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Transport of Rb and Sr to the ear in mature, excised shoots of wheat: Effects of temperature and stem length on Rb removal from the xylem

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Abstract

Wheat (*Triticum aestivum* L. cv. 'Arina') shoots grown in the field were excised post-anthesis and incubated in the laboratory for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. Strontium is a phloem-immobile, xylem-mobile element and indicates the distribution of the xylem sap in the plant. Rubidium is easily transported in the phloem and behaves similarly to the highly mobile K as far as the redistribution within the plant is concerned, although Rb cannot substitute physiologically or biochemically for K. The Sr contents in the ear were hardly affected by stem length or by steam-girdling (phloem-interruption). Rubidium on the other hand accumulated in the stem. A peduncle length of 5 cm was sufficient to decrease the Rb concentration in the xylem by more than 50% at 25°C. Only a minor quantity of Rb reached the ear after passing through 20 cm of stem without nodes and this transport was prevented by steam-girdling. A remarkable flux of Rb into the ear was observed in shoots with a vascular connection between the flag leaf lamina and the ear. Our results suggest that Sr was transported with the transpiration stream, while Rb was rapidly eliminated from the xylem and reached the ear via the phloem. The temperature optimum for the removal of Rb from the xylem was around 35°C. The nodes may further contribute, but are not prerequisites for this redistribution. The observed transfer processes could allow a solute specific transport via the xylem and phloem of maturing cereals and may be an important factor influencing the nutrient economy in the field.

Introduction

Solutes can be exchanged between the xylem and the phloem and such interactions may be important for the regulation of solute distribution in intact plants (Dickson *et al.*, 1985; McNeil, 1980; Pate, 1975; Peel, 1963; Simpson, 1986; Wolterbeek and de Bruin, 1986). Transfer cells, which have been identified in the stem and in leaf veins of several plant species, may be involved in the unloading of solutes from the xylem (Kuo *et al.*, 1980; Pate, 1975). The phloem (symplast) and the xylem (apoplast) are separated by at least one membrane. The carrier proteins present in the membrane(s) may determine the specificity of the solute transfer (Klotz and Erdei, 1988). Potassium flux into maturing cereal grains was found to be regulated by a transfer from the xylem to the phloem in the stem (Haeder and Beringer, 1984a; b). The energy status and membrane potentials are likely to be important factors for solute transfer from xylem to phloem (de Boer *et al.*, 1985; Kuppelwieser and Feller, 1990; Marschner, 1986). However, the precise localization of the transfer processes mentioned, the mechanisms involved and the regulatory properties are still largely unknown.

Potassium is one of the most mobile elements in higher plants and is easily translocated in the

xylem and in the phloem (Marschner, 1986; Pate, 1975). Trace concentrations of Rb behave similarly to the macronutrient K in cereals and Rb is frequently used for tracer studies (Erdei and Zsoldos, 1977; Haeder and Beringer, 1984a, b; Marschner and Schimansky, 1971; Martin, 1982). Potassium (Haeder and Beringer, 1984a) and Rb (Feller, 1989; Haeder and Beringer, 1984a, b) are transferred from the xylem to the phloem in the stem of cereals. In contrast, Ca and Sr, which are merely immobile in the phloem, are transported in the xylem with the transpiration stream (Hylmö, 1953) and are essentially not redistributed afterwards (Marschner, 1986; Mengel and Kirkby, 1987). Calcium and Sr can be retained in the stem by ion exchange (exchangeable fraction) or irreversibly (non-exchangeable fraction). In bean stems the ion exchange was found to be completed within a few hours and contributed only a minor percentage to the total Ca within the corresponding stem section (Biddulph *et al.*, 1961). The velocity of this ion exchange depended on the concentrations of ions available to the plants (Bell and Biddulph, 1963). Labelled Ca was replaced easily by unlabelled Ca, Sr and Mg, while K ions were far less effective (Bell and Biddulph, 1963).

The xylem and the phloem contents are both transported upwards in the peduncle to the maturing ear of wheat. A phloem interruption in the stem of cereals is easily achieved by steam-girdling (Martin, 1982). We used this system to investigate the effectiveness of the removal from the xylem in wheat internodes and the temperature dependence of this unloading in order to contribute to the physiological characterization of interactions between xylem and phloem.

