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Mercurial-Sensitive Water Transport in Barley Roots

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An isolated barley root was partitioned into the apical and basal part across the partition wall of the double-chamber osmometer. Transroot water movement was induced by subjecting the apical part to a sorbitol solution, while the basal part with the cut end was in artificial pond water. The rate of transroot osmosis was first low but enhanced by two means, infiltration of roots by pressurization and repetition of osmosis. Both effects acted additively. The radial hydraulic conductivity (Lp_r) was calculated by dividing the initial flow rate with the surface area of the apical part of the root, to which sorbitol was applied, and the osmotic gradient between the apical and basal part of the root. Lp_r which was first 0.02-0.04 pm s^{-1} Pa^{-1} increased up to 0.25-0.4 pm s^{-1} Pa^{-1} after enhancement. Enhancement is assumed to be caused by an increase of the area of the plasma membrane which is available to osmotic water movement. The inceased Lp_r is in the same order of magnitude as the hydraulic conductivity (Lp) of epidermal and cortical cells of barley roots obtained by Steudle and Jeschke (1983). $HgCl_2$, a potent inhibitor of water channels, suppressed Lp_r of non-infiltrated and infiltrated roots down to 17% and 8% of control values, respectively. A high sensitivity of Lp_r to $HgCl_2$ suggests that water channels constitute the most conductive pathway for osmotic radial water movement in barley roots.

Key words: Barley root — $HgCl_2$ — Hydraulic conductivity — Infiltration — Transroot osmosis — Water channels

In the soil-plant-atmosphere continuum the root offers the second largest resistance to water transport next to the stomata (Steudle *et al.* 1987). In barley roots the number of xylem vessels are seven. This whole set of vessels are observed at the basal part of the root more than 40 mm apart from the tip (Ohya 1996). In such roots in which the xylem vessels are fully developed the resistance to water transport locates not in the longitudinal direction but in the radial direction (Steudle and Jeschke 1983).

Steudle and Jeschke (1983) set the isolated barley root to the pressure probe and induced water movement either by applying the hydrostatic pressure or by changing the osmotic pressure of the bathing solution. The radial hydraulic conductivity (Lp_r) could be measured either by the rate of initial water flow or by the pressure relaxation curve. It amounted to 0.003-0.04 pm s^{-1} Pa^{-1}. On the other hand, applying the pressure probe directly to cortical cells of the root, they found the value of L_p to be 0.08-0.22 pm s^{-1} Pa^{-1} which was about 10 times larger than Lp_r. Based on the fact that six cell layers exist in series from the endodermis to the rhizodermis, they reasonably assumed that the main path of radial water transport through the root may be cell to cell or transcellular. According to intense studies by Peterson *et al.* (1993) Lp_r of maize roots was not changed by giving injury to the epidermis and the cortical layer, and also to the Casparian band. They concluded that the main barrier to the water transport in roots is evenly distributed over the entire tissue.

Recently, water channel proteins, aquaporins, which were firstly identified in animal cells (Preston *et al.* 1992), have been demonstrated to exist also in plant plasma membrane and tonoplast (Maeshima 1992, Maurel *et al.* 1993, Kammerloher *et al.* 1994, Kaldenhoff *et al.* 1995, Yamada *et al.* 1995, Maeshima *et al.* 1996). Most of aquaporins are inhibited by mercurial agents like $HgCl_2$. In characean cells the hydraulic conductivity is sensitive to *p*-chloromercuribenzenesulfonic acid (Wayne and Tazawa 1990) and to $HgCl_2$ (Henzler and Steudle 1995, Tazawa *et al.* 1996). In tomato roots the pressure-induced water flux was inhibited by $HgCl_2$. The inhibition was reversed by ME (Maggio and Joly 1995).

The objective of the present study is to know whether and how much water transport across the barley root is inhibited by $HgCl_2$. Under the assumption that mercurial inhibition of water transport is via inhibition of water channels, the strength of inhibition may indicate to which extent the transcellular water movement is involved in the radial transport of water across the barley root.

Materials and Methods

Plant materials

The plant material used was barley, *Hordeum vulgare* L.

