Plant Physiol. (1991) 97, 990–997
0032-0889/91/97/0990/08/$01.00/0

Received for publication March 27, 1991
Accepted June 26, 1991

Ion Relations of Symplastic and Apoplastic Space in Leaves from *Spinacia oleracea* L. and *Pisum sativum* L. under Salinity[1]

Michael Speer and Werner M. Kaiser*

Lehrstuhl Botanik I, Universität Würzburg, Mittlerer Dallenbergweg 64, 8700 Würzburg, Federal Republic of Germany

ABSTRACT

Salt tolerant spinach (*Spinacia oleracea*) and salt sensitive pea (*Pisum sativum*) plants were exposed to mild salinity under identical growth conditions. In order to compare the ability of the two species for extra- and intracellular solute compartmentation in leaves, various solutes were determined in intercellular washing fluids and in aqueously isolated intact chloroplasts. In pea plants exposed to 100 millimolar NaCl for 14 days, apoplastic salt concentrations in leaflets increased continuously with time up to 204 (Cl^-) and 87 millimolar (Na^+), whereas the two ions reached a steady concentration of only 13 and 7 millimolar, respectively, in spinach leaves. In isolated intact chloroplasts from both species, sodium concentrations were not much different, but chloride concentrations were significantly higher in pea than in spinach. Together with data from whole leaf extracts, these measurements permitted an estimation of apoplastic, cytoplasmic, and vacuolar solute concentrations. Sodium and chloride concentration gradients across the tonoplast were rather similar in both species, but spinach was able to maintain much steeper sodium gradients across the plasmamembrane compared with peas. Between day 12 and day 17, concentrations of other inorganic ions in the pea leaf apoplast increased abruptly, indicating the onset of cell disintegration. It is concluded that the differential salt sensitivity of pea and spinach cannot be traced back to a single plant performance. Major differences appear to be the inability of pea to control salt accumulation in the shoot, to maintain steep ion gradients across the leaf cell plasmalemma, and to synthesize compatible solutes. Perhaps less important is a lower selectivity of pea for K^+/Na^+ and NO_3^-/Cl^- uptake by roots.

Reduced growth and death of plants in response to salinity can be explained in terms of enzyme inhibition arising from salt accumulation in metabolic compartments. An additional explanation, originally proposed by Oertli (20), could be a build-up of high salt concentrations in the apoplastic space of the leaves. If transport of salt into the leaf exceeds uptake by cells, apoplastic salt concentrations will rise. Consequences of increased apoplastic salt concentrations may be a partial dehydration of cells and turgor loss (6, 18, 20), or damage of the plasma membrane surface (4).

Compared with the large body of literature on salt stress, only a few studies have dealt with Oertli's hypothesis (*e.g.* 6, 7, 13, 18). Experimental support was given more recently by Munns and Passioura (18), who analyzed the osmotic pressure of xylem sap from the tip of the primary leaf of an intact transpiring barley plant under salt stress (up to 200 mM). They argued that the sap reflected the apoplastic ion concentration of the mesophyll cells, because it had traversed the whole leaf before its collection. They observed that about 1 week after the highest external salt concentration was reached, the osmotic pressure of the xylem sap rose dramatically, followed by leaf death.

In the following, we describe changes of ion concentrations in the symplast and apoplast of pea leaflets and spinach leaves under salinity. Solute concentrations in isolated chloroplasts were also measured and used for estimating concentrations in the cytoplasm. Finally we try to evaluate the relative importance for salt tolerance of intracellular solute compartmentation in relation to overall salt fluxes.

MATERIALS AND METHODS

Plant Material

Pea (*Pisum sativum* L., var Kleine Rheinländerin) and spinach (*Spinacia oleracea* L., Hybrid 102, Yates) were germinated in moist vermiculite. After 14 (spinach) or 5 (pea) d, seedlings were transferred into hydroponic culture solution, containing 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 2 mM KH_2PO_4, and trace elements according to Johnson *et al.* (10). Plants were grown in a growth chamber at 350 $\mu E\ m^{-2}\ s^{-1}$ (HQi 400 W, Schreder Winterbach, FRG) with a light/dark period of 11/13 h and a day/night temperature of 22/17°C. Relative humidity was in the range of 50 to 85%.

Assays were started by a one-step addition of 100 mM NaCl to the nutrient solution when peas had developed three fully expanded leaflets and spinach leaf blades had a length of 5 to 8 cm.

For the 300 mM NaCl treatment of spinach, the external salt concentration was raised in three steps of 100 mM salt within 6 d. The first sampling was done just before the last increment. Samples were taken 5.5 to 6.5 h after the beginning of the light period.

Analysis was restricted to the three oldest leaves of both species (ignoring primary leaves of spinach), which were pooled to give one sample.

[1] This work was supported by the Deutsche Forschungsgemeinschaft (DFG), Sonderforschungsbereich 251.

