Hydraulic Conductivity and Aquaporins of Cortical Cells in Gravitropically Bending Roots of *Pisum sativum* L.

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**Abstract**: We examined the differential elongation of gravitropically bending roots of *Pisum sativum* L. in terms of cell enlargement and water uptake by cells in the growing tissue. Hydraulic conductivity between the elongating and mature tissues (Lp) was estimated from the equation \( G = A \times Lp \times \Delta \Psi \), where \( G \) is the water-uptake rate, \( A \) is the surface area of a single cell and \( \Delta \Psi \) is the driving force. The rate of entry of water into a cell was estimated from the rate of increase in the volumes of cells in the outer cortex, which were calculated from longitudinal sections at given times. Gravitropic bending occurred 1 h after the application of gravistimulation and the curvature increased rapidly for the next 3 h. The biggest difference in the partial elongation rate between opposite sides of a root was found in the region 3 to 4 mm from the root tip at the start of stimulation. Cell enlargement rate was 2.8 to 3.8 times greater on the upper side of the root than on the lower side. The water potential and the osmotic potential, in both the elongating and mature tissues, were the same on both sides of the root. Therefore, there was no difference in the driving force for water flow. Hydraulic conductivity was 2.3 to 4.2 times greater on the upper side of the root than on the lower side. There was no difference between the upper and lower sides of the root in the amounts of 19-kD and 24-kD proteins in membrane fractions, which we assumed to be aquaporins (putative aquaporins), as estimated with two preparations of polyclonal antibodies.

The differential elongation that occurred during root gravitropism was caused by a difference in Lp. However, the difference in Lp did not appear to be regulated by the concentration in cell membranes of the putative aquaporins.

**Key words**: Aquaporin, Cell elongation, Gravitropism, Hydraulic conductivity, *Pisum sativum* L., Water uptake.

In crop plants, the shape and size of the root system are very important since the roots have access to available water and nutrients and provide them to the aboveground parts of the plant. The direction of elongation, length, and branching of individual roots affect the shape and size of the root system. The tropisms of roots determine the direction of their elongation. Under normal field conditions, gravitropism is very important in this respect, and hydrotropism is also important under certain conditions (Tsuda et al., 2003). The gravitropic response is initiated when the root tip senses the force of gravity and the root bends as a result of the differential elongation of the elongating tissue, which is away from the root tip (Ishikawa et al., 1991; Mullen et al., 1998). Muday (2001) demonstrated that acid-induced loosening of the cell wall induces this differential elongation. The increase in volume of plant cells is due mainly to the increase in the volume of vacuoles. However, little information is available on the rate of water uptake by cells, even though there is some evidence that water uptake by cells plays an important role in the elongation of the plant cells (Nonami and Boyer, 1990, 1993; Miyamoto et al., 2002). Therefore, in the present study, we investigated water uptake in the gravitropically bending roots of *Pisum sativum* L.

The rate of increase in volume of a plant cell (\( G; \text{m}^{-3} \text{s}^{-1} \)) can be described by the following equation (Lockhart, 1965; Boyer et al., 1985; Passioura and Fry 1992):

\[
G = \frac{dV_c}{dt} = A \times Lp \times \Delta \Psi,
\]

where \( A \) is the surface area of the cell (\( \text{m}^2 \)), \( V_c \) is the water volume of the cell (\( \text{m}^3 \)), \( Lp \) is the hydraulic conductivity (\( \text{m}^2 \text{~m}^2 \text{s}^{-1} \text{MPa}^{-1} \)), and \( \Delta \Psi \) is the difference in water potential between the water source and the cell whose volume is increasing (\( \text{MPa} \)).

