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Cation fluxes in excised and intact roots in relation to specific and varietal differences

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Summary In this short survey differences between species and varieties in the four major mechanisms that affect selective uptake of potassium and sodium to the plant within the root are considered. These include influx selectivity, K^+/Na^+ exchange at the plasmalemma, and selectivity at the tonoplast as well as at the symplasm-xylem boundary. The affinity of various plants for potassium influx in system 1 is rather uniform although varietal differences in barley have been observed. Differences are much more pronounced for sodium influx, for which Helianthus showed rather high and Fagopyrum rather low affinity. There is substantial variation between species in the efficiency of K^+/Na^+ exchange at the plasmalemma of cortical root cells; the three cereals Hordeum, Triticum, and Secale were highly efficient while K^+/Na^+ exchange in Atriplex, Helianthus and Allium was poor, even if the cytoplasmic sodium content was accounted for. Apparently there was no direct relation between salt tolerance and K^+/Na^+ exchange. The observed differences in the efficiency of K^+-dependent sodium extrusion or K^+/Na^+ exchange were not due to the use of excised roots, they were observed also when roots of whole seedlings were investigated. At the tonoplast a 1 : 1 exchange of vacuolar potassium for sodium has been observed in roots of Hordeum. By this exchange sodium ions are removed from the symplasm and potassium ions are recovered from vacuoles and thus made available for transport to the shoot. Indications for specific differences in this exchange have been observed; the exchange appears to be more efficient in Helianthus than in Hordeum roots. More comparative studies are needed here. At the boundary between symplasm and xylem vessels selectivity can be set up during xylem release of cations and there are reports that suggest a preference for sodium (*Lycopersicum cheesemanii, Solanum pennellii*, and Suaeda) and for varietal differences amongst tomatoes. Selectivity at this boundary, the plasmalemma of the xylem parenchyma cells was described in this paper by the selectivity ratio of transport that relates the rates of xylem transport to the cytoplasmic sodium and potassium concentrations. Based on this ratio *Atriplex hortensis* was shown to discriminate for sodium during xylem release while there was little selectivity in Hordeum and possibly some discrimination in favour of K^+ in Allium roots. The data are shortly discussed in relation to salt tolerance and to the breeding of salt-tolerant crop varieties.

Introduction

In his well-known comparative study on the K^+-Na^+ selectivity in different plant species Collander has shown that whole plants behaved rather uniformly with respect to K^+ uptake but varied widely in their capability to take up or exclude Na^+ ions. These differences in Na^+ ionic relations are reflected in the wide spectrum of the ability of plants to cope with salinity which in most cases relates to excessive concentrations of sodium salts. Genotypic variations in salt tolerance of crop species have been studied[3,11,37,38] and reviewed recently[12,27].

Another important aspect of Na^+ salt relations and of the ability of plants to take up sodium ions is the need for an adequate sodium content in the shoots of plants when they are to be used as cattle feed. Whereas Na^+ ions are added in sufficient or overoptimal quantities to the human diet, this is not always true for fodder plants and pastural grasses[44]. Only a few extensive studies on the genotypic variation in Na^+ uptake by pastural grasses are available[44].

Apart from the basic scientific interest there are, therefore, two main reasons to study in a comparative way the properties of plants with respect to their Na^+ salt relations. Such studies may contribute to an understanding and improvement of agricultural plants for growth on saline soils and to an improvement or better selection of plants for cattle feed having sufficient Na^+ content for the demands of cattle.

Another practical aspect could justify such investigations. When studying uptake, xylem transport, and fluxes of Na^+ rather large differences – compared to fluxes of K^+ – between species or genotypes can be expected from an extrapolation of Collander's data. This is confirmed by the work of Rush and Epstein[38], although this is not to say that genotypic differences in K^+ ionic relations need to be small, as is seen from the prominent variations in K^+ influx rates in 10 genotypes of barley[9].

Whereas the prospects of investigating Na^+ ionic relations at first sight thus appear to be good, such studies are complicated by the fact that mere uptake data will provide little information when working with Na^+ ions. With K^+ ions Glass and Perley[9] obtained significant differences already from the kinetics of ion uptake and Glass *et al.*[10] showed that genotypic differences in K^+ nutrition of barley may be observed already by measuring pH changes.

In the case of Na^+ the ionic relations of a plant can be controlled decisively at least at three sites: a) at the plasmalemma and b) the tonoplast of the cortical cells, and c) at the plasmalemma of the xylem parenchyma cells in the root. At all three sites, therefore, genotypic differences may occur and for their understanding Na^+ fluxes at these three sites ought to be measured. In addition, such studies should include the effect of K^+ ions since the three transport sites may discriminate between K^+ and Na^+ ions.

In the present paper data on Na^+ relations in different plant species will be discussed. Emphasis shall be laid on the different strategies by which glycophytic plants maintain a high K^+-Na^+ selectivity in particular in their shoots or by which alternatively salt-tolerant species include Na^+ and have low selectivity for K^+.