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Abbreviations: APW, artificial pond water; J_v, rate of transroot osmosis; Lp, hydraulic conductivity of plasma membrane; Lp_r, radial hydraulic conductivity of root; ME, 2-mercaptoethanol

c.v. Akashinriki. Seeds weighing 10 g were sterilized with 10% H_2O_2 for 5 min and germinated in 0.5 l of deionized water under aeration at 25 C. After 24 hr germinated seeds were transferred onto a plastic net spanned in a pot in which about 3.5 l of 0.25 mM $CaSO_4$ solution was filled just to immerse seeds. A sheet of cotton cloth was placed on the net with its margin immersed in the solution to protect seeds from drying. Germinated seeds were cultured under aeration in darkness for 4–5 days. One day prior to the experiment the solution was replaced with the following culture medium (in mM): 4 KNO_3, 1 $NaNO_3$, 4 NaH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$ with microelements (in ppm) 1 Fe, 0.5 B, 0.5 Mn, 0.05 Zn, 0.02 Cu, and 0.01 Mo.

Seminal roots of more than 50 mm in length were isolated with scissors from the seedlings and placed in APW containing 0.1 mM each of KCl, NaCl and $CaCl_2$. Infiltration of roots was done by pressurization at 2–3 bars (Heydt and Steudle 1991).

Measurement of transroot osmosis

An isolated root was set into the double-chamber osmometer (Fig. 1) which was used for measuring transcellular osmosis in internodal cells of Characeae (Kamiya and Tazawa 1956, Tazawa *et al.* 1996). Normally, the apical part of the root was placed in the open pool A and the basal part was in the chamber B. At the partition wall the root was embedded into the narrow groove with a small amount of lanolin wax or a mixture of lanolin and vaseline to make the partition water tight. The osmometer with the root was placed under the microscope in such a position that the air bubble (b) in the capillary (C) was in the optical field.

In order to induce transroot osmosis from B to A, APW in pool A was replaced with a sorbitol solution of normally 0.2 M. The water flow was followed by dislocation of the air bubble (b). Then the initial rate of transroot osmosis (J_v) was calculated from the rate of movement of the air bubble which was measured directly with an ocular micrometer or recorded on the video tape via a video camera (SONY, DXC-108) equipped onto the microscope. The recordings were replayed and analyzed. After several minutes of osmosis the sorbitol solution was replaced with APW. Then the air bubble moved in the reverse direction from A to B and ceased to move after 10 min. Thereafter the next trial of osmosis was started. The osmosis could be repeated many times without giving injury to the root.

Calculation of radial hydraulic conductivity of root (Lp_r)

Since the longitudinal hydraulic resistance of the root part having mature xylem vessels was shown to be negligible in barley roots (Steudle and Jeschke 1983), the hydraulic resistance of the basal part of the root in B (Fig. 1) was neglected from the total resistance to transroot osmosis. Therefore, the main resistance to transroot osmosis under the present experimental condition is assumed to be offered by the apical part in A (Fig. 1). Since at the start of the experiment no transroot water movement was observed, the osmotic pressure of the xylem sap was assumed to be equal to that of APW. Since sorbitol was dissolved in APW, the driving force for transroot osmosis is equal to the osmotic pressure produced by sorbitol (π_s). Then the radial hydraulic conductivity of the root (Lp_r) was calculated by the following equation:

$$Lp_r \doteq J_v / A_a \pi_s \qquad (1)$$

where A_a is the surface area of the root in A. A_a was calculated from the length and diameter of the root in A which was assumed to be cylindrical. The diameter was measured at three loci, one at a point 5 mm apart from the apex, second at the middle and the third at the base.