First, we attempted to estimate the contribution of the hydraulic conductivity of the root tissue to differential elongation on the basis of equation (1). Then, we attempted to analyze water transport at the molecular level. Thus, we examined the levels of putative aquaporins in elongating cells. Aquaporins regulate the hydraulic conductivity of cell membranes (Chrispeels and Maurel, 1994; Maurel, 1997; Barrowclough et al., 2000) and are often expressed in rapidly growing tissues (Ludevid et al., 1992; Chaumont et al., 1998; Suga et al., 2001).
and in tissue in which the rate of water exchange is very high (Fleurat-Lessard et al., 1997; Barrieu et al., 1998). Aquaporins might play an important role in cell elongation by regulating water uptake.

Materials and Methods

1. Plant materials

Seeds of *Pisum sativum* L. (cv. Alaska) were allowed to germinate in darkness at 25 °C for 2 to 3 days, on filter paper wetted with deionized water. Seedlings with single straight roots of 20 to 35 mm in length were used for measurements.

2. Experimental setup

The cotyledons of a seedling were fixed with an insect pin to the edge of a block of polyurethane foam (245 mm × 100 mm × 50 mm) in an acrylic box (250 mm × 200 mm × 210 mm). The block was wrapped in three layers of cheesecloth, which were wetted with deionized water. The relative humidity inside the box was kept close to saturation by pieces of wet absorbent paper that were placed against the inner walls. The root axis of each seedling was oriented parallel to the direction of gravity. Cotyledons were wrapped with a piece of wet tissue (10 mm × 130 mm) to prevent desiccation. At the start of the experiment, the box was positioned to make the root axis horizontal (gravistimulation).

3. Determination of the partial elongation rate and curvature

The root surface was marked with thick India ink at approximately 1-mm intervals over a distance of 10 mm from the root tip after the seedling had been attached to the polyurethane block. The marks were made in a vapor-saturated cooler box. Images were recorded with a digital camera (Coolpix 990; Nikon Co. Ltd., Tokyo, Japan) at hourly intervals after the start of gravistimulation. Distances between marks were measured with appropriate software (SigmaScan; Sigma Co. Ltd, California, USA). A ruler was photographed together with the roots to allow precise measurements. The curvature of the root was measured with a protractor.
4. Measurements of the dimensions of elongating cells

After gravi-stimulation, seedlings were fixed in a mixture of formalin, acetic acid and 80% ethanol (5:5:90, v/v; FAA solution) and preserved for measurements of cell dimensions. A 10-mm portion from the tip of root was embedded in agar (50 g L\(^{-1}\)) and longitudinal sections of 30-µm thickness were cut with a micro-slicer (DTK-1000; Dohan EM Co. Ltd., Kyoto, Japan). Sections were stained with a solution of 10 g L\(^{-1}\) safranin. The diameters and lengths of 3 or 4 adjacent cortical cells in the three outmost layers were measured (9 to 12 cells in each root). The distances of the cortical cells from the root tip were estimated at given times after the start of stimulation by tracing the movement of the marks on the root surface.

Because the increase in cell volume is mainly due to the increase in volume of the water-filled vacuole, the rate of increase in volume per unit surface area of cortical cells can be taken as the rate of water uptake by a cell. We calculated the rate of increase by dividing the rate of increase in volume of cells by the average surface area (ASA). ASA was determined from equation (2), which assumes an exponential increase in surface area:

\[
ASA = \frac{(B-A)}{(\ln B - \ln A)} \tag{2}
\]

where \(A\) and \(B\) represent the surface area of a cell at 1 h and 3 h after the start of treatment, respectively.

5. Determination of water status and the hydraulic conductivity of tissues

Roots were cut into four pieces with a razor blade in a vapor-saturated cooler box 2 h after the start of gravi-stimulation. The apical 1 mm from the root tip was cut off and the remainder of the root was divided into two halves along the root axis to yield the upper and lower halves of the gravitropically bent root. Pieces from 1 to 8 mm and from 8 to 15 mm from the root tip, which are referred to as the elongating and the mature tissue, respectively, were cut from the halved roots and used for measurements of water potential. Each sample of elongating tissue included a small amount of mature tissue (from 6 to 8 mm from the root tip) to prevent any underestimation of the water potential of the elongating tissue (Boyer, 1995). For measurements of the osmotic potential, pieces from 1 to 6 mm and 8 to 13 mm from the root tip were used as the elongating and mature tissue, respectively. The water potential and osmotic potential were measured with an isopiestic psychrometer (Boyer and Knipling, 1965; Boyer, 1995; Hirasawa et al., 1997). We measured the water status of a group of vertically elongating roots (with no bending) at the same time as a control. Four to seven pieces of tissue were used for each measurement.