Sites and mechanisms of K^+-Na^+ selectivity

Until recently K^+-Na^+ selectivity has been studied mainly using a few species and the following characteristic features have been found for barley. As the primary selective barrier within the root the plasmalemma of the cortical cells controls selectivity in two ways. Firstly it favours influx and net uptake of K^+ over that of Na^+ at low external concentrations, in the range of system 1[35]. Na^+ influx is specifically inhibited by low concentrations of K^+ and a substantial uptake of Na^+ is possible only when K^+ ions are missing or very low in concentration[16,41] although Na^+ may be accumulated in vacuoles even in the presence of low concentrations of K^+, see below and Fig. 1. Secondly, the plasmalemma is the site of an efficient K^+-Na^+ exchange system by the operation of which Na^+ ions are extruded from the cytoplasm to the external solution in exchange for K^+ (or Rb^+)[16,17,20]. This exchange of Na^+ for K^+ is mediated by proton fluxes[20,21,36] and appears to be powered by active proton extrusion. Influx and exchange selectivity were suggested to be manifestations of the same membrane transport system[20]. The minimal components of this system appear to be a) a proton pump, b) a transport site mediating influx having high affinity for K^+ and a low one for Na^+, and c) a transport site permitting net Na^+ efflux and having only negligible affinity for K^+. Genotypic differences can be expected to occur in the secificity of the uptake site and in the presence or the properties of an efflux site.

The second membrane barrier that controls K^+-Na^+ selectivity is the tonoplast of cortical and possibly also the stelar parenchyma cells in the root. In some respect the tonoplast appears to be a mirror image of the plasmalemma. In barley it has been shown to favour uptake of Na^+ ions from the cytoplasm to the vacuoles[18,34]. In the opposite direction, net efflux of K^+ from vacuoles is possible[18], while Na^+ ions appear to be almost irreversibly sequestered in the vacuole[29]. Due to these properties of the tonoplast an exchange of vacuolar K^+ for cytoplasmic Na^+ is possible and for barley roots net exchange of these ions across the tonoplast has been observed[18].

Again, genotypic differences in the ability of plants to transfer cytoplasmic Na^+ to vacuoles can be expected. It should be noted, that by an influx across the tonoplast not only Na^+ ions are removed and K^+ is made available as a cytoplasmic constituent but that Na^+ together with anions like Cl^- may function as vacuolar osmotica and thereby provide a means for salt tolerance[12,20,42]. Additionally, lowering of the cytoplasmic Na^+ concentration will facilitate Na^+ uptake. The tonoplast in this way to some extent can counteract the selective properties of the plasmalemma (see the uptake of Na^+ in the presence of K^+ in barley roots, compare Fig. 1, below).

Before ions enter the apoplastic phase within the xylem vessels and are then transported to the shoot together with the transpiration stream or the volume flow generated by root pressure they have to cross another biomembrane, the plasmalemma of the xylem parenchyma cells where they are secreted or released to the xylem vessels. At this site again cation selectivity can be affected, see below.

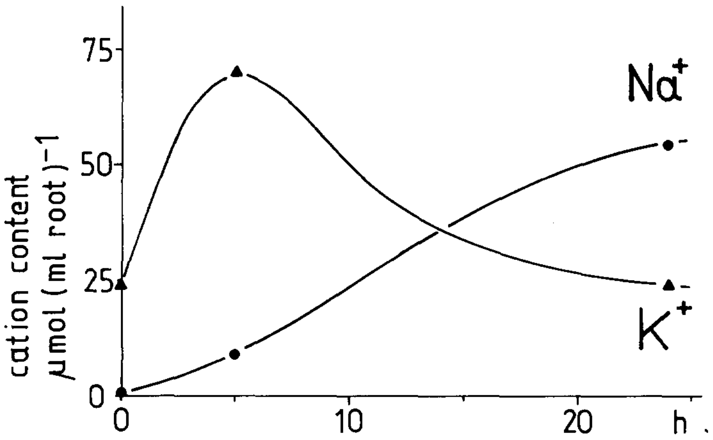

Fig. 1. Net changes in the volume content of K^+ (▲) and Na^+ (●) in excised barley (*Hordeum vulgare*, convar. distichon) roots during incubation in a solution containing 1 m*M* Na^+ and 0.2 m*M* K^+. The content at t = 0 is that of low-salt roots. Analysis was made by means of the flameless atomic absorption technique in 0.5 mm long sections taken 2.5 mm from the root tip. Composition of the incubation medium: 1 meq/l sodium phosphate buffer (pH 5.8); 0.2 m*M* KCl; 3 m*M* $CaSO_4$; 0.5 m*M* $MgSO_4$. For details of the ion profiles see Jeschke[18]; from Jeschke[20].