Extraction of Apoplastic Solutes

Leaf discs from spinach (6.2 cm^2) were briefly rinsed with deionized water, blotted dry, and weighed; from pea, whole leaflets were used. At the leaflet base, a small piece was cut off with a razor blade in order to create a cut surface of about 1 cm width. The material was placed into 30 mL of a sorbitol solution adjusted to the cryoscopically measured (Osmomat 030, Gonotec, Berlin, FRG) osmolality of the cell sap, and was gently vacuum-infiltrated for 3 min. Leaf discs/leaflets were then blotted dry and reweighed. The infiltrated material was placed into 20 mL vials with bottom holes. The cut surfaces of the leaflets were oriented toward the bottom of the perforated vials, which were transferred into a centrifuge tube and centrifuged (2°C) for 10 min at 400*g* (spinach) or 950*g* (pea). The amount of intercellular washing fluid, collected from the centrifuge tube, was determined by weighing, and the solution was stored frozen. Maximum time from the beginning of infiltration to the beginning of centrifugation was 7 min.

The remaining leaf disc/leaflet material was extracted for the analysis of total ion content as described elsewhere (22).

Symplastic ion concentrations were obtained by subtracting from the total extract the ion content of the intercellular washing fluid. The latter was calculated by assuming that apoplastic water volume remained constant.

Estimation of Apoplastic Water Volume

This technique is based on measuring the dilution of a [^{14}C] sorbitol-labeled solution by apoplastic water after vacuum-infiltration and centrifugation (see above). First, air space volume was determined by infiltrating leaf discs and leaflets with silicone oil (AR 200, Wacker Chemie, München, FRG) as proposed by Cosgrove & Cleland (3). Before infiltration, leaf discs (6.2 cm^2) or leaflets were preincubated overnight on water to ensure full hydration. The percentage of air space volume was calculated from the increase in weight and corrected for oil density. It was ensured that there was no oil adsorption on the cuticula after infiltration, which would have led to an overestimation of the air space volume.

Leaf discs/leaflets were then infiltrated with [^{14}C]sorbitol (Amersham Buchler, Braunschweig, FRG; 2×10^{-5} mM, giving an activity of 0.005 μCi/mL) and centrifuged as described above. The intercellular washing fluid was counted by liquid scintillation and compared with the activity of the medium before infiltration.

Aqueous Isolation and Purification of Chloroplasts

This was carried out as described previously (22), except that 20 mM *N*-[2-hydroxyethyl]-piperazine-*N'*-3-propane-sulfonic acid (adjusted to pH 8 with Tris) was used as buffer.

Chloroplast Volumes

Chloroplast volumes were determined by the ^3H_2O/[^{14}C] sorbitol method (9, 21).

Solute Determinations

Anions (Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, malate) were determined by isocratic ion chromatography (IC 1000, Biotronik, Maintal, FRG), fitted with an automatic sample injector, a conductivity meter, and a Shimadzu C-R1B integrator. Potassium and sodium were determined by flame photometry, and magnesium by atomic absorption spectroscopy.

RESULTS AND DISCUSSION

Overall Response of Pea and Spinach to Salinity

Under our standard growth conditions, spinach plants showed hardly any impairment of growth within a 17 d period after addition of salt (100 mM NaCl). In contrast, growth of pea shoots was drastically reduced. The oldest leaflets started to develop necrotic margins about 10 to 12 d after salt addition, and after 20 d, shoots were almost completely degenerated (not shown).

Salt concentrations in roots of both species remained below the external salt concentration. In spinach leaves, salt concentrations reached a rather low quasi steady-state after 4 d (Fig. 1). In contrast, pea leaves continued to accumulate salt, exceeding by far the concentration in the nutrient solution. Several other remarkable differences in the response to salinity of pea and spinach have been described earlier, a most striking effect being the total inhibition of nitrate transport from roots to shoot in peas (23, 24).

Apoplastic Solute Concentrations

Table I summarizes the basic data for the estimation of apoplastic solute concentrations, which is based on the following measurements, calculations and assumptions:

(a) Solutes were extracted from the leaf apoplastic space by infiltrating leaflets or leaf discs with isotonic sorbitol solutions, followed by low speed centrifugation and collection of some IWF.[2] Primary measured data are concentrations of solutes in IWF (mM). Concentrations can be converted into amounts per fresh weight (μmol g^{-1} fresh wt), by determining the volume of the infiltrated solution as total weight increase after infiltration (μL g^{-1} fresh wt). Only those leaflets were analyzed which, after infiltration, had become homogeneously translucent, indicating that all intercellular air spaces were filled with liquid. After centrifugation, 60 to 90% of the infiltrated solution was regained. The apoplastic water volume was calculated to be about fivefold diluted by the infiltrated solution (see below). The resulting redistribution of ions bound originally to the cation exchange groups of the cell wall might lead to a small overestimation of some cation concentrations in the water phase, but certainly not to an underestimation.

(b) To convert solute concentrations in IWF into solute concentrations in the apoplastic water phase, the ratio of the total V_{il} to V_{apo} had to be determined. This determination is based on the idea that the infiltration medium, when it is

[2] Abbreviations: IWF, intercellular washing fluid; V_{il}, infiltrated liquid volume; V_{apo}, apoplastic water volume; V_{air}, intercellular air space volume.

