explained by differences in root growth and morphology (Epstein, 1972). In the case of K nutrition, such results might well be expected since the amount of surface area the roots expose to the soil solution is a primary determinant of K uptake (Barber, 1978). Unfortunately, genotypes of Capeweed [*Artotheca calendula* (L.) Levyns] (Asher and Ozanne, 1977) and corn (*Zea mays* L.) (Baligar and Barber, 1979) with low K tended to have low growth rates. This association between low K and low growth rates provides the breeder with an additional challenge in developing productive forages with low K.

Since the early 1960s, substantial evidence has been compiled from a variety of organisms including higher plants that the transport of ions can be directly linked to ATP hydrolysis by a group of enzymes collectively called transport ATPases (Eddy, 1978; Mitchell, 1967; Skou, 1965). Understanding the function of the plasma membrane transport ATPase from higher plants may provide insight into genetic manipulations of K nutrition because of the suspected role of this transporter in K uptake (Briskin, 1986; Hodges, 1973; Sze, 1984). The involvement of a plasma membrane transport ATPase in the uptake of K has been indicated from electropotential studies (Cheeseman and Hanson, 1979), which suggest that K influx was coupled to an expenditure of ATP, probably via a transport ATPase. Hodges (1973) argued, based on correlative relationships, that the Mg-dependent, KCl-stimulated plasma membrane ATPase was involved with K uptake. The kinetics of K uptake were strikingly similar to that of the kinetics of K stimulation of the ATPase (Fisher et al., 1970). The K-stimulated ATPase activity was highly correlated with rates of K uptake among species of cereal grains (Fisher et al., 1970). The addition of diethylstilbesterol inhibited both K uptake and the ATPase in a similar fashion (Balke and Hodges, 1979).

The correlation between K uptake and the activity of K-stimulated ATPase indicated that the activity of this enzyme could be used to select genotypes differing in K uptake. Genotypic differences in salinity tolerance have been associated with changes in the stimulation of the transport ATPase by monovalent cations (Braun et al., 1986; Erdei and Kuiper, 1980; Hassidim et al., 1986; Lerner et al., 1983). Therefore, it may be possible to predict the K uptake potential of a genotype by the stimulation of the plasma membrane transport ATPase by monovalent cations. Characteristics of cation uptake, growth, and plasma membrane ATPase activity of two selections of *Lolium* × *Festuca* hybrid derivatives were compared to assess the role of the plasma membrane ATPase in regulating the leaf blade K concentration.

## MATERIALS AND METHODS

*Plant Materials. Lolium* × *Festuca* hybrid derivatives were provided by Dr. R.C. Buckner USDA-ARS, University of Kentucky, Lexington. Selections 13–1 and 78–5 were identified as being characteristically low and high, respectively, in leaf blade K concentration in field trials at Lexington and Quicksand, KY (Buckner et al., 1981). Buckner et al., (1961) described the breeding program used to produce the hybrid population from which 78–5 and 13–1 were selected. Tillers of these selections were potted in soil and vegetatively propagated in the greenhouse.

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*Uptake by Excised Roots.* Four sets of 10 tillers of each selection placed between two halves of a no. 12 foam stopper fitted within a plastic retention ring were suspended over 20 L of half-strength Hoagland's solution (Hoagland and Arnon, 1950). Nutrient solutions were vigorously aerated and changed weekly. Every 2 wk, roots were rinsed in deionized water and harvested. Primary roots exceeding 10 cm were selected and the apical 6 cm portion was used in the uptake experiments. Ten segments (approximately 0.25 g) were incubated for 30 min at room temperature with aeration in 25 mL of 0.2 m*M* $CaSO_4$ (pH 6.0) containing 0.01 to 10 m*M* RbCl supplemented with $^{86}Rb$. Free space $^{86}Rb$ was removed by submerging the roots in unlabeled solution for 5 min. Roots were collected on a Buchner funnel, packed into preweighed counting tubes, subjected to gamma scintillation counting, and reweighed to determine a fresh wt. The Rb accumulation was calculated on the basis of the specific activity of solutions at the beginning of the uptake period and the radioactivity of the sample. The uptake of $^{86}Rb$ from Rb solutions was measured as an estimate of K uptake based on the observation that the kinetics of Rb uptake were similar to that of K uptake from K solutions (Smith and Epstein, 1964).