Comparative studies of sodium and potassium ion fluxes

a) Influx selectivity

Epstein, who pioneered the investigations on the kinetics of ion influx, has compiled[4,5] the kinetic constants for K^+ and Na^+ influx in several species and some of these data together with some more recent ones are shown in Table 1. These data show remarkably uniform values for the Michaelis constants K_m of K^+ influx in excised low-salt roots, all being around 0.2 m*M* (exception Agropyrum, 0.008 m*M*) and this is confirmed by the data obtained for Triticum, Helianthus and Fagopyrum (Table 1). At first sight this uniformity does not suggest a possibility for high genotypic variation in the affinity of plant roots towards K^+. It is most interesting, therefore, that Glass and Perley[9] found the K_m values of K^+ influx to vary in a wide range in a series of 10 varieties of barley. The K_m data, some of which are contained in Table 1, ranged between 0.011 and 0.024 m*M* and there were corresponding differences in the V_{max} values. High affinities (low K_m) were correlated with high maximal rates of influx, V_{max}.

Kinetic constants for influx by low-salt roots might appear to be of little significance for the performance of plants under field conditions under which they are normally in a high-salt state. As is seen from Table 1, K_m values for high-K^+ roots were considerably higher, as was observed earlier and was attributed to a regulation of influx by the internal K^+ concentration in the root[8]. Also these K_m values varied over a wide range, but the ranking between the varieties differed from that obtained for low-salt roots (Table 1). Nevertheless, the observed variation in the kinetic data of low-K^+ roots were shown to relate

Table 1. Kinetics of K^+ and Na^+ influx from solutions of low ion concentrations (System 1)*.

		K^+		Na^+	
Species	Roots	K_m	V_{max}	K_m	V_{max}
Hordeum vulgare	Low salt	0.021[a]		0.32[b]	
Helianthus annuus[c]	Low salt	0.023	1.0	0.16	7.3
Helianthus annuus	High salt	0.17	0.94	0.86	1.7
Triticum aestivum[c] cv. Carstacht	Low salt	0.023	6.7	0.4	1.28
Fagopyrum esculentum[d]	Low salt	0.02	3.2	1.56	0.63
Hordeum vulgare[e]					
cv. Fergus	Low salt	0.0109	12.06	–	–
cv. Hector	Low salt	0.0128	10.91	–	–
cv. Conquest	Low salt	0.0241	9.76		
cv. Fergus	High salt	0.065	0.77	–	–
cv. Conquest	High salt	0.111	0.118	–	–
cv. Hector	High salt	0.188	2.12	–	–

[a] Epstein[4], [b] Rains and Epstein[35], [c] S. Kolibius, unpublished, [d] Eggers, this volume, [e] Glass and Perley[9].

* K_m apparent Michaelis constant in mM; V_{max} maximal rate of influx in μmol g^{-1} FW.

also to differences in the performance of the barley varieties. This was indicated by growth measurements under limiting K^+ supply or under conditions of competition between the varieties[9]. This study clearly shows the potential of kinetic measurements for finding varieties that can produce good yield also with decreased supply of fertilizers.

Although fewer data are available, the variation between species in the kinetic constants for Na^+ influx (low-salt roots) appears to be higher than those for K^+ (Table 1) and differences in K_m and V_{max} between varieties of tomato have been observed[30]. Clearly the affinity of roots for Na^+ is much lower than for K^+, but apart from Fagopyrum the K_m values ranged between 0.16 and 0.4 mM and are thus within the limits of system 1 of ion uptake (Table 1). As found for K^+ the apparent Michaelis constant was higher for high-salt roots (see the data for Helianthus, Table 1) indicating that Na^+ influx in the absence of K^+ is regulated in a similar way as was suggested for K^+ influx[8].

The kinetic data obtained for Fagopyrum roots, the species that was found to discriminate most strongly between K^+ and Na^+ amongst the plants studied by Collander[1] appear noteworthy. The apparent K_m for Na^+ influx (1.56 mM) is beyond the normal range of system 1 concentrations and as shown by the low V_{max}, buckwheat has virtually no capability to take up Na^+ from solutions of concentrations below 1 mM even when K^+ ions are absent. Whereas barley roots accumulate considerable amounts of Na^+ (final content about 75 μmol g^{-1}FW at 1 mM Na^+) buckwheat roots took up as little as 0.5 μmol g^{-1}FW from solutions containing 1 mM Na^+ (Eggers, unpublished). Apparently this species excludes Na^+ almost fully from its cells and appears to achieve selectivity mainly by means of a highly efficient influx selectivity (see also the contribution by H. Eggers to this volume).

By contrast to Fagopyrum, Helianthus roots showed a relatively high affinity (low K_m) and capacity (V_{max}) for Na^+ influx (Table 1) although this species was shown to be almost as highly $K^+ - Na^+$ selective as Fagopyrum[1]. Furthermore, Na^+ influx was much less inhibited by the presence of K^+ ions than it was in barley (refs.[17,35,41] and Table 3) or in Triticum roots (Kolibius, unpublished). In agreement with this observation K^+ influx by Helianthus roots was inhibited by the presence of Na^+ ions. In Helianthus roots $K^+ - Na^+$ discrimination, therefore, cannot be attributed to influx selectivity.