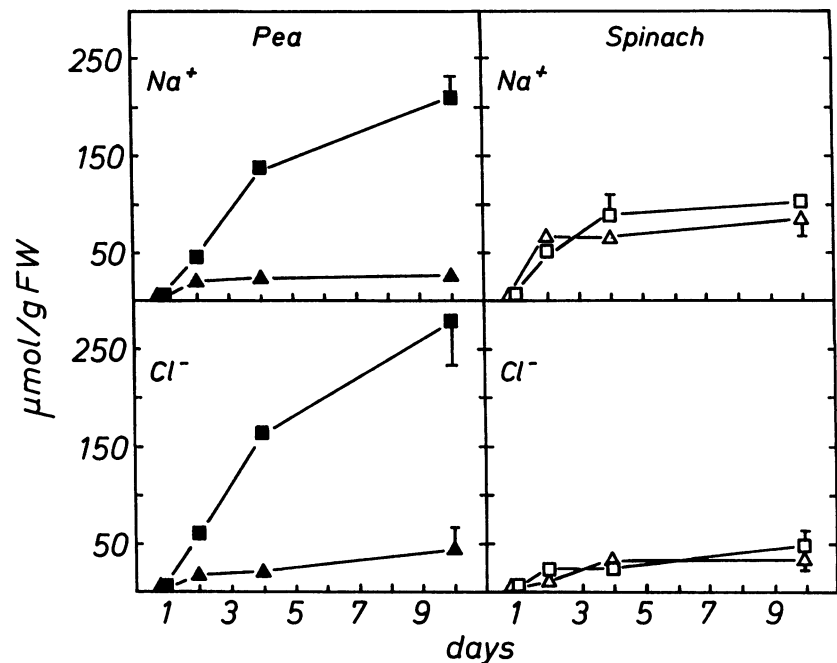

Figure 1. Total sodium and chloride concentrations of leaves and roots from pea and spinach during a 10-d salinity treatment (100 mM NaCl). Pea: (▲) root, (■) leaves; spinach: (△) root, (□) leaves. Each point represents the mean of six replicates. Bars depict maximum SD of each curve.

regained by centrifugation, should be diluted by apoplastic water. To determine the degree of dilution as precisely as possible, the infiltration medium was labeled with [^{14}C]sorbitol, which does not permeate the plasmalemma of cells within the time span of the experiment. Radioactivity was measured in the infiltration medium and in IWF after centrifugation. This determination was carried out only with fully hydrated leaf discs or leaflets, in order to minimize water uptake from the hypotonic [^{14}C]sorbitol medium. It was assumed that the resulting apoplastic water space remained constant under all further treatments, which is not necessarily true (1).

(c) The total V_{il} was usually determined as the difference in fresh weight before and after infiltration. If no water fluxes into or out of cells would occur, V_{il} should be identical with the V_{air}. As a control, V_{il} was determined not only by infiltration with hypotonic solution, but also by infiltrating silicone oil. The latter does not withdraw any water from the cells, and should give (after correction for oil density) the actual

Table I. *Basic Data for the Estimation of Apoplastic Solute Concentrations of Spinach and Pea*

For these measurements, fully hydrated leaf discs/leaflets were used to minimize water uptake from the hypotonic [^{14}C]sorbitol medium.

Method	Spinach	Pea
	air space volume (% of fresh wt)	
Silicone oil infiltration	4[illegible].5 ± 6.6 (*n* = 12)	42.3 ± 2.9 (*n* = 20)
Infiltration with hypotonic [^{14}C]sorbitol	45.7 ± 4.7 (*n* = 12)	46 ± 2 (*n* = 12)
	apoplastic water volume (% of fresh wt)	
[^{14}C]Sorbitol dilution	10.9 ± 1.4 (*n* = 12)	12.2 ± 2 (*n* = 18)

V_{air}. Table I shows that the two volumes were not far from each other. Both in pea and in spinach leaves, V_{air} was 42 to 46% of total tissue volume (see Table I).

V_{apo} was calculated according to Equations 1 and 2:

$$\frac{V_{air} + V_{apo}}{V_{air}} = \frac{dpm_{med}}{dpm_{IWF}} \times F \quad (1)$$

whereas dpm_{med} and dpm_{IWF} give the radioactivity per unit volume of the infiltration medium (before infiltration) and in the intercellular washing fluid, respectively. F represents the ratio of V_{air} measured by infiltrating hypotonic solution to V_{air} measured by silicone oil infiltration. F corrects for the concentrating effect of water uptake by the cells, which would have led to a slight underestimation of [^{14}C]sorbitol dilution.

Conversion of Equation 1 allows the calculation of V_{apo}:

$$V_{apo} = \frac{dpm_{med} \times V_{air}}{dpm_{IWF}} - V_{air} \times F \quad (2)$$

As mentioned above, IWF was usually obtained by infiltration of leaves with isotonic sorbitol media, whereas volumes were obtained with hypotonic sorbitol solutions. However, it has been shown earlier that V_{air} in mesophytic leaves is constant over a wide range of osmolalities (11).

The values determined for V_{apo} are consistent with those obtained by others with different methods: 7.2% in cotyledons of *Phaseolus vulgaris* (2), 5.4% in *Suaeda maritima* leaves (8), and 14% in *Citrus sinensis* leaves (14). In general, air space volume of leaves occupies up to 50% of the tissue volume (19), which is in good agreement with our values for spinach and pea. For the calculation of apoplastic ion concentrations on a molar basis, we assumed V_{apo} to remain constant during the time of salt treatment, although there are indications that it will decrease by 20 (8) to 50% (14) under salinity. Thus, there is only a danger for overestimating V_{apo} by our

method, and this would even strengthen our argument (see below).