*Hydroponic Experiment.* A sufficient number of tillers to comprise 1.00 ± 0.05 g of fresh wt (5–10) was placed between two halves of a no. 12 foam stopper and inserted into a plastic retention ring. Two rings of each selection were suspended over 20 L of nutrient solution. Three experiments were conducted in which the solution K, Na, or K and Na concentrations were varied. For all treatments, the solution contained 1 m*M* $Ca(NO_3)_2$, 0.5 m*M* $MgSO_4$, 8 $\mu$M Fe as N-carboxymethyl-N″-2-hydroxyethyl-N-N′-ethylenediglycine chelate titrated to pH 6.0, and micronutrients as found in Hoagland's solution (Hoagland and Arnon, 1950). In the first experiment, 0.2 m*M* $NaH_2PO_4$ and 0.075 m*M* $Na_2HPO_4$ were supplied and the KCl concentration was varied from 0.005 to 1.0 m*M*. In the next experiment, 0.2 m*M* $KH_2PO_4$ and 0.075 m*M* $K_2HPO_4$ were supplied and the NaCl concentration was varied from 0.1 to 1.1 m*M*. In the last experiment, Na and K were added from 0.2 *M* chloride, 0.45 M $H_2PO_4^{-1}$, and 0.075 *M* $HPO_4^{-2}$ stocks to yield a constant concentration of Na plus K of 0.5 m*M* with K:Na ratios of 3.17, 2.57, 1.78, 1.08, 0.92, 0.47, 0.19, 0.087, or 0.042. The K and Na concentrations of the nutrient solutions were monitored by atomic absorption spectrophotometry to insure that depletion did not exceed 10% of initial levels. Solutions were changed at least weekly.

Plants were allowed to grow in their respective solutions for 70 d, at which time roots and leaf blades were excised. After this initial harvest, roots and leaf blades were collected at 28-d intervals. Leaf blades and roots were oven-dried at 65 °C to constant mass (usually 2d) and digested with 9 to 1 (v/v) nitric to perchloric acid. The residue was dissolved in 1.0 *M* HCl containing 0.13 m*M* $SrCl_2$. Potassium, Ca, Mg, and Na were determined by atomic absorption spectrophotometry using an air-acetylene flame.

The mineral concentrations and dry wt. of both tissues from the 28-d harvests were used to calculate the extent of cation uptake, which was expressed as $mmol_c$ accumulated in both leaf blades and root tissues on a root dry wt. basis. Accumulation of dry matter and minerals in excised blades and roots from 28-d harvests represented in excess of 90% of the total whole plant retention based on data from destructive samplings of whole plants (data not shown). Such an observation is consistent with other results indicating that diameter and height of individual stem bases/crowns were relatively constant from harvest to harvest (data not shown).

General treatment effects were assessed by analysis of variance. The experimental design was a split-split plot with three replications. The main plots comprising a 20-L container was first split by selections. Each of these subplots were further subdivided into two sets of tillers. Combined analysis of variance for two consecutive 28-d harvests was performed as described by McIntosh (1983) assuming harvests as a fixed effect. Results of the analysis of variance are presented in detail elsewhere (Brauer, 1985). Cation accumulation data in these experiments represented a wide range of values and the magnitude of standard errors associated with means having low rates of accumulation were considerably smaller than the variance of means having high rates of retention. Therefore, the standard error of the mean was computed for each mean representing selection × nutrient treatment interaction to compare the effects of selection on cation uptake.

*Short-Term Uptake by Intact Tillers.* Plants were grown for 3 wk in solutions containing 1.0 m*M* $Ca(NO_3)_2$, 0.5 m*M* $MgSO_4$, 0.1 m*M*KCl, 0.225 m*M* $NaH_2PO_4$, 0.075 m*M* $Na_2HPO_4$, and micronutrients as described above; followed by 1 wk in 0.2 m*M* $CaSO_4$. Prior to the initiation of uptake, roots from one set of tillers for each selection from each 20-L container were harvested to estimate initial levels of K and Na. After 0.5 to 12 h in nutrient solutions, roots were harvested and the concentrations of K and Na were determined by atomic absorption spectrophotometry as described above. Uptake rates were determined by subtracting initial concentrations from values at subsequent harvests. Data from three replicates of each selection were averaged and significance was assessed by comparing means and their associated standard errors.