As follows from this comparison between the results obtained for sunflower and buckwheat roots, kinetic studies of Na^+ can be expected to reveal high genotypic variations but they do not by themselves allow to predict the performance of a plant in the discrimination between K^+ and Na^+ ions.

b) $K^+ - Na^+$ exchange at the plasmalemma

In barley roots a major source of $K^+ - Na^+$ selectivity has been shown to be K^+-dependent Na^+ efflux across the plasmalemma of their cortical cells[17]. In the presence of external K^+ net extrusion of Na^+ can be observed with sodium-loaded, excised roots[25]; the $K^+ - Na^+$ exchange system that is responsible for Na^+ extrusion operates in differentiated as well as in meristematic tissues of barley roots[20]. It must be asked whether this exchange system operates in other species, too.

Table 2 shows the sodium efflux ϕ_{co}, the cytoplasmic sodium content Q_c, and the potassium-dependent sodium extrusion $\phi_{co}(K^+\text{-dep})$ for a number of species. There are marked differences between species and a relatively high Q_c was found for barley, wheat and Atriplex roots. However, a prominent K^+-dependent sodium extrusion $\phi_{co}(K^+\text{-dep})$ was found only for the three cereal grasses wheat, barley and rye, indicating these species to possess an efficient $K^+ - Na^+$ exchange system. When the rate of sodium extrusion induced by 0.2 mM K^+ is related to the cytoplasmic sodium content Q_c and when the ratio $\phi_{co}(K^+\text{-dep})/Q_c$ is taken as a measure of exchange performance, wheat roots appear to be even more efficient in potassium- sodium exchange than barley roots (Table 2, last column).

In the other species tested so far, in *Fagopyrum esculentum*, *Helianthus annuus*, *Atriplex hortensis*, and *Allium cepa* only a small or no K^+-dependent Na^+ extrusion could be observed (Table 2). The ratio ϕ_{co} $(K^+\text{-dep})/Q_c$ in all these cases was low, indicating that it was not cytoplasmic Na^+ content Q_c that limited

Table 2. Effect of $\mathrm{K^+}$ on the plasmalemma $\mathrm{Na^+}$ efflux from cortical cells of roots in different species: Roots were equilibrated with 1 m*M* $\mathrm{Na^+}$ solution and then the steady state fluxes were measured by means of compartmental analysis – only the plasmalemma efflux ϕ_{co} and the cytoplasmic $\mathrm{Na^+}$ content Q_c are shown –; in the steady state 0.2 m*M* $\mathrm{K^+}$ was then added and the transient, $\mathrm{K^+}$-dependent $\mathrm{Na^+}$ efflux ϕ_{co} ($\mathrm{K^+}$-dep) was measured*

Conditions	$\mathrm{Na^+}$-loaded roots, (1*M* $\mathrm{Na^+}$), steady state		Transient $\mathrm{Na^+}$efflux after addition of 0.2 m*M* $\mathrm{K^+}$	
Parameter	ϕ_{co}	Q_c	ϕ_{co} ($\mathrm{K^+}$-dep)	ϕ_{co}($\mathrm{K^+}$-dep)/Q_c
Allium cepa[a]	0.21	0.34	0.03	0.08
Helianthus anuus[a]	1.1	1.08	0.3	0.28
Triticum aestivum[a]	2.0	2.2	7.0	3.2
Hordeum vulgare[b]	2.1	3.6	8.2	2.3
Secale careale[c]	1.2	1.3	1.9	1.5
Atriplex hortensis[d]	2.5	4.5	0.9	0.2
Fagopyrum esculentum[e]	0.07	0.13	n.d.**	–

* Fluxes in μmol g^{-1} FW h^{-1}; cytoplasmic content in μmol g^{-1} FW.

** n.d. = not detectable.

[a] Jeschke and Nassery[2]; [b] Jeschke[19]; [c] E. Moreth, unpublished; [d] Stelter[40]; ($\mathrm{K^+}$ = 1m*M*); [e] H. Eggers, unpublished.

$\mathrm{K^+-Na^+}$ exchange. Only for roots of Fagopyrum, in which no $\mathrm{K^+}$-dependent $\mathrm{Na^+}$ efflux could be detected, it cannot be excluded that this was due to the very low cytoplasmic $\mathrm{Na^+}$ content (Table 2). However, since these roots almost totally exclude $\mathrm{Na^+}$, see above, the apparent absence of $\mathrm{K^+-Na^+}$ exchange is understandable. For Helianthus, on the other hand, which is also highly $\mathrm{K^+-Na^+}$ selective in its shoots[1], the apparent absence of $\mathrm{K^+-Na^+}$ exchange appears remarkable. Possibly this low level of $\mathrm{K^+-Na^+}$ exchange is related to the low degree of influx selectivity in this species (see above) since influx selectivity and $\mathrm{K^+-Na^+}$ exchange have been suggested to be achievements of the same membrane transport system[20]. By contrast to Fagopyrum and Helianthus, *Atriplex hortensis* contains more $\mathrm{Na^+}$ than $\mathrm{K^+}$ in its leaves[1] and the low degree of $\mathrm{K^+-Na^+}$ selectivity at the plasmalemma (Table 2) was to be expected, therefore. This species apparently includes $\mathrm{Na^+}$ rather than to exclude it and this undoubtedly is related to the salt tolerance of Atriplex. It should be noted however, that $\mathrm{Na^+}$ inclusion does by no means pertain to the cytoplasm. Atriplex roots have been shown to keep the cytoplasmic $\mathrm{Na^+}$ level similarly low as do barley roots[25].