The hydraulic conductivity of the tissue (L\(_p\)) was calculated by dividing the rate of water uptake by the cell by \(\Delta \Psi\), according to equation (1). We determined \(\Delta \Psi\) from the difference in water potential between the mature (water source) and the elongating tissue.

6. Isolation of membrane fractions

Soluble protein, crude membranes, and three membrane fractions were isolated according to the scheme shown in Fig 1. Samples of root tissue (30 g) were homogenized with an ice-cold mortar and pestle in 200 mL of grinding buffer [0.25 M mannitol, 25 mM HEPES-Tris (pH 7.5), 2 mM ethylene glycol-bis(β-amino-ethylether)-N,N,N’,N’-tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 1 g L\(^{-1}\) bovine serum albumin] and filtered through four layers of cheesecloth. Then the filtrate was centrifuged at 1,500 \(\times\) g for 10 min. The supernatant was centrifuged at 13,000 \(\times\) g for 15 min. The resultant supernatant (the soluble-protein fraction) was centrifuged at 80,000 \(\times\) g for 30 min. The pellet (crude membrane fraction) was suspended in approximately 5 mL of suspension.

Fig. 2. Amino acid sequences of aquaporins from various plant species. Antibodies, designated anti-MIP and anti-PIP, were raised against the polypeptides within the shaded rectangles outlined by a solid line and with a dotted line, respectively. Accession numbers, with plant names in parentheses, are: AtPIP1;3 = CAA53476 (Arabidopsis thaliana); AtPIP2;1 = CAB67649 (Arabidopsis thaliana); VfPIP = CAB93959 (Vicia faba); NePIP = BAA20076 (Nicotiana excelsior); OsPIP = BAA24016 (Oryza sativa); HvPIP1;3 = BAA23745 (Hordeum vulgare subsp. vulgare); PsPIP = CAB45651 (Pisum sativum); PsTIP = CAB45653 (Pisum sativum); AtPIP1;1 = CAA51171 (Arabidopsis thaliana); and AtPIP2;2 = CAB10515 (Arabidopsis thaliana).
buffer [0.25 M mannitol, 10 mM MES-Tris (pH 7.3), 1 mM EGTA, and 1 mM DTT] and subjected to stepwise sucrose density centrifugation at 95,000 × g for 2.5 h. Bands of material between the layers of 20% (w/w) and 25% sucrose, 25% and 30% sucrose, and 30% and 34% sucrose (fractions I, II and III, respectively) were collected. Each fraction was diluted with suspension buffer and centrifuged at 80,000 × g for 40 min. Each pellet was resuspended in the appropriate amount of the same buffer. The concentration of protein in each fraction was determined by the Bradford’s method (Bradford, 1976) with protein assay solution (Bio-Rad, Tokyo, Japan).

7. Antibodies

There are 35 genes for individual aquaporins in Arabidopsis thaliana (Johanson et al., 2001) and 31 in maize (Chaumont et al., 2001). In order to estimate the total amount of all aquaporins simultaneously but, also, to distinguish between plasma-membrane-specific and tonoplast-specific aquaporins (PIPs and TIPs, respectively), we used two preparations of polyclonal antibodies (BML Co. Ltd., Naogoya, Japan). One was directed against membrane intrinsic protein (anti-MIP) and the preparation contained antibodies raised against the synthetic oligopeptides SGGHINPAVT and SGGHVNPAT, both of which include a motif that is conserved in all aquaporins (the NPA motif). The other was directed against plasma membrane intrinsic protein (anti-PIP) and contained antibodies raised against peptides FSATDPKRNARDSH, FSATDAKRNARDSH, FSATDPKRSARDSH, and FSATDAKRSARDSH. We postulated that these antibodies would recognize plasma-membrane-specific aquaporins (Fig. 2). The aquaporins reported from Pisum sativum L. contain these amino acid sequences (PsPIP and PsTIP in Fig. 2).