*ATPase Assay.* The activity of the plasma membrane ATPase was determined by the protocol of Brauer et al., (1982), with each assay being performed in triplicate. Microsomal membranes were purified by differential centrifugation at 0 to 4 °C (Brauer et al., 1982). Thirty grams fresh wt. of roots were rinsed with tap water and homogenized using a mortar and pestle in the presence of 3 g of polyvinylpolypyrrolidone and 120 mL of extracting buffer containing 0.25 *M* sucrose, 50 m*M* tris(hydroxymethyl)aminomethane titrated to pH 7.8 with N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 2 m*M* sodium ethylenediamine-tetraacetate, and 2 m*M* dithiothreitol. The homogenate was filtered through cheesecloth and centrifuged at 13 000 × *g* for 10 min. Microsomal membranes were collected by centrifugation at 40 000 × *g* for 60 min. The resulting pellet was suspended in 10 mL of extracting buffer containing 0.2 *M* NaCl. Microsomal membranes were collected by repeating the 40 000 × *g* centrifugation. This final pellet was suspended in 4 mL of extracting buffer and aliquots were used for the ATPase assay. Protein concentration was determined by a modification of the Lowry method after precipitation by trichloroacetic acid in the presence of deoxycholate (Peterson, 1977).

## RESULTS AND DISCUSSION

*Rb Uptake by Excised Roots.* Preliminary experiments indicated that over a wide range of Rb concentrations, uptake increased linearly as a function of time for a minimum of 60 min (data not shown). Also, a desorption time of less than or equal to 5 min but more than 1 min was sufficient to remove apoplasmic $^{86}Rb$ (Balke and Hodges, 1979). Kinetics of Rb uptake were determined using an uptake period of 30 min from solutions containing 0.012 to 10.0 m*M* RbCl in 0.2 m*M* $CaSO_4$ as an indicator of the kinetics of K

uptake. At all of the tested concentrations, Rb uptake by excised roots of the high-K selection, 78–5, was greater than that of 13–1 (Fig. 1). The magnitude of the difference between selections was greater at the lower Rb concentrations. At 0.2 m*M* Rb, root segments of 78–5 accumulated Rb at a rate that was about 100% greater than that by 13–1, 0.055 vs. 0.022 mmol Rb kg root$^{-1}$ (fresh wt.) h$^{-1}$. At 4.0 m*M* Rb, the rate of Rb uptake by 78–5 was only 45% greater than by 13–1, 0.95 vs. 0.62 mmol Rb kg root$^{-1}$ (fresh wt.) h$^{-1}$. Differences in Rb uptake were of sufficient magnitude to explain the 20 to 50% difference in leaf blade K concentration observed in field trials (Buckner et al., 1981). Such differences in Rb uptake by excised roots suggested that part of the difference in leaf blade K concentration could be attributed to an alteration in K uptake by roots.

Root segments prepared as described for Rb uptake experiments were found to have similar physical characteristics. Root lengths, volumes, radii, and surface area were determined essentially as described by Peterson and Barber (1981). Length per g of roots averaged 409 ± 58 (standard deviation) and 445 ± 67 cm for 13–1 and 78–5, respectively, on a fresh wt. basis; and 4844 ± 507 (standard deviation) and 5298 ± 610 cm, respectively, on a dry wt. basis. Root radius averaged 0.008 cm with both selections. Values for surface area per g root were similar, averaging 20.6 and 22.4 cm$^2$ per g fresh wt. of root for 13–1 and 78–5, respectively.

*Hydroponic Experiments.* Cation uptake by intact tillers was assessed to determine if the difference in Rb uptake by excised roots between the two selections persisted for K uptake by intact plants. Preliminary experiments indicated that the two selections differed in both K and Na uptake (Fig. 2). When plants were grown in variable concentrations of K, tillers of the high-K selection, 78–5, accumulated more K than 13–1. Analysis of variance indicated that KCl levels, selections, and selection by nutrient treatment interaction significantly ($P < 0.001$) affected K accumulation (data not shown). The difference between selections was greater at lower solution concentrations. At concentrations below 0.03 m*M* K, uptake by the high-K selection 78–5 was about 100% greater than by 13–1. At 1.0 m*M* K, K accumulation by 78–5 was only 40% greater than by 13–1. Data from the experiment of growing tillers in solutions of varying KCl indicated that the low-K selection, 13–1, tended to accumulate more Na (data not shown). Therefore, in the next experiment, the effect of varying the solution Na concentration was tested. Analysis of variance indicated that Na treatments and selections significantly ($P < 0.001$) affected Na uptake (data not shown). The selection × nutrient treatment interaction was significant at $P = 0.05$ (data not shown). From 0.1 to 1.1 m*M* Na, the low-K selection, 13–1, accumulated more Na than 78–5 (Fig. 2). The results from these two preliminary experiments indicated that an alteration in the selectivity of cation uptake processes was at least partially responsible for the difference in blade K concentration.