Methods

Winter wheat (*Triticum aestivum* L. cv. 'Arina') was grown in a field near Bern. At different stages after ear emergence plants were detached above the soil surface, recut submerged in distilled water and transported to the laboratory standing in distilled water. Some shoots were steam-girdled below the ear according to Martin (1982). Prior to starting the experiments the submerged stem was cut with a razor blade to the desired length.

A preliminary experiment to examine variation of the incubation time was performed shortly after ear emergence (around anthesis), when the basal part of the peduncle was still soft. The first set of experiments (comparison of shoots with steam-girdled and ungirdled peduncles) was started one week after anthesis. The individual shoots were incubated for 72 hours standing in small flasks with a sufficient volume of feeding solution (2 m*M* RbCl + 2 m*M* $SrCl_2$) in a light/dark cycle (14 h 120 μE m^{-2} sec^{-1} from 4 Philips TL 40 W/33 and 2 Osram Fluora fluorescent tubes, 24–26°C and 10 h dark, 21–23°C). The experiment with shoots detached above the third leaf from the top was carried out 2 weeks after anthesis under the same conditions.

The influence of stem temperature on solute fluxes was investigated 3 weeks after anthesis using detached ears with 18 cm stem, which were fixed with foam rubber below the ear in glass tubes with 10 mL feeding solution (2 m*M* RbCl + 2 m*M* $SrCl_2$). The cut end of the stem reached 4 cm into the feeding solution, the upper part of the stem was surrounded by air in the glass tube and the ear was outside the tube in ambient air. The tubes were placed in water baths with constant temperatures (0, 24, 30, 35 or 40°C) and the plants were incubated for 72 h in a light/dark cycle as mentioned above except that the photosynthetic active radiation was reduced to 100 μE m^{-2} sec^{-1} (Philips TL 40 W/33 fluorescent tubes only). The room temperature (relevant for the ear) was 22–23°C in the light and 20–21°C in the dark phase.

Evapotranspiration was determined gravimetrically (repeated weighings of the flasks with the feeding solution and the plant). In order to calculate net transpiration of plants the evaporation was measured in flasks of the same size with the same quantity of feeding solution and subtracted from the evapotranspiration. The evaporation was always far below the transpiration.

Five separately incubated shoots were analyzed for each treatment in the main experiments. The separated plant parts were dried at 105°C and analyzed for Rb and Sr contents by

atomic absorption spectrophotometry as described previously (Feller, 1989).

Results and discussion

Strontium and Rb fed into the cut xylem of detached wheat shoots were distributed differently (Fig. 1, Table 1). The appearance of Sr in the ear after 1 day indicates that the exchangeable fraction in the 25 cm peduncle was already saturated and Sr entered the ear with the transpiration stream. Strontium accumulated in a very similar manner in the ear of shoots detached above or below the flag leaf node. The amounts of Sr in the stem and in the ear were similar except during the initial phase (ion exchange in the peduncle). Highest Sr contents were detected in the flag leaf and presumably reflect a high transpiration rate of this organ. Rubidium behaved quite differently from Sr. The smaller Rb contents in shoots detached above the flag leaf node were consistent with a transfer of Rb to the phloem and the release of small amounts of phloem sap into the feeding solution as proposed previously (Feller, 1989). Total amounts of Rb and Sr were similar in shoots cut below the flag leaf node suggesting that the node might act as a valve. Only minor quantities of Rb reached the ear of shoots detached above the node. This preliminary experiment and observations reported earlier (Feller, 1989) were the basis for a more detailed investigation of the Rb removal from the xylem sap in the peduncle.