8. Extraction of proteins from root tissue

Individual roots were sectioned with a razor blade into four fragments (from 1 to 6 mm and from 8 to 13 mm from the root tip, and the upper and lower sides of each piece) in a vapor-saturated cooler box either 1 h or 3 h after the start of gravi-stimulation. Each sample was immediately wrapped in a small piece of aluminum foil and frozen in liquid nitrogen. Samples were stored at −75°C in a freezer. Crude protein was extracted from sets of 20 similar fragments (about 40 mg) in 360 µL of grinding buffer [0.3 M sucrose, 50 mM Tris (pH 7.8), 8.0 mM ethylenediaminetetraacetic acid (EDTA), 4 mM DTT, 2 mM phenylmethylsulfonyl fluoride (PMSF)]. The suspension was centrifuged at 10,000 × g for 10 min and the supernatant was collected. The protein content of the supernatant was measured by Bradford’s method, as described above.

9. Quantitation of putative aquaporins

Samples of crude protein (20 µg of protein) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 5% stacking gel and 13% separation gel) and blotted onto a polyvinylidene difluoride (PVDF) membrane (Clear Blot Membrane-PAE-6666; Atto Co. Ltd., Tokyo, Japan). Putative aquaporins were allowed to react with either anti-MIP or anti-PIP as the first antibody and alkaline-
phosphatase-conjugated goat antibodies against rabbit IgG as the second antibody and then visualized with development reagent (Immun-Blot Assay Kit; BIO-RAD, Tokyo, Japan). The densities of bands of immunoreactive proteins were measured with densitometric software (Densitograph ver. 4.0 software for Macintosh; Atto Co. Ltd., Tokyo, Japan).

**Results**

1. Curvature and partial elongation rate

Gravitropic bending began 1 h after the start of gravi-stimulation (Fig. 3). The curvature of the root increased rapidly for the next 3 h. The average curvature was 46° at 5 h after the start of measurements.

Fig. 4 shows the elongation rates of the root surface on the upper and lower sides at 3 h after the start of gravi-stimulation. The average elongation rates on the upper and the lower sides of the root were 0.50 mm h\(^{-1}\) and 0.31 mm h\(^{-1}\), respectively. The elongation rate was highest in the region that was 3 to 4 mm from the root tip at the start of stimulation. The elongation rate was significantly higher on the upper side than on the lower side in the region that had originally been 2 to 4 mm from the tip. Thus, the differential gravitropic elongation was caused by differences in this region.

2. Cell dimensions

There was no difference in cell dimensions between the upper and lower sides of the root during the first hour after the start of treatment (Table 1). By contrast, cell dimensions were larger on the upper side than on the lower side at 3 h. The increase of cell volume was 2.8 to 3.8 times greater on the upper side than on the lower side (Table 2).

3. Water status and hydraulic conductivity of tissues

In gravitropically bending roots, there were no differences in water and osmotic potential between the

| Table 1. Changes in the dimensions\(^1\) of cortical cells in roots after the application of gravi-stimulation. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Position \(^2\) | Time after stimulation | Side | Diameter \((10^6 \text{ m})\) | Length \((10^6 \text{ m})\) | Surface area \((10^9 \text{ m}^2)\) | Volume \((10^{15} \text{ m}^3)\) |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 3 mm | 1 h | Upper | 25 ± 1 a | 84 ± 3 a | 8 ± 1 a | 43 ± 6 a |
| | | Lower | 24 ± 0 a | 77 ± 5 a | 7 ± 0 a | 37 ± 3 a |
| | 3 h | Upper | 28 ± 0 a | 112 ± 4 a | 11 ± 0 a | 72 ± 4 a |
| | | Lower | 26 ± 1 b | 89 ± 5 a | 8 ± 0 b | 48 ± 1 b |
| 4 mm | 1 h | Upper | 24 ± 2 a | 156 ± 11 a | 13 ± 1 a | 73 ± 12 a |
| | | Lower | 25 ± 2 a | 151 ± 3 a | 13 ± 1 a | 80 ± 13 a |
| | 3 h | Upper | 28 ± 1 a | 166 ± 5 a | 16 ± 1 a | 96 ± 12 a |
| | | Lower | 28 ± 1 a | 140 ± 4 b | 14 ± 1 a | 85 ± 12 a |