Selectivity of uptake to K and Na was assessed by growing tillers at nine different K:Na ratios at a fixed concentration of K plus Na of 0.5 m*M*. The effect of nutrient supply on plant growth was evaluated by dry wt. per set of tillers at each harvest (Table 1). Analysis of variance indicated that there was no significant effect of nutrient treatments or selection on dry matter accumulation. The selection × nutrient treatment interaction was significant at $P = 0.05$. At the three highest solution ratios of K:Na, tillers of 13–1 and 78–5 yielded between 620 and 640 mg of roots and leaf blades (dry wt.) in 28 d (Table 1). Growth decreased slightly as the solution K:Na ratio was decreased below 0.47. The decline in dry wt. was more pronounced

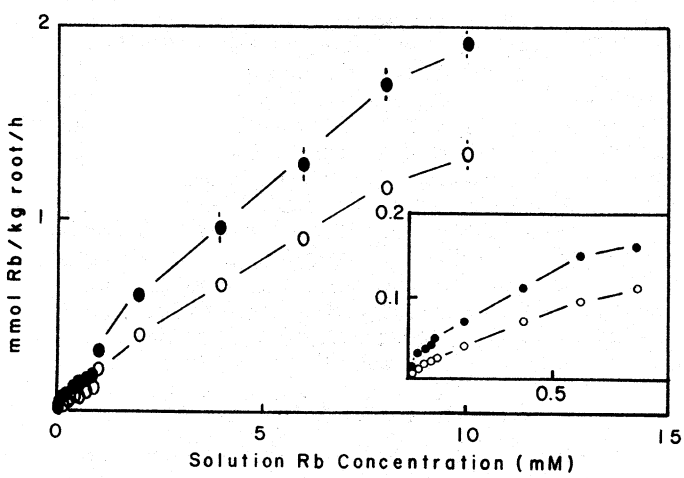


Fig. 1. Concentration dependence of Rb uptake by excised root segments. The Rb uptake by root segments from RbCl solutions in 0.2 m*M* $CaSO_4$ (pH 6.0) was determined using an incubation period of 30 min. Data represented by open and closed symbols are from the low and high-K selections, 13–1 and 78–5, respectively. The insert focuses on uptake from lower Rb solutions. Data points are the mean of three to six observations, and standard error of the mean is presented as a bar where the range exceeds the size of the symbol.

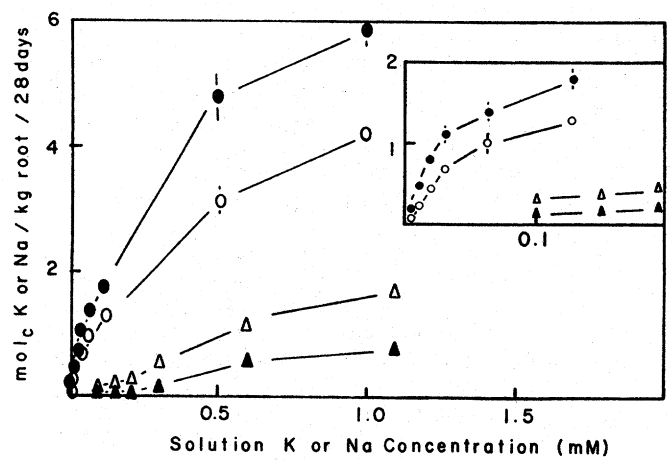


Fig. 2. Concentration dependence of K and Na uptake by growing tillers. K, and Na uptake (○ and △, respectively) were determined in separate experiments by measuring the accumulation of cations in the growth of roots and leaf blades during 28-d periods. Data are expressed as accumulation in both roots and leaf blades per kg of roots (dry wt. basis) with open and closed symbols representing results from selections 13–1 and 78–5, respectively. Data are the average from two consecutive harvests containing three replications each ($n = 12$). Standard error of the mean is presented as a bar where the range exceeds the size of the symbol. The insert focuses on K and Na uptake from solution concentrations below 0.2 m*M*.

with 78–5. At K:Na ratio of 0.042, 13–1 and 78–5 yielded 582 and 554 mg, respectively. These results indicated that the selections were similar in productivity and there was a slight tendency for dry wt. per set of tillers to decline when the K:Na ratio was reduced below 0.47, especially with 78–5.