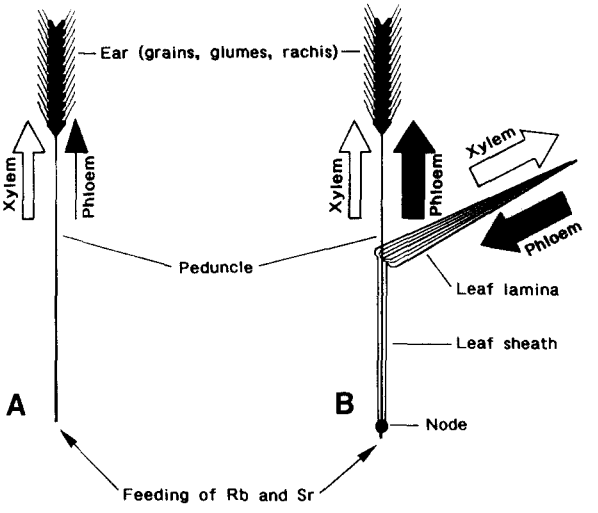

Fig. 1. Scheme for xylem and phloem fluxes in wheat shoots detached above (**A**) or below (**B**) the flag leaf node. The xylem transport in the peduncle (stem) is not affected by the node in short-term experiments. The flag leaf can act as a phloem source. Therefore the phloem transport in the peduncle of detached shoots strongly depends on the presence of the node allowing the redistribution of solutes from the leaf to the ear.

The Rb contents in the ear of wheat shoots detached above the flag leaf node strongly depended on the peduncle length, while the Sr accumulation was relatively constant (Fig. 2). The Sr contents of the whole shoots were well correlated with the transpiration (Table 2). Accumulation of Sr in the stem was most likely a result of transpiration from the stem itself and to a minor extent of ion exchange processes in the xylem. Initially exchangeable Ca previously present in xylem walls may be replaced by Sr thereby decreasing the Sr concentration in the xylem sap entering the ear. It was assumed that the transpi-

Table 1. Distribution of Rb and Sr in detached wheat shoots fed through the cut xylem with 2 m*M* RbCl + 2 m*M* $SrCl_2$. The plants were collected around anthesis from the field and incubated in a culture room (14 h light/10 h dark). The flag leaf node was eliminated by cutting 25 cm below the ear. Means of 2 independent replicates are shown

Part of the shoot	Cut above the flag leaf node[a]				Cut below the flag leaf node	
	1 day incubated		4 days incubated		4 days incubated	
	Rb (μg plant^{-1})	Sr	Rb	Sr	Rb	Sr
Ear	6	32	64	368	355	357
Stem	32	116	109	364	854	422
Leaf (sheath + lamina)	–	–	–	–	871	1464
Total	38	148	173	732	2080	2243

[a] The peduncle 25 cm below the ear.

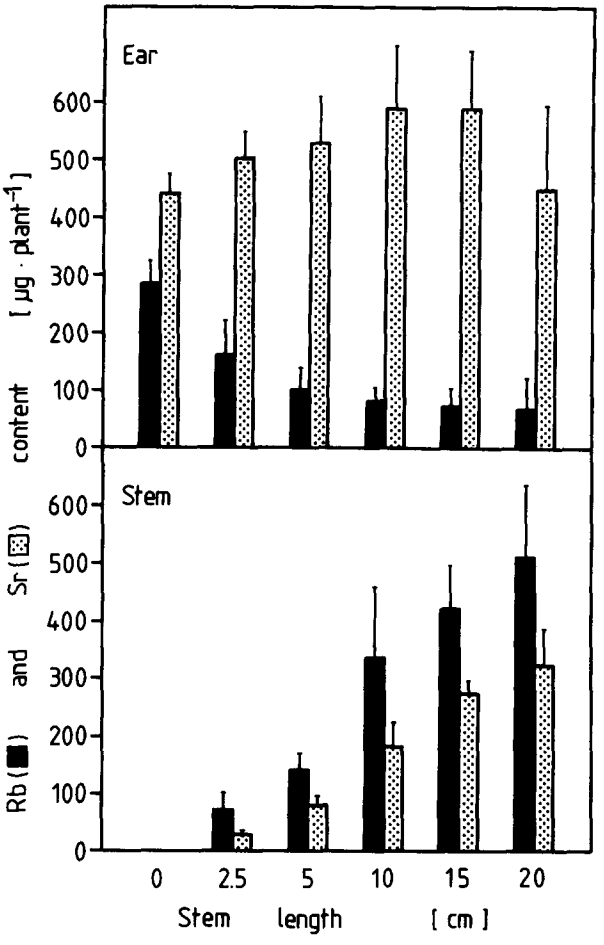

Fig. 2. Fluxes of Rb and Sr in ungirdled wheat. The shoots were detached one week after anthesis at different distances from the ear and incubated for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. Rubidium and Sr contents were analyzed in the ear (containing the uppermost 2 cm stem) and in the remaining stem (length indicated). Means and standard deviations of 5 replicates are shown.

ration of the ear was not affected by the stem length and therefore the increased transpiration was caused by the transpiration of the longer stem. From these calculated transpiration values for the stem and from the Sr concentration in the feeding solution theoretical Sr contents were computed for a distribution via the transpiration stream. The measured contents were always slightly higher and it appears likely that ion exchange processes (Sr replacing Ca) in the cell walls contributed to this difference. Therefore Sr content may be used as an indicator for the cumulative transpiration from the different parts of the shoots used in this system.