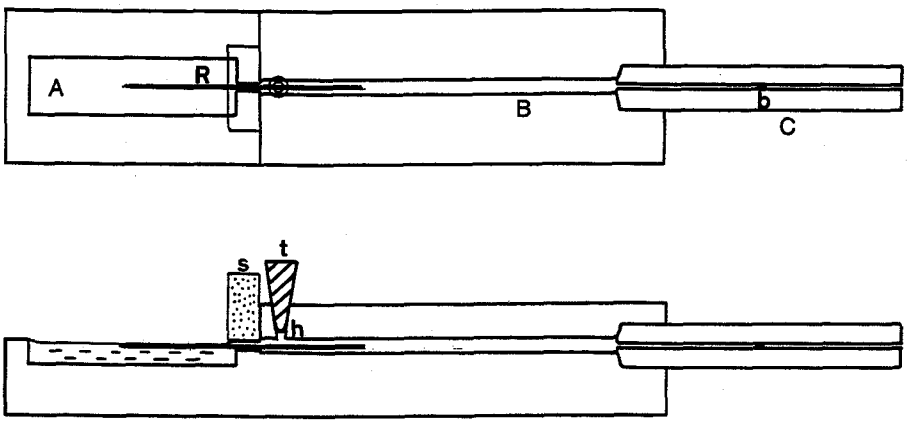

Fig. 1. Upper and side views of a double-chamber volumeter made of plexiglass for measuring the transroot osmotic water movement. Isolated root R was embedded in a groove with lanolin to be partitioned into two parts, the apical part in pool A and the basal part in chamber B through a sealing block S. After filling B with APW through the hole h and introducing an air bubble b in the capillary C, the hole was closed with a plastic tap t. Pool A was filled with APW. Transroot osmosis was induced by replacing APW in A with a sorbitol solution. Movement of the air bubble from B to A was enlarged through a microscope and imaged on a video monitor through a video camera.

Three values were averaged.

In order to see the effect of a water channel inhibitor $HgCl_2$ the apical part in A (Fig. 1) was treated with 100 μM $HgCl_2$ for more than 10 min after the control measurement.

Experiments were done at room temperature (22-26 C).

Results

Facilitation of transroot osmosis by infiltration of roots and repetition of osmosis

Figure 2 shows the time course of transroot water flow induced by subjecting an isolated root to osmotic gradient of 0.2 M sorbitol. The rate of osmosis was first low (ni). It was increased by about 700% by infiltration of the root (i-1). The rate was further increased by repeating osmosis. In the fifth osmosis after infiltration (i-5) the rate was about twice that of the first osmosis after infiltration (i-1). Table 1 summarizes the increase in Lp_r by infiltration and subsequent repetition of osmosis. Table 1 further suggests that duration of infiltration was not essential for the increase, since no difference in the effect of infiltration was found between 30 sec and 2 min infiltration.

The transroot osmosis was also enhanced in non-infiltrated roots with repetition of the osmosis. Figure 3 shows such an example. Comparing with the first osmosis, the initial rate of osmosis was increased to 4.4 times in the fifth osmosis. No further increase was observed in the sixth osmosis (data not shown). Table 2 summarizes the results of similar experiments in both non-infiltrated and infiltrated roots. In both roots Lp_r was increased by 2.4-2.5 times in the fifth osmosis.

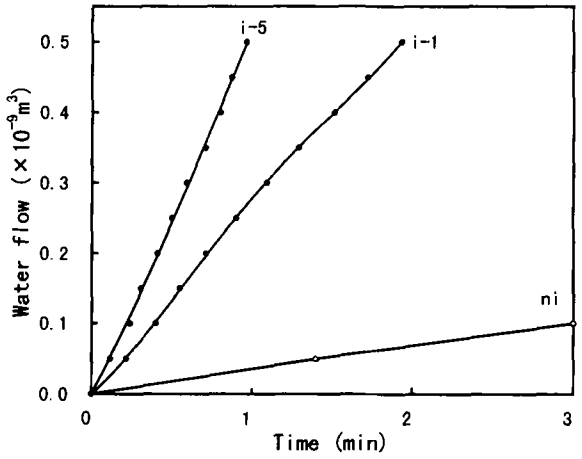

Fig. 2. Time courses of transroot water flow induced by 0.2 M sorbitol. The same root was used first without infiltration (ni) and then after infiltration (i). The first (i-1) and fifth (i-5) osmoses after infiltration were indicated.

Table 1. Radial hydraulic conductivity (Lp_r) of barley roots before and after infiltration

Root	Lp_r (pm s^{-1} Pa^{-1})		
	Before	After infiltration *	
		1st	3rd-5th**
1	0.021	0.20	0.42
2	0.037	0.26	0.48
3	0.025	0.22	0.35
4	0.025	0.17	0.26
Mean	0.027	0.21	0.38
±SEM	0.003	0.02	0.05

* Roots were infiltrated by pressurzation (about 2 bars) either for 30 sec (root 2, 4) or 2 min (root 1, 3).