Solution K:Na ratio treatments had a significant effect on the accumulation of K, Na, and Mg ($P < 0.001$, 0.01, and 0.01, respectively), whereas Ca accumulation was only slightly affected (data not shown). Selection differences in the uptake of all four cations were indicated by significant $F$-tests ($P < 0.01$). A significant selection × nutrient ratio treatment interaction was found only in the case of Na uptake (data not shown). Potassium uptake increased and Na uptake decreased with both selections as the K:Na ratio increased (Fig. 3). At each of the K:Na ratios, K uptake by the high-K selection, 78–5, was greater than the low-K selection, 13–1, and Na uptake was greater with 13–1 than 78–5. Therefore, the ratio of K:Na uptake was greater with 78–5 than 13–1, indicating a greater selectivity for K than Na. Increases in Na accumulation with decreases in the solution K:Na ratio followed an exponential curve with 78–5, whereas with 13–1 a biphasic increase was observed. This difference in the shape of curves relating Na accumulation to the solution ratio probably accounts for the highly significant selection × treatment interaction. Magnesium uptake by both selections decreased with increasing K:Na ratios, with the sharpest decline occurring when the ratio was increased from 0.042 to 0.19. Calcium uptake was only slightly affected by changes in the solution ratio. Both Ca and Mg uptake was greater by 13–1 than by 78–5.

These results indicated that the two selections differed in K uptake, however, alterations in the accumulation of Ca, Mg, and Na were also observed. The high-K selection, 78–5, had accumulated more K than selection 13–1, but less Ca, Mg, and Na than 13–1. The sum of the uptake of the four cations was not significantly different between selections ($P > 0.10$). The lack of a difference in total cation uptake between these selections indicated that the capacity to accumulate cations was not different. The difference in mineral nutrition appeared to be confined to that of the cations since differences in the accumulation of Cl, nitrate, and P were not detected (data not shown).

*Short-Term Uptake Studies with Intact Tillers.* Roots of tillers depleted of minerals by growing in dilute $CaSO_4$ for 1 wk rapidly accumulated K and Na when these nutrients were resupplied (Fig. 4) From

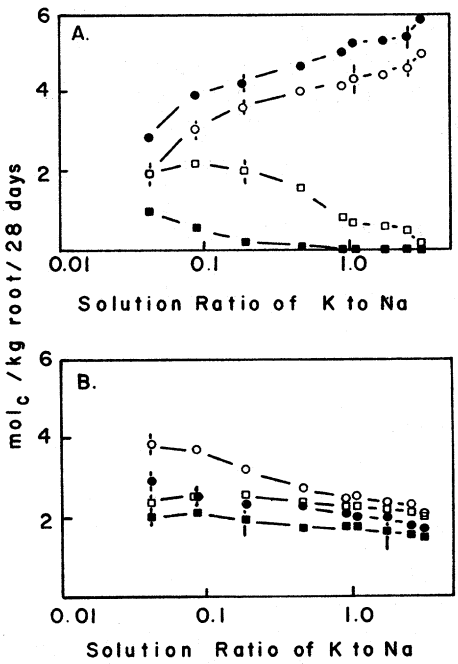


Fig. 3. Effect of the solution ratio of K:Na on the uptake of K, Na, Ca, and Mg by growing tillers. Uptake of K (panel A, ○), Na (panel A, □), Ca (panel B, ■), and Mg (panel B, ○) was determined by the accumulation of minerals in the roots and leaf blades during 28-d periods. Data from selections 13–1 and 78–5 represented by open and closed symbols, respectively, are the means from two consecutive harvests ($n = 12$). Standard error of the mean is presented as a bar where the range exceeds the size of the symbol.

Table 1. Effects of the solution K:Na ratio on dry matter production. Tillers of 13–1 and 78–5 were grown in nutrient solutions containing a combined concentration of K and Na of 0.5 m$M$ at nine solution ratios.

| Solution ratio | Genotype 13–1 | Genotype 78–5 |
|---|---|---|
| K:Na | — mg dry wt./28 d ± SE — | |
| 3.17 | 620 ± 32† | 640 ± 43 |
| 2.57 | 641 ± 46 | 633 ± 23 |
| 1.39 | 620 ± 27 | 629 ± 33 |
| 0.92 | 626 ± 33 | 626 ± 27 |
| 0.67 | 621 ± 29 | 620 ± 12 |
| 0.47 | 614 ± 42 | 617 ± 42 |
| 0.19 | 608 ± 23 | 601 ± 20 |
| 0.087 | 601 ± 17 | 582 ± 34 |
| 0.042 | 582 ± 32 | 554 ± 19 |

† The combined dry wt. of harvested leaf blades and roots from two successive harvests (mean of 12 observations).