From the prevailing results it can be concluded that efficient $\mathrm{K^+-Na^+}$ exchange is a property of certain species and so far has been found only for cereal grasses. In addition, some indications have been obtained that barley varieties differing in their salt tolerance also differ in $\mathrm{K^+-Na^+}$ exchange at the plasmalemma. Experiments are in progress to substantiate these preliminary results. It appears promising to investigate the capability of $K^+ - Na^+$ exchange when searching for genotypic differences in $K^+ - Na^+$ selectivity, in particular in Gramineae species.

c) Flux measurements with whole plants

Most experimental results on individual ion fluxes at the cellular membranes of root cells so far have been obtained with excised roots. The results, also those pertaining to $K^+ - Na^+$ selectivity, therefore, could be restricted to these roots, which lack the continuous supply of photosynthesis products from the shoots.

Some authors have studied the efflux behaviour of or have applied compartmental analysis to roots of whole plants[14,15]. During such measurements with labelled roots of whole plants tracer is not only removed from the roots by exchange with the external, unlabelled solution but also by transport to the shoot, together with the continuing flow of unlabelled ions through the xylem vessels. In previous studies xylem transport either was ignored as being small[14] or no attempt was made to evaluate the efflux data for obtaining ion fluxes at the cellular membranes[6,15].

Recently a method for studying ion fluxes in roots of whole plants has been developed, which makes allowance for the continuous transport of tracer to the shoot[23]. So far this method has been applied to Na^+ fluxes in Helianthus[23] and to K^+ and Na^+ fluxes in Hordeum seedlings[22] and some of the results are given in Table 3. As can be seen, fluxes obtained with whole plants and with excised roots compare well, only the tonoplast fluxes showed some differences (Table 3). Thus the tonoplast fluxes of Na^+ in excised roots of sunflower were significantly lower than those obtained with whole plants. As has been discussed[23] this appears to be related to the observation that intact sunflower roots – in contrast to excised ones – had not reached a level of saturation of sodium accumulation. In these roots a sizeable net Na^+ accumulation continued throughout the duration of efflux experiments. Accordingly the tonoplast influx ϕ_{cv} by far exceeded the efflux ϕ_{vc}, see Table 3.

Good agreement was found for $K^+ - Na^+$ exchange: with barley high rates of K^+-dependent net Na^+ extrusion could be induced by the addition of 0.2 mM K^+ to Na^+-loaded excised or intact roots[22]; with sunflower, on the other hand $K^+ - Na^+$ exchange was low, no matter whether excised or intact roots were used[23,24]. This shows that the low efficiency of $K^+ - Na^+$ exchange in sunflower roots (ref.[24] and Table 2) was not due to the use of excised roots and to their limited energy reserves.

A few details of Table 3 deserve to be noticed. Sodium influx ϕ_{oc} in sunflower roots was much less depressed by the continuous presence of 0.2 mM K^+ than was true for barley roots. Similarly K^+ inhibited the xylem transport of sodium much less in sunflower than in barley roots (Table 3); however, even in the absence of K^+ sodium transport across the xylem vessels ϕ_{cx} was low in

Table 3. Unidirectional steady state sodium and potassium fluxes and cytoplasmic or vacuolar contents in intact and excised roots of *Hordeum vulgare* and of *Helianthus annuus* in the absence and in the presence of K^+.[1]

Species	Hordeum[a]					Helianthus[b]		
Ion Conditions	Na^+ fluxes			K^+ fluxes		Na^+ fluxes		
	1 m*M* Na^+		1m*M* Na^+ 0.2 m*M* K^+	1 m*M* Na^+ 0.2 m*M* K^+		1 m*M* Na^+		1 m*M* Na^+ 0.2 m*M* K^+
Roots	intact	excised	intact	intact	excised	intact	excised	intact
ϕ_{oc}	5.5	4.4	1.05	13.6	15	2.4	3.5	1.1
ϕ_{co}	2.0	2.1	0.81	7.3	5	1.5	1.1	1.0
ϕ_{cv}	1.04	0.99	0.28	2.1	7.6	1.9	0.4	0.4
ϕ_{vc}	0.86	0.99	0.19	2.0	7.6	1.3	0.4	0.4
ϕ_{cx}	3.2	2.4	0.14	6.2	10	0.29	0.46	0.12
Q_c	3.3	3.6	0.59	11.5	21.7	1.5	1.1	0.46
Q_v	76	71	48	68	69.3	35	46	44
Q_v/Q_c	23	19.6	81	5.9	3.2	24	43	96

[1] ϕ_{oc}, ϕ_{co} = plasmalemma influx and efflux; ϕ_{cv},ϕ_{vc} = tonoplast influx and efflux; ϕ_{cx} = xylem transport; all fluxes in μmol g^{-1} FW h^{-1}. Q_c, Q_v = cytoplasmic and vacuolar ion content in μmol g^{-1} FW.