** Osmosis was repeated 3-5 times after infiltration.

Fig. 2 depicts the data from root 2.

Dependence of the rate of osmosis on the osmotic gradient

Figure 4 shows kinetics of transroot water flow induced by the osmotic gradient of 0.1 M, 0.2 M and 0.3 M, respectively. The root had been subjected to transroot osmosis four times beforehand in order to attain the steady hydraulic conductivity of the root (cf. Table 2). The initial flow rate (J_v) was approximately proportional to the osmotic pressure of sorbitol in pool A (π_s in Eq. (1)). The dependence of J_v on the osmotic gradient is similar to that found for the transcellular osmosis in the internodal cell of Characeae (Kamiya and Tazawa 1956). Namely, barley roots behaves like a single cell with respect to the osmotic water movement.

Effect of $HgCl_2$ on Lp_r in the absence and presence of 2-mercaptoethanol

If the radial water transport in the root occurs mainly via cell-to-cell pathways or transcellularly, water should cross the plasma membrane many times. However, if water moves mainly via symplasmic and/or apoplasmic pathway, water crosses the plasma membrane only few times, for instance at the endodermis and/or at the

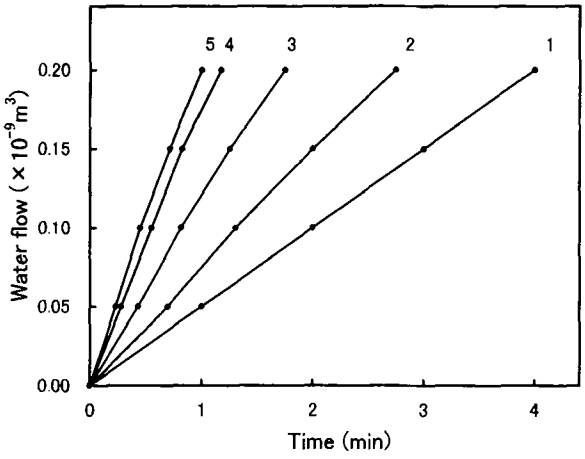

Fig. 3. Repetition of transroot osmosis induced by 0.2 M sorbitol in a non-infiltrated root in the order of 1-5.

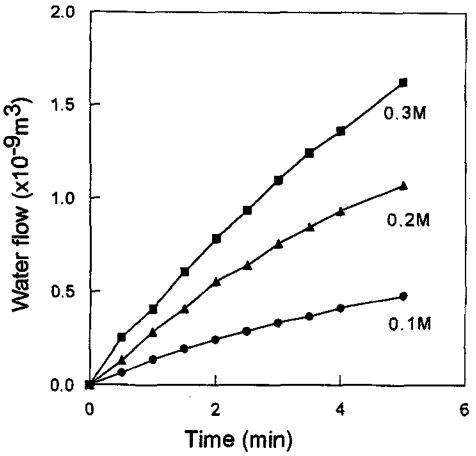

Fig. 4. Transroot osmosis induced sequentially by 0.1, 0.2 and 0.3 M sorbitol in an infiltrated root. Before the osmosis with 0.1 M sorbitol was started, roots had experienced four times transroot osmosis in the order of 0.1, 0.2, 0.3 and 0.2 M sorbitol.

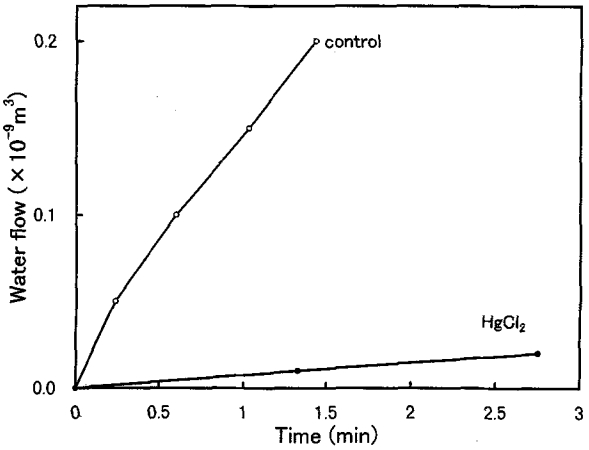

Fig. 5. Effect of $HgCl_2$ on transroot osmosis induced by 0.2 M sorbitol. After the control measurement the root was treated with 100 μM $HgCl_2$ for 20 min. Before the control measurement the root was subjected 4 times to osmosis.