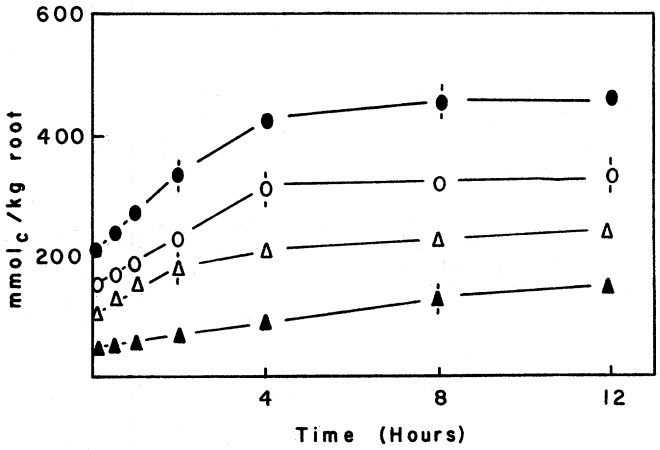


Fig. 4. Time course of K (○) and Na (△) uptake by roots of intact tillers after depleting internal concentration by growing on 0.2 m$M$ $CaSO_4$ for 1 wk. Roots were excised and allowed to reform while plants were grown on complete nutrient solution. After 3 wk, the nutrient solutions were replaced with 0.2 m$M$ $CaSO_4$ to deplete root levels of K and Na. At time zero, roots of tillers were placed in solutions containing 2.5 m$M$ NaCl, 2.5 m$M$ KCl, and 0.2 m$M$ $CaSO_4$. Uptake was determined from changes in K and Na concentration of roots. Open and closed symbols represent data from selection 13–1 and 78–5, respectively. Data are the mean of three replicates, and standard error of the mean is presented as a bar where the error exceeds the size of the symbol.

2.5 m$M$ Cl salts of both Na and K in 0.2 m$M$ $CaSO_4$, uptake proceeded at a linear rate for up to 2 h and reached steady-state levels between 8 and 12 h. Roots of 78–5 accumulated K at a greater initial rate, as reflected by the steeper rise in K concentration during the first hour of incubation, and reached a higher steady-state level as compared to 13–1. Roots of 13–1, however, had a greater initial rate of Na uptake and accumulated more Na than the other selection.

The degree to which the plasma membrane contributed to the differences between 13–1 and 78–5 in K and Na uptake was assessed by comparing the rates of K and Na uptake by control roots and roots that were treated to disturb the plasma membrane. Previous research has shown that the plasma membrane becomes perturbed and thus leaky when the intracellular space is depleted of Ca (Epstein, 1961; Leggett and Gilbert, 1967). This was accomplished by rinsing roots for 60s in water titrated to pH 4.0 with $H_2SO_4$ (Leggett and Gilbert, 1967) or for 10 min in 5.0 m$M$ EGTA. After the pretreatments, roots of intact tillers were placed in solutions containing a combined concentration of KCl and NaCl of 5.0 m$M$ with K:Na ratios of 9.0, 1.0, or 0.11 for 2 h. With control plants only, uptake solutions contained 0.2 m$M$ $CaSO_4$ in addition to KCl and NaCl. Under control conditions, roots of selection 78–5 had higher rates of K uptake and lower rates of Na uptake than 13–1 from the three K:Na ratios tested (Table 2). Perturbation of the plasma membrane by depleting the membranes of Ca by either acid or EGTA treatment resulted in the roots of both selections being equal in the rate of K uptake. Roots of both selections accumulated about 50 $mmol_c$ K kg of roots$^{-1}$ h$^{-1}$ from solutions with a ratio of K:Na of 9.0. At the other two K:Na ratios, net losses of K were observed with both selections. These results suggest that some function of the plasma membrane contributed to differences in K uptake by these selections.