Spinach and pea plants were exposed to salinity under otherwise identical growth conditions, but at different plant age (see "Materials and Methods").

Figures 2 and 3 illustrate the distribution of sodium and chloride between symplastic and apoplastic space of both species during a 17 d period after salt addition.

Oertli's (20) hypothesis claims that a rise in apoplastic ion concentrations should precede cell death, not be a result of it. This is partly confirmed by our results for Na^+ and Cl^-. Apoplastic salt concentrations in pea leaflets started to rise briefly after the onset of salt treatment (Figs. 2 and 3). Leaflets showed no visible symptoms of damage yet, when apolastic salt concentrations had already increased to about 100 mM Cl^- and 60 mM Na^+ after 10 d of salt treatment.

We have to emphasize that the data for pea from days 12 to 17 represent a rather optimistic estimation of salt accu-

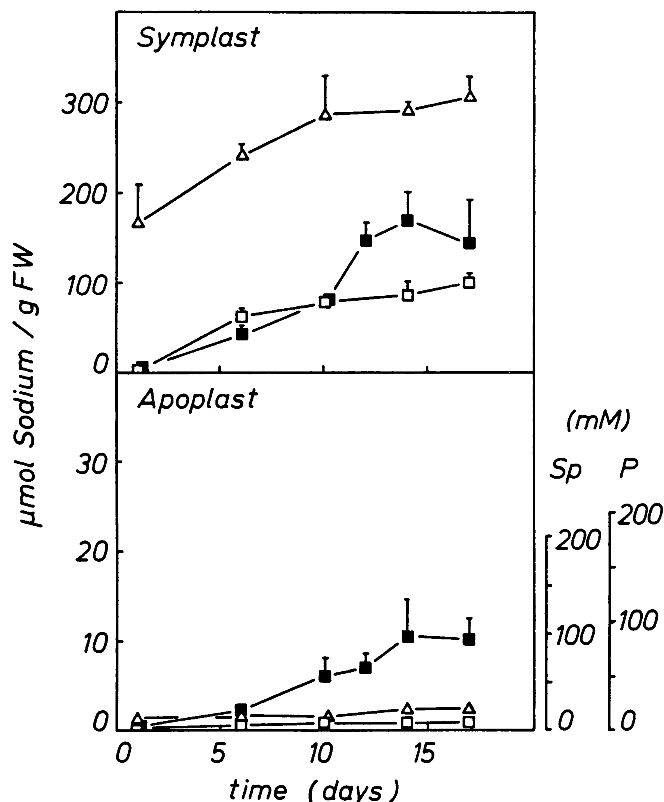

Figure 2. Sodium distribution between symplastic and apoplastic space of leaves from spinach and pea during a 17-d salinity treatment. Pea (■) and spinach (□) were stressed with 100 mM NaCl by a one-step addition to the nutrient solution on day 1. The first sampling of spinach at 300 mM external NaCl (△) was done just before the last salt concentration increment (see "Materials and Methods"). The right scale expresses the apolastic sodium concentrations on a molar basis for spinach (Sp) and pea (P) according to our estimation of apoplastic water volume. Each point represents the mean of at least five replicates ± SD. Maximum symplast (and apoplast) concentrations in control leaves of spinach were 0.63 (0.09), and in pea 0.2 (0.15) µmol/g fresh weight.

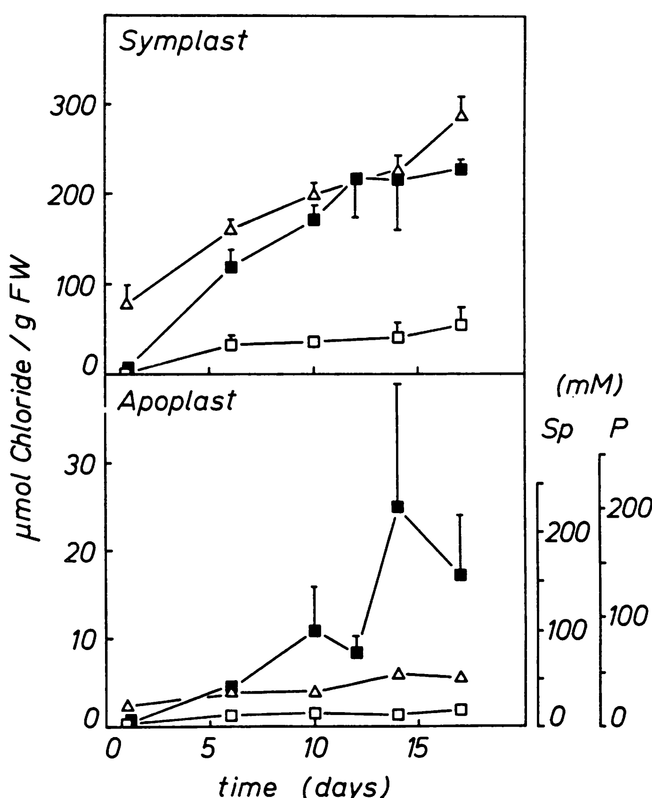

Figure 3. Chloride distribution between symplastic and apoplastic space of leaves from spinach and pea during a 17-d salinity treatment (for conditions and symbols, see Fig. 2). Each point represents the mean of at least five replicates ± SD. Maximum symplast (and apoplast) concentrations of control leaves of spinach were 1.45 (0.59), and in pea 3.9 (0.75) µmol/g fresh weight.