Rubidium on the other hand accumulated considerably in the stem (Fig. 2). Rubidium must have been rapidly eliminated from the xylem sap during its acropetal translocation in the xylem, since 2.5 cm stem were sufficient to decrease the Rb content of the ear by about 40%. A relatively constant Rb level was detected in the ears with 10, 15 or 20 cm stem. This Rb could derive from the xylem (incomplete removal of Rb from the xylem sap in the peduncle) or from the phloem (minor flux of phloem sap containing Rb into the ear). In order to distinguish between these possibilities, the phloem of some plants was interrupted as discussed below.

The interruption of the phloem below the ear (steam-girdling) caused no major changes in the Sr fluxes (Fig. 3 as compared to Fig. 2). In these shoots essentially no Rb entered the ear after passing through 20 cm stem. These results suggest that the Rb detected in the ears of ungirdled plants (Fig. 2) was transported via the phloem

Table 2. Transpiration and Sr contents in wheat stems (steam-girdled below the ear). The uppermost 2 cm stem including the girdling position were added to the ear and only the remaining stem length was analyzed. The transpiration of the stem was calculated by subtracting the transpiration of the ear with 0 cm stem from the transpiration of the shoots with a given stem length. Theoretical Sr contents were computed from the calculated transpiration of the stem and the Sr concentration in the feeding solution. Measurements are represented by the means ±SD of 5 replicates, while calculations are based only on the averages

Stem length (cm)	Transpiration (mL plant^{-1})		Strontium content in the stem (μg plant^{-1})	
	Shoot (stem + ear) Measured	Stem Calculated	Measured content	Calculated from transpiration data
0	2.87 ∓ 0.29	0	0	0
2.5	3.07 ± 0.51	0.20	41.0 ± 4.1	38.9
5.0	3.16 ± 0.58	0.29	79.1 ± 16.1	54.6
10.0	3.50 ± 0.30	0.64	175.6 ± 22.2	121.4
15.0	4.07 ± 0.42	1.20	282.4 ± 57.9	229.5
20.0	4.16 ± 0.42	1.29	342.5 ± 43.2	246.7