Table 2. Changes in radial hydraulic conductivity (Lp_r in pm s^{-1} Pa^{-1}) upon repetition of transroot osmosis in non-infiltrated (−) and infiltrated (+) barley roots

Infilter.		Number of osmosis 1st	2nd	3rd	4th	5th
−	Lp_r	0.043	0.057	0.066	0.088	0.102
	±SEM	0.006	0.009	0.009	0.016	0.021
	Ratio	1.0	1.3	1.5	2.1	2.4
	(n)	(10)	(10)	(10)	(6)	(5)
+	Lp_r	0.10	0.17	0.21	0.23	0.25
	±SEM	0.03	0.04	0.04	0.02	0.02
	Ratio	1.0	1.7	2.1	2.3	2.5
	(n)	(9)	(9)	(9)	(6)	(6)

n: number of roots used.

Table 3. Inhibition of radial hydraulic conductivity (Lp_r in pm s^{-1} Pa^{-1}) by 100 μM $HgCl_2$ in non-infiltrated (−) and infiltrated (+) barley roots

Infiltr.		Control	$HgCl_2$*
−	Lp_r	0.040	0.007
	±SEM	0.005	0.002
	%	100	17
	(n)	(8)	(8)
+	Lp_r	0.22	0.012
	±SEM	0.05	0.003
	%	100	8
	(n)	(6)	(6)

* Treatment time was 10-25 min.
n: number of roots used.

pericycle. It is then of significance to test whether or not the water movement across the radial cell layers of the root is inhibited by the inhibitor of water channels in the plasma membrane.

Figure 5 shows the transroot water movement induced by the osmotic gradient of 0.2 M sorbitol before and after 20 min treatment of the root with 100 μM $HgCl_2$, respectively. The water flow was strongly inhibited by $HgCl_2$. Table 3 shows that 100 μM $HgCl_2$ decreased Lp_r in both non-infiltrated and infiltrated roots. In non-infiltrated roots, Lp_r for the first osmosis was low (0.04 pm s^{-1} Pa^{-1}) as already shown in Table 2. In the second osmosis, Lp_r had to be increased by 30% (Table 2) but in the presence of $HgCl_2$ it was decreased down to 17% of the control value. In infiltrated roots, Lp_r, which had reached the steady high level (0.22 pm s^{-1} Pa^{-1}, cf. Table 2) after repetition of osmosis, was decreased down to 8% of the control value. Thus transroot osmosis in barley roots is very sensitive to $HgCl_2$ no matter whether the roots were infiltrated or not. Furthermore, the result indicates that the part of Lp_r increased by infiltration and repetition of osmosis is as sensitive to $HgCl_2$ as the non-enhanced part of Lp_r.

The decrease in Lp_r by $HgCl_2$ was not reversed by subsequent treatment of roots with ME. Lp_r, which was 0.17 pm s^{-1} Pa^{-1} on the average of three roots, was decreased down to 17% of the control. The decrease was not reversed by ME (10% of the control). However, when roots were incubated in $HgCl_2$ with ME, ME protected the inhibitory effect of $HgCl_2$. The inhibition was about 40% (Table 4) comparing with 90% without ME (Table 3). Subsequent application of $HgCl_2$ without ME increased the degree of inhibition (Table 4), but still a slight positive effect of ME was observed compared with the inhibition caused by $HgCl_2$ without preincubation with ME (Table 3). Thus presumptive water channel proteins are assumed to

Table 4. Protective effect of 2-mercaptoethanol (ME) on inhibition of Lp_r by 100 μM $HgCl_2$ in infiltrated barley roots

Root No.	Control Lp_r (pm/s/Pa)	Hg+ME* Time (min)	Hg+ME* Lp_r (pm/s/Pa)	Hg** Time (min)	Hg** Lp_r (pm/s/Pa)
1	0.58	10	0.10	10	0.006
2	0.19	5	0.19	8	0.006
3	0.11	15	0.19		
4	0.26	15	0.18	4	0.145
Mean	0.29		0.17		0.05
±SEM	0.10		0.02		0.06
(%)	(100)		(59)		(17)

* After control measurement each root had been pretreated with 100 μM $HgCl_2$±10 mM ME for the indicated time.