mulation for two reasons: (a) for proper infiltration we selected still rather healthy looking individual plants; (b) concentrations are calculated from the fresh weight of the whole leaflet. However, development of necrotic spots began almost exclusively at the leaflet margins. At day 14, the total Cl^- and Na^+ concentrations at the margins (roughly defined as the area about 5 mm from the leaflet edge surrounding the leaflet center) were 340 and 200 µmol/g fresh wt, respectively. In the center of the leaflet, the respective values were 195 µmol/g fresh wt Cl^- and 120 µmol/g fresh wt Na^+. It is justified to assume that apoplastic salt concentrations at the margins were also higher than shown by Figures 2 and 3. Salt accumulation in leaflet margins probably reflects a transpiration-mediated distribution pattern (17). The connection between high local salt contents and leaf burns was also noted by others (14, 17, 20, 26).

Even at high external salinity (300 mM), spinach leaves maintained a strong salt gradient between symplastic (vacuolar + cytosolic) and apoplastic space (Figs. 2 and 3). Ten days after the last increment of the external salt concentration (see "Materials and Methods"), the ratios of Cl^-_{sym}/Cl^-_{apo} and Na^+_{sym}/Na^+_{apo} (mM/mM; for calculation, see legend to Table II) were 7.09 and 27.7, respectively. In pea stressed only with

Table II. *Ion Concentrations (mM ± SD, n = 4–18) in Vacuoles (Vac), Cytoplasm (Cyt), and Apoplastic Space (Apo) in Leaves (C = Control; S = +Salt) of Pea and Spinach 10 d after Addition of 100 mM NaCl*

Measured values were concentrations in total leaf tissue, in the apoplast, and in isolated chloroplasts. Data for chloroplasts are not listed separately because they are considered to be identical with concentrations in the cytosol and, of course, in total cytoplasm. Vacuolar volume (per g fresh wt) was calculated by subtraction of (mg dry wt + μL Cyt + μL Apo)/g fresh wt. Cytosolic volume (per g fresh wt) was assumed to equal the chloroplastic volume (per g fresh wt), therefore $V_{cytoplasm} = 2 \times V_{chloro}$. The latter were 22 ± 4 μL/mg Chl (n = 4) (and 1.78 mg Chl/g fresh wt) for pea, and 25 ± 5 (n = 4) μL/mg Chl (and 1.44 mg Chl/g fresh wt) for spinach. See Table I for apoplastic water volume. Resulting vacuolar volumes were 682 μL in pea and 704 μL in spinach (per g fresh wt). The sums of positive and negative charges are expressed in meq/L. Data were collected from different experiments carried out over a period of 2 years. C = control; S = +NaCl.

Ion		Pea ± 100 mM NaCl			Spinach ± 100 mM NaCl		
		Vac	Cyt	Apo	Vac	Cyt	Apo
				mM ± SD[a]			
K^+	C	104 ± 23	53 ± 5	13 ± 4	260 ± 31	147 ± 25	10 ± 4
	S	42 ± 12	29 ± 17	14 ± 4	172 ± 33	78 ± 18	18 ± 4
Na^+	C	3 ± 1	19 ± 4	1.5 ± 1	0.5 ± 0.1	26 ± 16	1 ± 1
	S	114 ± 7	60 ± 19	50 ± 16	109 ± 15	43 ± 15	6 ± 1
Mg^{2+}	C	12 ± 3	6 ± 2	4 ± 2	37 ± 6	17 ± 6	2 ± 1
	S	6.5 ± 1	4 ± 3	2 ± 1	29 ± 5	15 ± 7	3 ± 1
Cl^-	C	4 ± 1	5 ± 3	6 ± 2	1 ± 1	6 ± 5	4 ± 1
	S	245 ± 34	60 ± 35	89 ± 67	50 ± 5	9 ± 4	14 ± 6
NO_3^-	C	33 ± 15	6 ± 2	2.1 ± 1.1	29 ± 13	10 ± 6	5 ± 1
	S	0	1 ± 1	0	19 ± 8	4 ± 1	6.6 ± 5
SO_4^{2-}	C	28 ± 15	7 ± 5	0.7 ± 0.16	2 ± 1	19 ± 11	0.45 ± 0.18
	S	15 ± 4	4 ± 1	1.6 ± 1.1	1.4 ± 0.5	10 ± 8	0.83 ± 0.2
HPO_4^{2-}	C	24 ± 5	12 ± 9	2 ± 1	16 ± 5	15 ± 11	1.3 ± 0.5
	S	6 ± 2	3 ± 0.5	1.6 ± 1.2	16 ± 3	9 ± 7	2 ± 0.9
Mal^{2-}	C	74 ± 10	3 ± 1	3.4 ± 1.7	3.6 ± 0.6	2 ± 2	0
	S	2.5 ± 5	1 ± 1	0.32 ± 0.5	1.2 ± 0.1	1 ± 2	0
$\sum Cat^+$	C	131	84	22.5	334.5	207	15
	S	169	97	68	339	151	30
$\sum An^-$	C	289	55	20.3	73.2	88	12.5
	S	292	77	96	106.2	53	26.3

[a] n = 4 to 18.