The results for Na uptake were quite different from those obtained for K uptake. Roots of 13–1 had greater rates of Na uptake even when the roots had been treated with either acid or EGTA. For example, control roots of 13–1 and 78–5 accumulated 162 and 9 $mmol_c$ Na kg roots$^{-1}$ h$^{-1}$, respectively, at a K:Na ratio of 0.11. After treating roots with EGTA, Na uptake rate increased to 221 and 54 $mmol_c$ kg roots$^{-1}$ h$^{-1}$, respectively. The lack of an effect of the acid and EGTA treatment on the differential accumulation of Na between selections indicated that functions of the plasma membranes did not significantly contribute to differences in Na accumulation. In addition, the acid and EGTA treatments increased Na uptake by both selections, especially at higher K:Na ratios. This observation indicates that some function of the plasma membrane restricted the accumulation of Na. No evidence has been found for a K-dependent Na efflux mechanism in the *Lolium* × *Festuca* selections (data not shown) as reported for barley (*Hordeum vulgare*, L.) by Jeschke (1980).

*ATPase Activity.* The hydrolysis of ATP by the plasma membrane transport ATPase from higher plant plasma membranes is characterized as being Mg dependent (Briskin, 1986; Hodges, 1973; Sze, 1984). Additionally, the Mg-dependent activity is stimulated by KCl, and KCl-stimulated activity is specifically inhibited by submillimolar levels of vanadate (Briskin, 1986; Sze, 1984). The effects of Mg, KCl, and vanadate on

**Table 2. Effects of EGTA and acid pretreatment on K and Na uptake by genotypes 13–1 and 78–5. Roots of intact tillers were either preincubated for 60 s in pH 4.0 water or 5.0 m$M$ EGTA for 10 min to deplete the external surface of the plasma membrane of Ca. Uptake of K and Na was determined as described in the text.**

| Treatment | Solution ratio | Genotype | | | |
|---|---|---|---|---|---|
| | | 13–1 | 78–5 | 13–1 | 78–5 |
| | | K uptake | | Na uptake | |
| | K:Na | $mmol_c$ kg root$^{-1}$ h$^{-1}$ ± SE | | | |
| Control | 9.00 | 32 ± 6† | 77 ± 10 | 10 ± 2 | −1 ± 1 |
| | 1.00 | 7 ± 2 | 37 ± 6 | 71 ± 18 | 2 ± 1 |
| | 0.11 | −6 ± 2 | 20 ± 5 | 162 ± 33 | 9 ± 1 |
| Acid | 9.00 | 51 ± 9 | 52 ± 8 | 43 ± 10 | 1 ± 1 |
| | 1.00 | 3 ± 1 | −2 ± 1 | 87 ± 24 | −2 ± 2 |
| | 0.11 | −22 ± 3 | −17 ± 6 | 200 ± 45 | 20 ± 4 |
| EGTA | 9.00 | 49 ± 8 | 51 ± 10 | 63 ± 15 | 15 ± 3 |
| | 1.00 | −2 ± 1 | −1 ± 1 | 93 ± 14 | 28 ± 7 |
| | 0.11 | −24 ± 6 | −20 ± 6 | 221 ± 22 | 54 ± 15 |

† Mean of 3 replicates.

**Table 3. Effect of Mg, KCl, and vanadate on the hydrolysis of ATP by microsomal membranes isolated from genotypes 13–1 and 78–5. The effect of 3 m$M$ $MgSO_4$, 50 m$M$ KCl, and 0.2 m$M$ vanadate on the hydrolysis of 3 m$M$ ATP was assessed at pH 6.5 using membranes from roots of 13–1 and 78–5 as described in materials and methods.**

| Assay condition | Genotype | | | |
|---|---|---|---|---|
| | 13–1 | | 78–5 | |
| | Control | + 0.2 m$M$ Vanadate | Control | +0.2 m$M$ Vanadate |
| | $\mu$mol mg protein$^{-1}$ h$^{-1}$ ± SE | | | |
| ATP alone | 1.06 + 0.05† | 1.02 + 0.04 | 1.00 ± 0.05 | 0.97 ± 0.04 |
| ATP + 50 m$M$ KCl | 1.08 + 0.10 | | 1.04 ± 0.08 | |
| 3 m$M$ Mg ATP | 1.86 + 0.11 | 1.09 + 0.05 | 1.80 ± 0.09 | 0.97 ± 0.06 |
| Mg-dependent | 0.80 | 0.07 | 0.76 | 0.00 |
| 3 m$M$ Mg ATP + KCl | 2.33 + 0.13 | 1.15 + 0.10 | 2.57 ± 0.12 | 0.96 ± 0.05 |
| KCl-stimulated | 0.47 | 0.06 | 0.77 | 0.00 |

† Mean of three experiments, each containing three replicates ($n$ = 9).