** After pretreatment the root was treated with 100 μM $HgCl_2$ for the indicated time.

The concentration of sorbitol for inducing transroot osmosis was either 100 mM (root 2-4) or 200 mM (root 1).

contain thiol groups sensitive to $HgCl_2$.

Radial hydraulic conductivity (Lp_r) along the root length

Under the assumption that the radial osmotic water transport in roots is driven by the difference in the osmotic pressure between the xylem vessel sap and the external medium, Lp_r of root segments may be influenced by the extent of development of xylem vessels at the segment. Lp_r of the apical segment having immature vessels may be smaller than that of the basal segment having mature vessels. In order to know whether the transroot osmotic water movement is influenced by the development of xylem vessels at the apical part of the root, the tip of the apical part in pool A was repeatedly cut, each time by 5 mm. Figure 6 shows an example how the transroot water flow was influenced by cutting the apical part of the root. When the root tip was intact, the osmosis proceeded almost linearly with time. Excision of the tip influenced the rate of osmosis at the initial stage not significantly but decreased it at the later stage. The decrease became more significant with the increase of the cut length. In order to assess the effect of excision the rate of water flow (J_v) was calculated by dividing the total amount of water flowed (0.2×10^{-9} m^3 in Fig. 6) with the time elapsed. Assuming that the whole surface of the root in pool A (Fig. 1) is equally available to radial water flow, J_v of the root from which the tip was cut, can be estimated from J_v of the root with the intact root. The estimated J_v indicated as $(J_v)_{est}$ is calculated by the following equation.

$$(J_v)_{est}=(J_v)_o\times L/L_o \qquad (2)$$

where $(J_v)_o$, L_o and L represent J_v of the root with the intact tip, the length of the intact root and the length of the root from which the tip was removed, respectively. If the cut portion of the root is not available to osmosis, the measured J_v should be larger than $(J_v)_{est}$. If it is equally available as the remaining portion, no difference would exist between the measured and estimated values. However, if the xylem vessels are open at the cut surface, both leak of xylem fluid and diffusion of sorbitol into xylem vessels from the cut surface may cause a decrease in J_v, in which the former effect may be significant already in the initial phase of osmosis and the latter effect in the later stage as shown in Fig. 6. In such a case the measured J_v should be less than the estimated J_v.

Table 5 shows the ratio between J_v and $(J_v)_{est}$ in % for intact roots and the roots from which tips were removed. When the tip was removed by 5 mm, the ratio decreased in five roots, remained nearly constant in one root and increased in two roots. On an average, the effect of removal of 5 mm was not so big. Removal of further 5 mm (−10 mm) decreased the ratio slightly. However, removal of 15 mm brought about a significant decrease of the ratio. It may be assumed that the xylem may be still undifferentiated at the site 5-10 mm from the root apex.

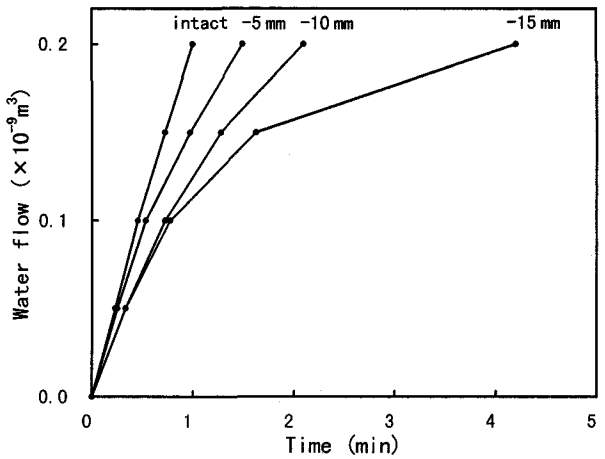

Fig. 6. Changes in the transroot water flow caused by stepwise cutting of the apical end, each time by 5 mm.