100 mM NaCl, the respective gradients were 2.81 and 4.30. Clearly, spinach has a much higher capacity to maintain salt concentration gradients between symplast and apoplast. In addition to the remarkably different symplastic and apoplastic salt concentrations, both species showed further characteristic differences in leaf ion relations.

Symplastic potassium concentrations (Fig. 4) decreased in salt treated plants to about 52% in spinach and 20% in pea relative to control leaves after 17 d. Ion uptake and/or transport competition under salinity has often been described (*e.g.* 5, 16, 22, 25). Obviously, pea has a less well developed selectivity for the ion pair K^+/Na^+, and also for NO_3^-/Cl^- (Table II). Apoplastic potassium concentrations in the pea mesophyll (Fig. 4) are in good agreement with those obtained by Long & Widders (15), who used an elution analysis of stripped pea leaflets. In both species, apoplastic potassium concentrations in salt treated leaves were above control levels, probably indicating a displacement of potassium from fixed anionic exchange sites in the cell wall. The normal K^+ gradient between symplastic and apoplastic space of pea leaflets was almost completely abolished after 17 d of salt treatment.

Figure 5 illustrates the distribution of phosphate between symplast and apoplast of both species. Symplastic phosphate concentration decreased in salt-treated pea leaves relative to controls, suggesting an unspecific ion uptake and/or transport competition at high external sodium and chloride concentrations. In salt-treated spinach leaves, symplastic phosphate concentration was even increased compared with controls. Apoplastic phosphate concentration in spinach leaves remained fairly constant over the whole assay period. The drastic increase of this solute in pea leaflets between days 12 to 17 is probably indicative for the onset of severe damage in parts of the mesophyll tissue. It is assumed that the increase results from a liberation of intracellular phosphate to the apoplast. We also measured apoplastic sulfate and magnesium concentrations, which behaved in a similar way (data not shown).

Partial disintegration of mesophyll cells might reflect

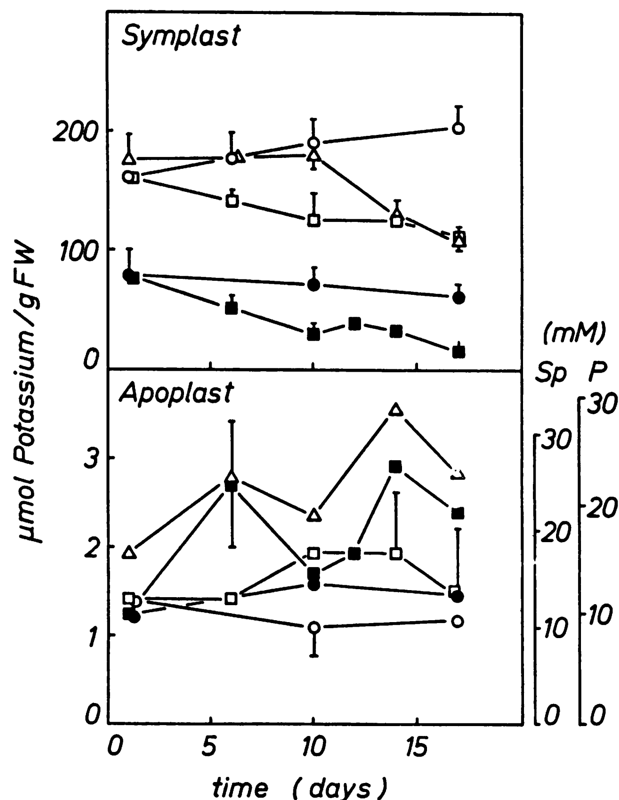

Figure 4. Potassium distribution between symplastic and apoplastic space of leaves from spinach and pea during a 17-d salinity treatment (conditions described in Fig. 2). Symbols: pea leaflets (●, control; ■, +100 mM NaCl); spinach leaves (○, control; □, +100 mM NaCl; △, +300 mM NaCl). Each point represents the mean of at least five replicates; bars (in the lower figure) depict the maximum SD of each curve.

plasma membrane damage from the outside by high apoplastic salt concentrations (4), or from inside by high salt concentrations in the cytoplasm.

Solute Distribution between Vacuole, Cytoplasm, and Apoplast

In Table II, we have combined data on solute concentrations in leaf tissues and apoplast with data from pea and spinach chloroplasts, isolated by aqueous procedures after 10 d of salinity treatment (100 mM NaCl). From these data, solute concentrations were calculated in the apoplast, cytoplasm, and vacuole of pea and spinach leaves (for details of the calculation see legend to Table II).