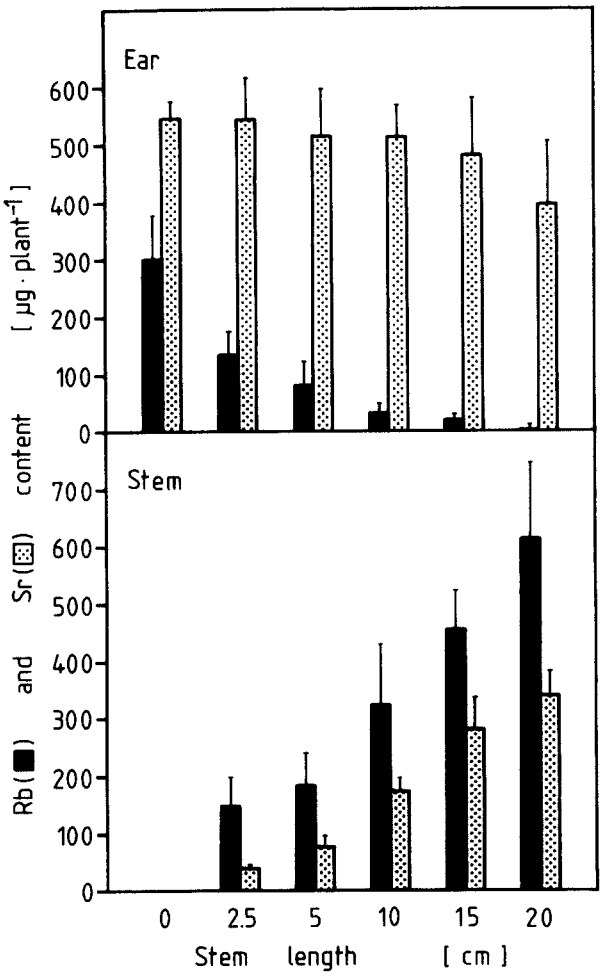

Fig. 3. Fluxes of Rb and Sr in wheat steam-girdled below the ear. The shoots were cut one week after anthesis at different distances from the ear and incubated for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. Rubidium and Sr contents were analyzed in the ear (containing the uppermost 2 cm stem including the girdling position) and in the remaining stem (length indicated). Means and standard deviations of 5 replicates are shown.

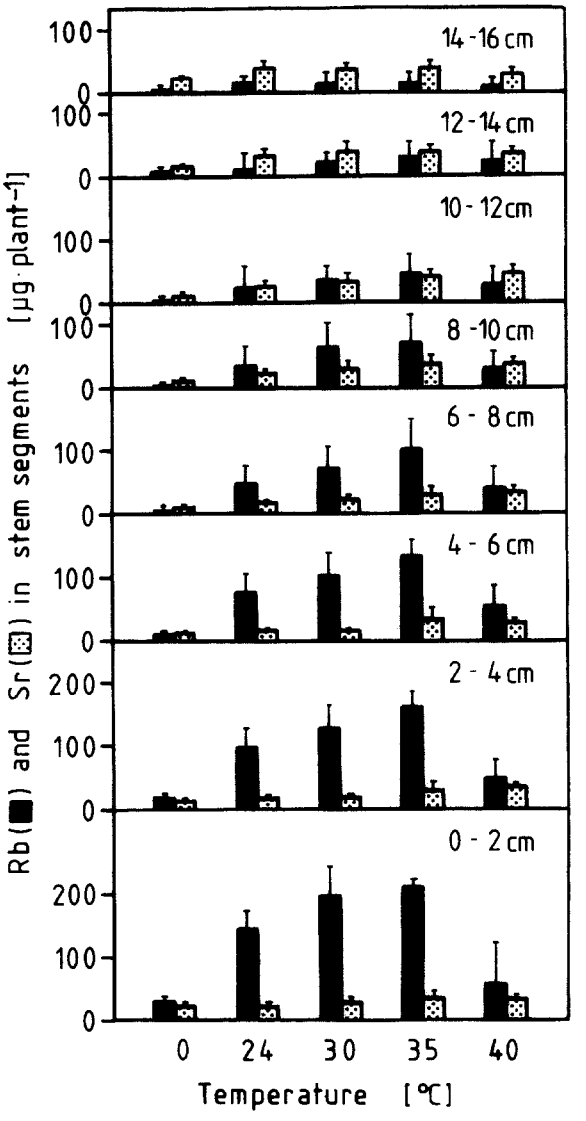

Fig. 4. Influence of the temperature on Rb accumulation in the stem of detached wheat shoots. Ears with 18 cm stem (steam-girdled below the ear) were incubated 3 weeks after anthesis for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. The ear was kept at ambient temperature (20–23°C) and only the stem was subjected to the temperature indicated. Rubidium and Sr contents in stem segments (beginning with 0–2 cm at the base) were analyzed. Means and standard deviations of 5 replicates are shown.

rather than in the xylem into the rachis. Apparently an effective and selective solute transfer from the xylem to the phloem must be present in wheat internodes, although it is not yet clear which tissues are involved in this transfer process. The nodes may contribute to this unloading, but are not a prerequisite for it.

More detailed information was obtained by analyzing 2 cm-segments of the stem and by dissection of the ear into rachis, glumes and grains (Figs. 4 and 5). Since these plants were collected at a different stage of maturation than those used for the experiments mentioned above, the transpiration of the ear and the Sr fluxes to the ear were altered. This fact may also explain the appearance of a small amount of Rb in ears of steam-girdled shoots kept at root temperature