[a] *Hordeum vulgare*, convar. distichon; data from Jeschke[22]; [b] *Helianthus annuus*; data from Jeschke and Jambor[23].

sunflower compared to barley roots. The relatively small effect of K^+ on sodium transport found for sunflower, therefore, is not at variance with the finding of a high $K^+ - Na^+$ selectivity in the shoots of sunflower plants. Furthermore, the small response of sodium influx ϕ_{oc} to the presence of K^+ as found with compartmental analysis (Table 3) is consistent with the low influx selectivity observed with tracer influx studies for this species, see above. It must be asked, therefore, by which mechanism(s) the high K^+/Na^+ ratio in sunflower shoots[1] is established since neither influx nor exchange selectivity at the plasmalemma appear to be responsible. As will be shown below, selectivity at the tonoplast could be responsible.

Although as yet no data on genotypic variations in individual ion fluxes are available, it appears that the method using roots of whole plants is promising for obtaining such data. Not only does this method allow an investigation of ion flux properties of roots *in situ* on the whole plant. But it is applicable also to such species which have small roots and are, therefore, not suited for experiments with excised roots. Moreover the use of roots of whole plants allows to study effects of the shoot and of transpiration on ion fluxes in the root as well as to investigate a shoot-dependent regulation of ion fluxes in the root.

d) Selectivity at the tonoplast

Selective properties of the tonoplast generally have to be derived from indirect measurements since the tonoplast as an internal membrane is accessible to experimentation only via the plasmalemma and the cytoplasm. Compartmental analysis yields data about the steady-state distribution of K^+ and Na^+ between cytoplasm and vacuoles and by this method sodium was shown to be preferentially localised in vacuoles and potassium more so in the cytoplasm in several species, barley[34], maize[31], onion[28], (see also Table 3). This pattern of distribution was found also by measurements of the longitudinal distribution of K^+ and Na^+ along the root axis in Hordeum and Atriplex and by calculating the cytoplasmic and vacuolar ion concentrations on the basis of the high cytoplasmic volume fraction in meristematic and the high vacuolar fraction in differentiated tissues[25]. Recently electron microprobe measurements have been applied to the distribution of K^+ and Na^+ between cytoplasm and vacuoles in barley roots[33]. The resulting data confirm those obtained with the other methods, but in addition the ratio K^+/Na^+ was shown to vary radially within the root and to be higher in the stele than in the cortex, a result that cannot be obtained by the other methods.

While thus the stationary distribution between cytoplasm and vacuole seems well documented, little direct information on time-dependent changes in the distribution is available. Indications for an exchange with time of vacuolar K^+ for Na^+ were obtained from the decrease in K^+ and increase in Na^+ content in barley leaves with increasing age[11]. With the method of longitudinal ion profiles net exchange of vacuolar K^+ for Na^+ was shown to occur under appropriate experimental conditions[18]. Fig. 1 shows as an example the changes in K^+ and Na^+ contents in barley roots during incubation in a solution containing 1 mM Na^+ and 0.2 mM K^+. An initial rapid increase in K^+ content was followed by a massive decrease between 5 and 24 hrs. that was mirrored by a continuous increase in sodium content. From the magnitude of K^+ loss and Na^+ uptake it follows that K^+ ions had been accumulated in vacuoles and were then exchanged for Na^+ at a stoichiometry close to 1. The K^+ ions originating from vacuoles were then available within the cytoplasm whence they were transported through the xylem vessels; Na^+ ions were taken up from the external solution and apparently were transported via cytoplasm and tonoplast to the vacuole.

The predominant vacuolar localization of Na^+ can be seen also from the data of Table 3. Here the vacuolar contents of K^+ and Na^+ were related to their contents in the cytoplasm. The ratio Q_v/Q_c for Na^+ was much higher than for K^+ and significantly higher when both K^+ and Na^+ were present externally than in solutions containing only Na^+ besides Ca^{++} and Mg^{++}. Furthermore, the ratio Q_v/Q_c (Na^+) was somewhat higher for sunflower than for barley roots (Table 3). The data suggest than Na^+ ions are expelled from the cytoplasm to vacuoles when K^+ ions are present and that the Na^+-K^+ selective system at the tonoplast that is responsible for this distribution may be more efficient in sunflower than in barley roots. Sequestration of Na^+ ions in vacuoles, therefore, could be one of the mechanisms by which sunflower roots achieve the high $K^+ - Na^+$ selectivity in the shoots. Na^+ ions taken up by the roots thereby are included in vacuoles within the root and prevented from reaching the shoot.