As expected, concentrations of most inorganic solutes were highest in the vacuole, medium in the cytoplasm, and lowest in the apoplast. In spinach, we usually found a large cation over anion excess. This is a common observation (22, 23) and can be explained by the presence of anionic macromolecules and by acidic amino acids, carrying a net negative charge (22). In peas, the situation was different. Here, the vacuole contained a large anion excess. In control plants, it was caused mainly by the very high malate concentration. However, due to a pK_a of 5.11 for malate and 7.21 for phosphate, at the low vacuolar pH many of these ions will be protonized and will thus not count as much for charge balance considerations. Under salinity, vacuolar malate, nitrate, and phosphate levels decreased, but the excess uptake of chloride over sodium again caused anion excess. How charge balance was maintained under these conditions remains obscure. However, the comparison between leaf sap osmolality and the sum of measured solutes (Table III) indicates that all major osmotically relevant solutes of both species were detected by our analysis.

Concentration gradients across the plasmalemma were usually much larger than for the tonoplast. However, there were some remarkable exceptions. In nonsalinized, low Na^+ plants (both species), Na^+ appeared to be accumulated in the cytoplasm, but Cl^- was almost equally distributed between the three spaces. A cytosolic sulfate accumulation was observed in spinach, but not in pea. In peas, magnesium was rather evenly distributed among the three compartments. Nitrate

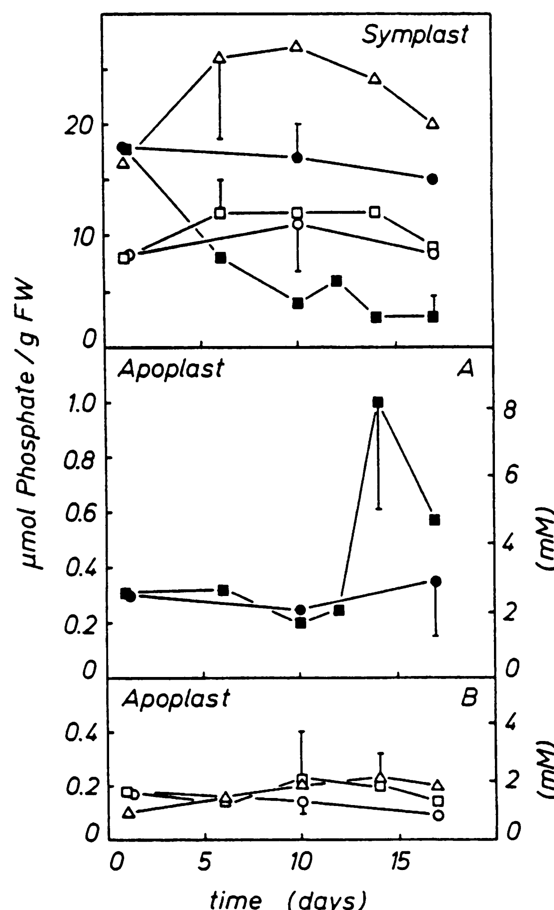

Figure 5. Phosphate distribution between symplastic and apoplastic space of leaves from pea (A) and spinach (B) during a 17-d salinity treatment (conditions as in Fig. 2; symbols, see Fig. 4). Means of at least five replicates; bars depict the maximum SD of each curve.

Table III. *Comparison between Measured Osmolality (mosmol/kg) and the Sum (mol/L) of Measured Solutes (without Amino Acids and Sugars) in Pea and Spinach Leaves 10 d after the Addition of 100 mm NaCl to the Nutrient Solution*

C = control; S = +NaCl. Amino acid concentrations in leaf extracts are approximately 25 μmol/g fresh wt in pea and spinach. Concentrations of reducing sugars for pea and spinach are approximately 30 and approximately 15 to 40 μmol/g fresh wt, respectively.

	Pea		Spinach	
	Osmolality	Sum of solutes	Osmolality	Sum of solutes
C	333	291	410	367
S	535	446	555	411

was preferentially accumulated in the vacuole. For nitrate, we observed a very strong depletion in all compartments of salt-treated pea leaves, which confirms earlier findings (23, 24).

Thus, concerning salt gradients across plasmalemma and tonoplast of the mesophyll, two aspects appear most remarkable (see Tables II and IV):

Under salinity, Na^+ and Cl^- gradients of similar magnitude were built up across the tonoplast in both pea and spinach. But whereas spinach maintained a low sodium and chloride concentration in the apoplast, and also a low chloride concentration in the cytoplasm, peas were not able to do so.

Therefore, we believe that the differential salt tolerance of pea and spinach is not only—or perhaps not at all—due to a differential ability to maintain concentration gradients across the tonoplast, but to an ability to control total salt fluxes into the leaf apoplast, and from there into the cells. During prolonged salt treatment, the highly loaded pea apoplast favors salt flux into the symplast. Even if intracellular salt gradients (shown in Tables II and IV) were maintained during days 10 to 17 after salt addition, absolute salt concentrations (especially Cl^-) in the cytoplasm will rise and cause enzyme inhibition (12) and breakdown of metabolism.

It is well known that under salinity, spinach accumulates quarternary ammonium compounds in metabolic compartments (22). Until now, we found no organic solute that could play a similar role in peas, except a slight accumulation of sugars and amino acids (24). Pinitol as a possible candidate in peas was not accumulated under our conditions (M. Popp, personal communication). Thus, insufficient intracellular osmotic compensation by compatible solutes might be another decisive factor for the high salt sensitivity of pea.

Table IV. *Concentration Gradients across the Plasmalemma ([Cyt]/[Apo]) and the Tonoplast ([Vac]/[Cyt]) of Cl^- and Na^+ in Leaves of Spinach and Pea 10 d after the Addition of 100 mm NaCl to the Nutrient Solution*

C = control; S = +NaCl. Data from Table II.