Table 5. Ratio between observed (J_v) and estimated ($(J_v)_{est}$) rats of transroot osmotic flow in roots with intact tips and in roots from which apical part was removed stepwise, each time by 5 mm

Root	$J_v/(J_v)_{est}$ (%) Intact	−5 mm	−10 mm	−15 mm
1	100	78	70	44
2	100	120	81	59
3	100	125	108	11
4	100	95	86	40
5	100	84	83	90
6	100	51	59	42
7	100	58	12	
8	100	26		
Mean	100	80	71	47
±SEM		12	11	10

Osmosis was induced with 0.2 M sorbitol.

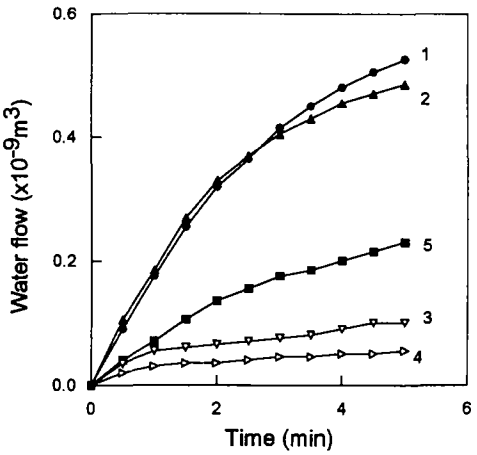

Fig. 7. Transroot osmosis influenced by removal of the apical end and by resealing of the cut surface with lanolin. Osmosis was induced by 0.2 M sorbitol. The initial length (L) of the apical part of the root in pool A was 24 mm. First, osmosis was repeated in the root having intact apex (osmosis 1 and 2). After excision of the apical 12 mm the third osmosis was done (osmosis 3, L=12 mm). The root was further shortened by excision of 4.5 mm (osmosis 4, L=7.5 mm). After osmosis 4 the cut end was covered with lanolin over 3 mm (osmosis 5, effective L=4.5 mm).

In other words, the apical region of up to 10 mm is assumed to be relatively indifferent to the radial water transport as pointed out by Steudle and Jeschke (1983) in barley roots.

In the above we assumed that the decrease of the ratio between J_v and $(J_v)_{est}$ by removal of the root tip may be due to a leak of the xylem fluid and diffusion of sorbitol into xylem vessels. Actually removal of 16.5 mm tip segment from the 24 mm long root reduced the rate of osmosis drastically down to 10% of the original value (Fig. 7). The once decreased Lp_r was greatly increased up to 46% of the original value by sealing the cut surface with lanolin. The result supports the assumption that free ending of vessels caused by cutting acts to attenuate the osmotic driving force of sorbitol.

Discussion

Pathways for radial water transport

When the transroot-osmosis is induced by replacing APW in A (Fig. 1) with a sorbitol solution, water in B enters into the opened xylem vessel of the basal part, transported to the xylem vessel of the apical root in A and escapes to the bathing medium radially through the root tissue. There may be three possible routes for the radial water transport in the root. The first route is a combination of apoplasmic and transcellular pathways, the second one is the transcellular pathways in series, and the third one is a combination of symplasmic and transcellular pathways (Steudle and Jeschke 1983). In any route the transcellular pathway is involved in series with the other pathways. Therefore, if water channels are the main hydraulic pathway of the plasma membrane, the radial water transport in roots is assumed to be severely reduced by the water channel blocker. Actually Lp_r was reduced by $HgCl_2$ down to 17% of the control value in non-infiltrated roots and to 8% in infiltrated roots which is in the same order of magnitude as that of decrease in the hydraulic conductivity of characean cells (Tazawa *et al.* 1996) and exceedingly larger than the inhibition observed in roots of tomato seedlings (Maggio and Joly 1995) in which Lp_r was reduced to about 50% of the control value by 30 min treatment with 500 μM $HgCl_2$. Comparing with 83-92% decrease in Lp_r with 100 μM $HgCl_2$ in barley roots, tomato roots are less sensitive to $HgCl_2$. This may mean the difference in contribution of other water transport pathways other than the transcellular one for the radial water transport in tomato roots or may suggest less contribution of water channels across the plasma membrane in tomato roots than in barley roots.