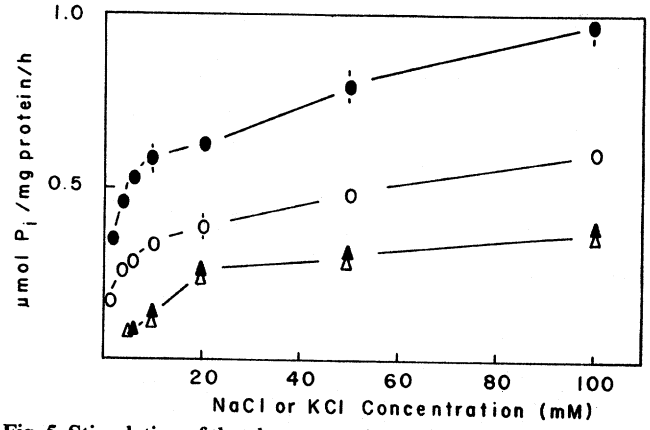


**Fig. 5. Stimulation of the plasma membrane ATPase activity by KCl and NaCl. Effect of increasing KCl (○) or NaCl (△) concentration from 2.0 to 100 m$M$ on Mg-dependent ATP hydrolysis was assessed with microsomes from roots of 13–1 and 78–5 (open and closed symbols, respectively). Data are the mean of three replications, and standard error of the mean is presented as a bar where the range exceeds the size of the symbol.**

ATP hydrolysis by microsomal membranes from *Lolium* × *Festuca* roots were assessed to determine if the activity of the plasma membrane transport ATPase from the *Lolium* × *Festuca* hybrids was similar to the activities from other higher plants (Table 3). Magnesium-dependent ATPase activity was approximately the same with both selections, 0.8 $\mu$mol mg protein$^{-1}$ h$^{-1}$. Addition of 50 m*M* KCl stimulated ATP hydrolysis only in the presence of Mg. The KCl stimulation was about 50% greater in favor of 78–5 (0.77 vs. 0.47 $\mu$mol mg protein$^{-1}$ h$^{-1}$. Both the Mg-dependent and the KCl-stimulated activities were strongly inhibited by 0.2 m*M* sodium vanadate. Therefore, KCl-stimulated, Mg-dependent ATP hydrolysis by microsomal membranes from these *Lolium* × *Festuca* hybrids had the characteristics reported for other higher plant plasma membrane transport ATPases (Briskin, 1986; Hodges, 1973; Sze, 1984).

Stimulation of Mg ATP hydrolysis by NaCl and KCl was assessed to determine if differences in Na and K uptake between these selections were associated with similar differences in cation stimulation of the ATPase activity. The KCl stimulation of ATPase activity was greater with the high-K selection, 78–5, than with 13–1 (Fig. 5). The magnitude of the differences in KCl stimulation was of similar magnitude as differences in K and Rb uptake. Stimulation of ATP hydrolysis was less with NaCl as compared to KCl with the same degree of stimulation for both selections. Therefore, there was no association between selection differences in Na uptake and Na stimulation of the transport ATPase. Similar results were found when plasma membrane-enriched vesicles were purified by density sucrose centrifugation from roots of 28-d-old seedlings (data not shown).

In conclusion, the difference between selections in leaf blade K concentration, at least in part, could be attributed to a dissimilarity in a plasma membrane process involving K transport. Differences in Rb uptake by excised roots (Fig. 1) and in K uptake by intact tillers (Fig. 2) were of sufficient magnitude to explain the 20 to 50% difference in leaf blade K concentration. Differences in K uptake reflected a change in the selectivity for cation uptake. Perturbation of the plasma membrane by treating roots with either acid or chelators to reduce the Ca from external surface of the plasma membrane (Epstein 1961; Leggett and Gilbert, 1967) eliminated the differences in K uptake between the two selections (Table 2). Thus, some function of the plasma membrane was probably responsible for the variation in K uptake. There was a strong association between selection differences in K uptake and K stimulation of the plasma membrane ATPase (Fig. 5), suggesting that the transport ATPase had a role in the selection difference in K uptake. When the plasma membrane was perturbed, the difference between selections in Na uptake persisted. These results indicated that some other membrane besides the plasma membrane was responsible for the difference in Na uptake between the two selections, with the most likely candidate being the tonoplast. Therefore, the differences between 13–1 and 78–5 in the magnitude of the selectivity of uptake of K over Na were due to the interaction of at least two processes.

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