		Pea		Spinach	
		Plasmalemma	Tonoplast	Plasmalemma	Tonoplast
Cl^-	C	0.83	0.8	1.5	0.17
	S	0.67	4.1	0.64	5.6
Na^+	C	12.7	0.16	26	0.02
	S	1.2	1.9	7.2	2.53

LITERATURE CITED

1. **Andersen MN, Jensen CR, Lösch R** (1991) Derivation of pressure-volume curves by a nonlinear regression procedure and determination of apoplastic water. J Exp Bot **42:** 159–165
2. **Briarty LG** (1980) Stereological analysis of cotyledon cell development in *Phaseolus* I. Analysis of a cell model. J Exp Bot **31:** 1379–1386
3. **Cosgrove DJ, Cleland RE** (1983) Solutes in the free space of growing stem tissues. Plant Physiol **72:** 326–331
4. **Cramer GR, Läuchli A, Polito VS** (1985) Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells. Plant Physiol **79:** 207–211
5. **Erdei L, Kuiper PJC** (1979) The effect of salinity on growth, cation content, Na^+-uptake and translocation in salt-sensitive and salt-tolerant *Plantago* species. Physiol Plant **47:** 95–99
6. **Flowers TJ, Yeo AR** (1986) Ion relations of plants under drought and salinity. Aust J Plant Physiol **13:** 75–91
7. **Greenway H, Munns R** (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol **31:** 149–190
8. **Hajibagheri MA, Hall JL, Flowers TJ** (1984) Stereological analysis of leaf cells of the halophyte *Suaeda maritima* L. Dum. J Exp Bot **35:** 1547–1557
9. **Heldt HW, Werdan K, Milovancev M, Geller G** (1973) Alkalization of the chloroplast stroma caused by light dependent proton flux into the thylakoid space. Biochim Biophys Acta **314:** 240–241
10. **Johnson C, Stout P, Broyer T, Carlton A** (1957) Comparative chlorine requirements of different plant species. Plant Soil **8:** 337–353
11. **Kaiser WM** (1982) Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso- and xerophytes under osmotic stress. Planta **154:** 538–545
12. **Kaiser WM, Schröppel-Meier G, Wirth E** (1986) Enzyme activities in an artificial stroma medium. Planta **167:** 292–299
13. **Leigh RA, Tomos AD** (1983) An attempt to use isolated vacuoles to determine the distribution of sodium and potassium in cells of storage roots of red beet (*Beta vulgaris* L.). Planta **159:** 469–475
14. **Lloyd J, Kriedemann PE, Aspinall D** (1990) Contrasts between *Citrus* species in response to salinisation: an analysis of photosynthesis and water relations for different rootstock-scion combinations. Physiol Plant **78:** 236–246
15. **Long JM, Widders IE** (1990) Quantification of apoplastic potassium content by elution analysis of leaf lamina tissue from pea (*Pisum sativum* L. cv Argenteum). Plant Physiol **94:** 1040–1047
16. **Lynch J, Läuchli A** (1984) Potassium transport in salt-stressed barley roots. Planta **161:** 295–301
17. **Marschner H** (1986) Mineral Nutrition of Higher Plants. Academic Press, London
18. **Munns R, Passioura J** (1984) Effect of prolonged exposure to NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring barley plants. Aust J Plant Physiol **11:** 497–507
19. **Nobel PS** (1974) Introduction to Biophysical Plant Physiology. Freeman & Co, San Francisco
20. **Oertli JJ** (1968) Extracellular salt accumulation, a possible mechanism of salt injury in plants. Agrochimica **12:** 461–469

21. **Robinson SP** (1985) Osmotic adjustment by intact isolated chloroplasts in response to osmotic stress and its effect on photosynthesis and chloroplast volume. Plant Physiol **79:** 996–1002
22. **Schroeppel-Meier G, Kaiser WM** (1988) Ion homeostasis in chloroplasts under salinity and mineral deficiency. I. Solute concentrations in leaves and chloroplasts from spinach plants under NaCl or $NaNO_3$ salinity. Plant Physiol **87:** 822–827
23. **Schroeppel-Meier G, Schmidt W, Kaiser WM** (1988) Solute distribution between chloroplasts and extrachloroplastic space in leaves from a glycophyte (*Pisum sativum*) under salinity. Plant Physiol (Life Sci Adv) **7:** 217–222
24. **Speer M, Schmidt W, Kaiser WM** (1989) Solute concentrations in roots, leaves and chloroplasts of peas and spinach under salinity. *In* M Tazawa, M Katsumi, Y Masuda, H Okamoto, eds, Plant Water Relations and Growth under Stress, Yamada Conference. Osaka and Myu KK, Tokyo, pp 101–108
25. **Subbarao GV, Johansen C, Jana MK, Kuma Rao JVDK** (1990) Physiological basis of difference in salinity tolerance of pigeonpea and its related wild species. J Plant Physiol **137:** 64–71
26. **Zid E, Grignon C** (1985) Sodium-calcium interactions in leaves of *Citrus aurantium* grown in the presence of NaCl. Physiol Veg **23:** 895–903