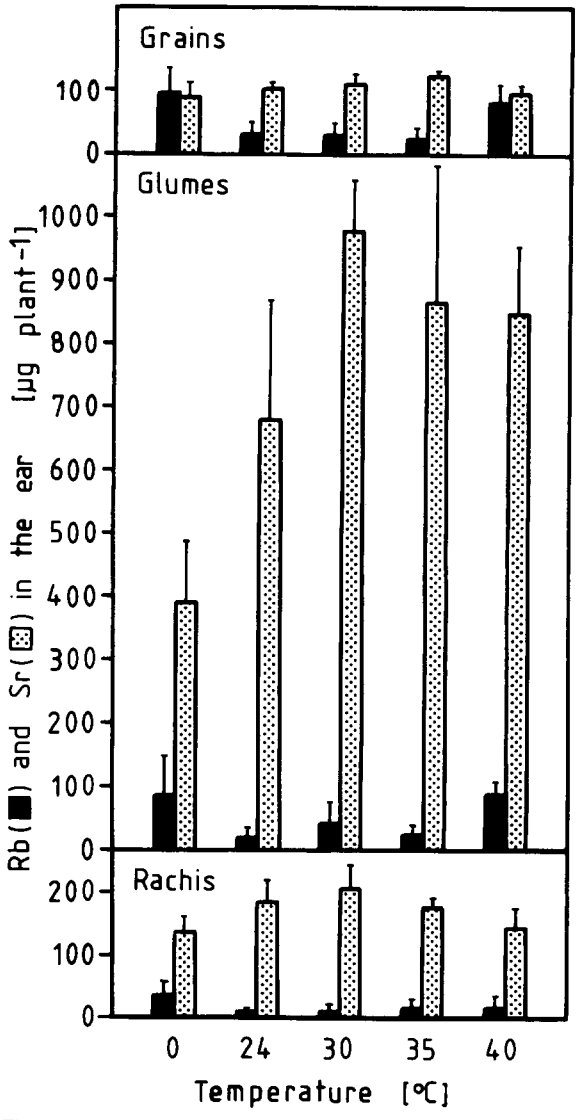

Fig. 5. Influence of the temperature on Rb and Sr fluxes into ears of detached wheat shoots. Ears with 18 cm stem (steam-girdled below the ear) were incubated 3 weeks after anthesis for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. The ear was kept at ambient temperature (20–23°C) and only the stem was subjected to the temperature indicated. The ear was divided into grains, glumes (containing glumes, lemmas and paleas) and rachis (including the uppermost 2 cm stem). Means and standard deviations of 5 replicates are shown.

(Fig. 5). The Rb accumulation in the stem strongly depended on the temperature with a maximal activity in the range of 30–35°C. At 0 or 40°C only minor quantities of Rb were retained in the stem (Fig. 4) and higher contents appeared in the ear. Steam-girdled plants were used for these experiments in order to interrupt the Rb flux via the phloem into the ear. Rubidium detected in rachis, glumes and grains of these shoots must have entered the ear via the xylem. In the optimal temperature range most of the Rb was accumulated in the 3 basal stem segments. Obviously, the elimination of Rb from the xylem takes place immediately below the ear (Fig. 3) or in lower parts of the same internode (Fig. 4). The higher Sr contents in the stem segments at elevated temperatures could be a consequence of a stimulated transpiration in the stem as suggested by the water consumption data in this experiment (Table 3).

Within the ear Sr accumulated mainly in the glumes (Fig. 5), while a considerable part of the Rb entering the ear was detected in the grains. Since the phloem was interrupted, Rb must have entered the ear with the transpiration stream. Some Rb may directly reach the grains via the xylem. A further redistribution within the ear is possible either by a transfer from the xylem to the phloem in the rachis or by the export from the glumes via the phloem. The variation of the stem temperature affected to some extent the Sr fluxes into the glumes, most likely by affecting their transpiration. However, this observation does not invalidate our conclusion that the Rb elimination from the xylem strongly depends on ambient temperature. At 0°C the transpiration and therefore the velocity of solute flow in the xylem were lower than at 24–35°C. Nevertheless, more Rb entered the ear via the xylem and much less accumulated in the stem at 0°C compared to 24–35°C (Figs. 4 and 5). The exchange of solutes between the xylem and the phloem in the uppermost internodes of maturing wheat may allow a specific transport of solutes to the grains

Table 3. Transpiration of detached wheat shoots incubated at different temperatures. Ears with 18 cm stem (steam-girdled below the ear) were incubated for 72 h standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$. The ear was kept at ambient temperature (20–23°C) and only the stem was subjected to the temperature indicated. Means ±SD of 5 replicates are shown