Besides providing a mechanism of $K^+ - Na^+$ selectivity, sequestration of Na^+ in vacuoles is thought to be a means by which salt-tolerant species take up Na^+ and use this ion as a 'cheap' vacuolar osmoticum[12,20,42]. It appears that much more comparative information about the capability of vacuoles to store Na^+ and of the tonoplast to discriminate between K^+ and Na^+ should be gathered.

e) Selectivity at the symplasm—xylem boundary

As a third site the plasmalemma of the xylem parenchyma cells can determine the overall selectivity of the plant in two ways. Firstly when released or secreted to the xylem vessels K^+ or Na^+ could be favoured or retarded and secondly Na^+ ions can be reabsorbed from the xylem fluid. This latter process has been found in some plant species like beans, maize and soybeans, which are highly efficient in excluding sodium from their shoots[13,26,39]. This reabsorption of sodium is mediated by xylem parenchyma cells in the basal parts of roots or shoots and in some cases these cells have specialized as transfer cells (Kramer, this volume). Indications have been obtained (Kolibius, unpublished) that reabsorption of Na^+ occurs also in sunflower roots.

In glycophytic species having high K^+/Na^+ ratios in their shoots a preference for K^+ during xylem release could improve the overall selectivity, indications for such a preference are limited, however (ref.[28] and below). The situation is different in halophytes[7] and salt-tolerant nonhalophytes[12] which include Na^+ in their shoots[1] rather than to prevent its uptake or to retain it in their roots. Sodium content in the leaves of halophytes is high[7] and salt-tolerant barley varieties[11] or tomato species[37] accumulate Na^+ in their leaves. In these plants the release of ions to the xylem vessels could prefer Na^+ over K^+.

In the salt-tolerant tomato *Lycopersicum cheesmanii* grown on 50 m*M* KCl or NaCl ($+0.5$ m*M* $CaSO_4$) more Na^+ than K^+ was translocated to the shoot while the salt-sensitive species *L. esculentum* translocated similar amounts of K^+ but very little Na^+ (ref.[37]). Although selectivity cannot be deduced directly from such data obtained with one-salt experiments, they suggest a preference for Na^+ transport in the salt-tolerant species. When growing tomatoes on Hoagland solution salinized up to 200 m*M* NaCl, Dehan and Tal[2] found much lower K^+/Na^+ ratios in leaves compared to roots in the salt-tolerant species *Solanum pennellii* but higher ones in the leaves of the salt-sensitive species *L. esculentum*. This result strongly suggests a preferred release of Na^+ to the xylem vessels in the salt-tolerant species and contrasts to those obtained for barley[32,34] according to which the K^+/Na^+ ratio in the leaves were higher than in the root but equalled that in the root cytoplasm suggesting little if any selectivity during alkali cation release to the xylem vessels, see below.

Evidence for a preference for Na^+ in xylem transport has been obtained also for the halophyte *Suaeda maritima*[7,43]. In this case K^+ increased the transport of Na^+ (5 m*M* Na^+) in the xylem in particular at high external K^+ concentrations.

However, K^+/Na^+ selectivity at the symplasm-xylem boundary can be described unequivocally only when the concentration or at least the content of K^+ and Na^+ in the cytoplasm and their rates of xylem transport are known. These data are available for roots of Allium[28], Hordeum (Table 3) and *Atriplex hortensis*[40] and in Table 4 the selectivity of the release of K^+ and Na^+ to the xylem vessels was calculated from the ratios of xylem transport ϕ_{cx} (or J_{cx}) and of cytoplasmic content Q_c:

$$S(\text{transport}) = \frac{\phi_{cx}(K)/\phi_{cx}(Na)}{Q_c(K)/Q_c(Na)}$$

For Allium roots this selectivity ratio was 2.8, possibly suggesting some preference for K^+, similarly for Hordeum a selectivity of 2.3 for K^+ in xylem release was calculated from the flux data obtained for whole plants (Tables 3, 4). Keeping in mind the experimental errors of determining the cytoplasmic content by means of compartmental analysis I would suggest that S(transport) is close enough to 1 and that there is little discrimination between K^+ and Na^+ during release to the xylem vessels in barley. Selectivity is set up at the plasmalemma and the tonoplast in this species.

Quite different results were obtained from measurements of xylem transport and longitudinal ion profiles in roots of the salt-tolerant *Atriplex hortensis*[40] (Table 4). Under all conditions tested S(transport) was clearly below 1 indicating a preference for Na^+. In the Table also the data obtained with low-salt roots and with 1 m*M* Na^+ and 1 m*M* K^+ (and Ca^{++}) are included, since S(transport) could be calculated on the basis of the cytoplasmic ion concentrations and the endogenous transport of the ions that were not present externally.

The low values of S(transport) found for *Atriplex hortensis* clearly show a discrimination in favour of Na^+ during xylem release in this halotolerant species. Preferential xylem transport and in addition vacuolar accumulation of Na^+ appear to be the mechanisms by which Atriplex removes K^+ from the root cytoplasm ($K^+ - Na^+$ exchange was low, Table 2). In addition, Na^+ transport in the xylem aids in the osmotic adjustment of the shoot to a saline environment. As a special adaptation and similar to other Atriplex species, *A. hortensis* can excrete excessive quantities of Na^+ into bladder hairs on the epidermis of its leaves.

Taken together, there is clear evidence for cation selectivity at the symplasm-xylem boundary only for preferred release (Suaeda, Atriplex) or reabsorption (Phaseolus, Zea) of sodium while a possible discrimination in favour of K^+ in other species (Hordeum, Allium) is still ambiguous. As follows from the results of Dehan and Tal[2] and Rush and Epstein[38] genotypic differences with respect to selectivity at this site can be expected to be found also for other species.