Enhancement of radial water transport

The value of Lp_r of non-infiltrated roots obtained in the first osmosis dispersed between 0.017 and 0.07 pm s^{-1} Pa^{-1} with the mean value of 0.043 pm s^{-1} Pa^{-1} and was slightly larger than that (0.003-0.043 pm s^{-1} Pa^{-1}) obtained by Steudle and Jeschke (1983) in barley roots. In the present study we found that transroot osmosis was greatly enhanced by two means, infiltration of roots and repetition of osmosis. After infiltration and subsequent repetition of osmosis Lp_r increased from 0.027 to 0.38 pm s^{-1} Pa^{-1} (Table 1). The increase in Lp_r can be interpreted in terms of an increase in the effectve area of the plasma membrane making contact with sorbitol solution. This interpretation is supported by the fact that most of the increased part of Lp_r was reduced by $HgCl_2$ (infiltrated roots in Table 3). Furthermore, we observed that a root having a high Lp_r after repetition of osmosis looked more or less transparent, suggesting an infiltration effect of repeating osmosis. However, infiltration by pressurization was not enough to reach the maximum Lp_r value. Prolongation of pressurization time from 30 sec to 2 min did not cause a further increase. We assume that repetition of osmosis has two effects, infiltration effect and an unkown effect. One possibility for the unknown effect is that cell walls forming the apoplasmic pathway are originally not hydrophilic (cf. p. 259 in Passioura 1988) but becomes hydrophilic with repetition of osmosis with the result of increased hydraulic conductivity of the apoplasmic pathway. It is supposed that some hydrophobic area at the cell wall-plasma membrane interface (Peterson *et al.* 1993) may block access of sorbitol solution to the surface of cells which are the main barrier to the radial water transport. Repetition of radial water movement would anyhow remove such a hydrophobic area so that solutions containing osmotic solutes become accessible to the plasma membrane of cells.

Interpretation of high Lp_r

The mean value of Lp_r after enhancement was about

0.3 pm s^{-1} Pa^{-1} (Tables 1, 2). This value is in the same order of magnitude of the hydraulic conductivity (0.08–0.22 pm s^{-1} Pa^{-1}) of the plasma membrane of epidermal and cortical cells of barley roots (Steudle and Jeschke 1983). Then it may be assumed that a single layer of cells surrounding the root may offer the main conductive pathway for the radial water transport. As a candidate of such a structure, the endodermis is assumed, since the Casparian band is assumed to be relatively impermeable to water and solutes in the apoplast (Kramer 1983). Assuming that the endodermal cell layer provides the main pathway for the radial water movement, Lp of endodermal cells can be estimated from Lp_r. The transcellular hydraulic conductivity which is one half of Lp can be obtained by multiplying Lp_r with 3–4, since the outer surface area of the endodermal cell layer is 1/3–1/4 of the surface area of the root (Ohya 1996, Steudle and Jeschke 1983). Then Lp of the endodermal cell can be obtained by multiplying Lp_r by 6–8. The value thus obtained amounts to about 2 pm s^{-1} Pa^{-1} which is 10 times or more than the value of Lp experimentally measured by Steudle and Jeschke (1983). The conclusion drawn from this discrepancy is that the cells available for the transcellular osmosis in roots are not restricted to the endodermal cells but includes cortical and epidermal cells.

Peterson *et al.* (1993), using young maize roots, found that wounding of the epidermis and cortex did not cause any significant increase in Lp_r. Injury of the endodermis increased Lp_r in proportion to the injured area. They assumed that the Casparian band without suberin lamellae is fairly permeable to water and infer that the pathway of the radial water transport does not localize at the endodermis with the Casparian band but at the plasma membranes and the apoplast of all the living tissues. The same conclusion was obtained also by puncturing the endodermis of maize roots (Steudle *et al.* 1993). However, strong inhibition of osmotic water transport by $HgCl_2$ found in the present study suggests that in barley roots the bypass of water movement via the Casparian band is negligible comparing with the main route which is transcellular.

Conclusion

In conclusion, the radial osmotic water transport in barley roots cultivated under aeration is low but can be enhanced about ten times by infiltration and repetition of osmosis. This means that in non-treated roots the original "wet" area of the plasma membrane used for osmosis is only about 10% of the total area which becomes more available after enhancing treatments. The radial water transport in both non-enhanced and enhanced roots was very sensitive to $HgCl_2$, suggesting that water moves radially across the root mainly via water channels in the plasma membrane.

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