Stem temperature	Transpiration (mL plant^{-1})
0	4.02 ± 0.99
24	6.51 ± 1.14
30	8.15 ± 0.87
35	8.09 ± 0.89
40	7.03 ± 0.74

(phloem sinks) or to the glumes (phloem sources). Xylem sap is delivered to all parts of the ear and the fluxes depend on the relative transpiration rates. Since temperature strongly affects the unloading of Rb from the xylem, this external factor may considerably influence solute fluxes. Suboptimal temperatures might be especially relevant for plants in the field. Furthermore, net photosynthesis (important for source strength), grain filling (sink strength) and transfer processes during translocation may depend differently on ambient temperature.

In the experiments discussed above it remained open, whether the elimination of Rb from the xylem sap is restricted to the peduncle or takes place also in lower parts of the stem. Therefore plants were detached above the third leaf node from the top and the leaf laminas were removed in order to eliminate the major phloem sources (Fig. 6). From the distribution of Rb and Sr in these shoots it was evident that Rb can also be eliminated from the xylem sap in more basal internodes. Taking into account the age of the plants, the Sr contents in the ear were comparable to those detected in shorter systems (Figs. 2, 3, 5 and 6).

In trees, legumes or tomato plants the rays play a major role in the xylem-to-phloem transfer of solutes (Van Bel, 1990 and references therein). The symplastic transport from a xylem vessel to the phloem includes the transport across a membrane, the translocation within the ray and finally the release into the sieve tube/companion cell complex (Van Bel, 1990). In cereals the situation is quite different as far as the anatomy of the vascular bundles and therefore also the route for the xylem-to-phloem transfer are concerned. No ray cells are involved in such transfer processes in closed bundles with only a few xylem vessels and sieve tubes in close proximity. It must be borne in mind that trees and dicotyledons with secondary growth on one side and monocotyledons (*e.g.* cereals) with no secondary growth on the other side may differ in their interactions between xylem and phloem (tissues involved, mechanisms, regulatory aspects).

Selective transfers of solutes from the xylem to the phloem in internodes of cereals must be considered as important events for nutrient economy in the field and for grain filling. It remains a challenge for future experiments to identify basic control mechanisms involved in the regulation of xylem/phloem interactions by internal and external factors.

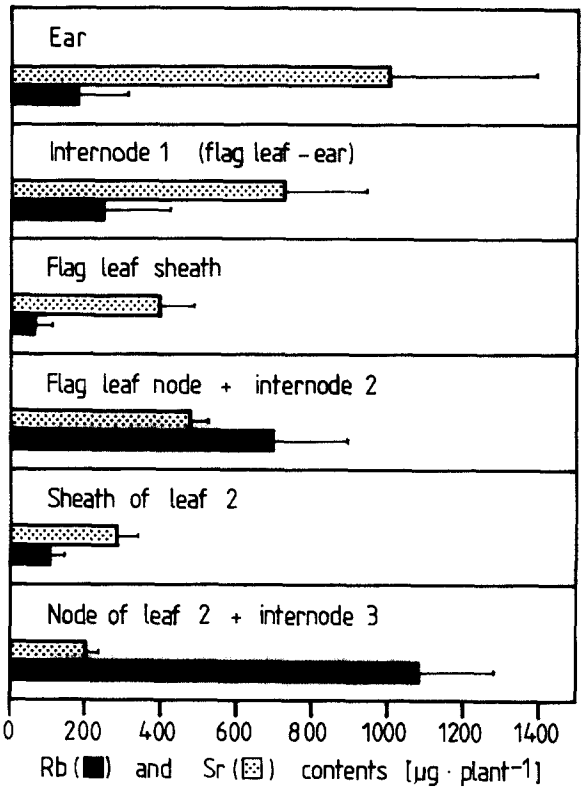

Fig. 6. Distribution of Rb and Sr in ungirdled wheat shoots cut above the third leaf node from the top. The plants were detached 2 weeks after anthesis, the leaf laminas were removed and the shoots were incubated standing in 2 m*M* RbCl + 2 m*M* $SrCl_2$ for 72 h. Leaves, nodes and internodes are numbered from the top. Means and standard deviations of 5 replicates are shown.

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