Table 4. Cytoplasmic Na^+ and K^+ contents or concentrations, xylem transport ϕ_{cx} of K^+ and Na^+, and selectivity ratio of xylem transport S(transport)[3] in different species.

Species	External[4] solution	Xylem transport[1]		Cytopl. content[2] or concentration		S(transport)[3]
		K^+	Na^+	K^+	Na^+	
Allium cepa[a]	1 m*M* Na, 1 m*M* K	21.6	0.65	8.5	0.76	2.8
Hordeum vulgare[b]	1 m*M* Na, 0.2 m*M* K	6.2	0.14	11.5	0.59	2.3
Atriplex hortensis[c]	0Na, 0K, (low salt)	0.8*	0.9*	90	2	0.02
Atriplex hortensis[c]	1 m*M* Na	3.7*	8.7	46	15	0.14
Atriplex hortensis[c]	1 m*M* K	8.3	0.7*	91	0.5	0.06
Atriplex hortensis[c]	1 m*M* Na, 1 m*M* K	5.8	5.4	81	2.5	0.033

[1] In μmol g^{-1} FW h^{-1}; [2] cytoplasmic content Q_c in μmol g^{-1} FW or cytoplasmic K^+ or Na^+ concentration in m*M*; [3] $S(\text{transport}) = \phi_{cx}(K)\ Q_c(Na)/\phi_{cx}(Na)\ Q_c(K)$ or $S(\text{transport}) = \phi_{cx}(K)\ [Na]_c/\phi_{cx}(Na)\ [K]_c$;

[4] concentrations of alkali cations are given only, for full composition, see refs;

[a] Macklon, 1975, Q_c and xylem transport ϕ_{cx} from flux measurements with excised roots;

[b] Jeschke, 1982, Q_c and xylem transport ϕ_{cx} from flux measurements with intact roots;

[c] Stelter, 1979, cytoplasmic K^+ and Na^+ concentrations in m*M* from longitudinal ion profiles of *Atriplex* roots, see Jeschke and Stelter, 1976, ϕ_{cx} from transport measurements; the transport data designated by a star* refer to the transport of endogenous reserves.

Concluding remarks

What emerges from this short comparative survey of the mechanisms and strategies of $K^+ - Na^+$ selectivity is that the quantitative variation in selectivity observed by Collander[1] in fact is due to prominent qualitative differences between the species. Even plants like Fagopyrum and Helianthus that appear quantitatively similar according to Collanders data achieve the same degree of selectivity in quite different ways.

High K^+/Na^+ ratios in shoots, apparently of evolutionary advantage in glycophytes, relate to different mechanisms or strategies: to K^+ selectivity during influx in Fagopyrum, to influx selectivity, efficient $K^+ - Na^+$ exchange and to preferred vacuolar accumulation of Na^+ in cereal grasses (Hordeum, Triticum and Secale), to low influx and exchange selectivity, but Na^+ accumulation in vacuoles and possibly reabsorption of Na^+ from the xylem sap in Helianthus, and to low Na^+ influx (influx selectivity) and Na^+ reabsorption from the xylem vessels in Phaseolus and Zea.

Low K^+/Na^+ ratios in shoots combined with the accumulation of Na^+ in leaves of some halophytes being of evolutionary and ecological advantage in the saline environment, on the other hand, appear to be related to low influx selectivity, to a preference for Na^+ during xylem release (Suaeda, Atriplex), and to a high $Na^+ - K^+$ selectivity at the tonoplast (Atriplex)[40].

Only one feature appears to be common to glycophytes and to halophytes and salt-tolerant nonhalophytes. This is the ability to exclude Na^+ from the cytoplasm[20,42]. This again is achieved in different ways, by low influx of Na^+ (Fagopyrum), by the combination of influx selectivity, $K^+ - Na^+$ exchange and vacuolar sequestration of Na^+ (Hordeum) or by vacuolar sequestration of Na^+ in combination with preferred release of Na^+ to the xylem sap (roots of Atriplex).

When searching for species or varieties having higher salt-tolerance, the selective properties of the tonoplast seem most important and a low selectivity at the plasmalemma appears to be mandatory for species having a high degree of salt tolerance. Special interest should be paid also to the selectivity at the symplasm-xylem boundary. High preference for K^+ in the release to the xylem vessels at this site, no matter whether it is achieved by a retention of Na^+ in the root cytoplasm or by reabsorption of Na^+ from the xylem sap., appears to be counterproductive for salt tolerance.

It appears worth noticing that similar arguments apply to species that tend to include Na^+ and are, therefore, suitable as species which contain sufficient amounts of Na^+ for the needs of cattle even when the soil is rich in K^+ due to fertilization. In this connection it would be interesting to investigate the $K^+ - Na^+$ selective properties of those grass species like *Holcus lanatus*, *Lolium perenne* and *Anthoxanthum odoratum* that have a high Na^+ content in their leaves[44].

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