\(^1\) Three to four adjacent cells in the outer first through third layers of the cortex were measured (9–12 cells at each position) in each root. Cell shape was assumed to be cylindrical and surface area and volume were calculated from the measured diameter and length. Longitudinal sections were prepared for measurements. Values are means ± standard errors. When values are followed by same letter of the alphabet, there is no significant difference between the upper side and the lower side of the root (\(n = 3\); paired t-test, 5% level).

\(^2\) The distance from the root tip at the beginning of the treatment, which is applied to the following Tables correspondingly.

| Table 2. Rate of increase in cell volume from 1 to 3 h after the start of gravi-stimulation. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Position | Side | Rate of increase in volume \((10^6 \text{ m}^3 \text{ h}^{-1})\) | Average surface area \((10^9 \text{ m}^2)\) | Rate of increase in volume per unit surface area \((10^6 \text{ m}^3 \text{ m}^{-2} \text{ h}^{-1})\) | Ratio\(^1\) |
|----------------|----------------|----------------|----------------|----------------|----------------|
| 3 mm | Upper | 14.42 | 9.39 | 1.54 | 2.8 |
| | Lower | 5.36 | 7.62 | 0.74 | 1.0 |
| 4 mm | Upper | 11.49 | 14.43 | 0.80 | 3.8 |
| | Lower | 2.80 | 13.41 | 0.21 | 1.0 |

\(^1\) Ratio of the rate of increase in volume per unit surface area on the upper side to that on the lower side.
opposite sides of the roots either in the elongating or the mature tissue (Table 3). Therefore, the calculated turgor pressure was the same on both sides. However, since the osmotic potential of the elongating tissue was lower than that of the mature tissue, the water potential of the former was lower than that of the latter. The mean difference in water potential between the two sides of the root was approximately 0.2 MPa (Table 3). In vertically elongating roots (with no bending), there were no differences in the water or osmotic potential between opposite sides of the root as in the gravitropically bending roots (Table 3).

The hydraulic conductivity of the tissue was 2.3 to 4.2 times greater on the upper side of the root than on the lower side (Table 4). This difference was responsible for the differential elongation, which was followed by gravitropic bending.

4. Localization of membrane proteins that reacted to anti-MIP and anti-PIP

Staining with Coomassie Brilliant Blue revealed many bands of protein in the crude membrane fraction (Fig. 5B). After sucrose density centrifugation and similar analysis, bands of 19-kD and 24-kD proteins were observed in fraction I, and well-defined bands of 24-kD and 55-kD proteins were observed in fractions II and III.

Anti-PIP yielded intense bands of 24-kD and 55-kD proteins and many other bands, including that of a 19-kD protein in the crude membrane fraction (Fig. 5C). Intense bands of 24-kD and 55-kD proteins were observed in fraction I, and such bands were more clearly defined in fractions II and III.

| Tissue        | Side  | WP (MPa) | OP (MPa) | TP (MPa) |
|---------------|-------|----------|----------|----------|
| Bending roots |       |          |          |          |
| Elongating tissue | Upper | – 0.44 ± 0.06 a | – 0.95 ± 0.06 a | 0.51 |
|                | Lower | – 0.45 ± 0.04 a | – 0.96 ± 0.06 a | 0.51 |
| Mature tissue  | Upper | – 0.23 ± 0.02 a | – 0.79 ± 0.08 a | 0.56 |
|                | Lower | – 0.22 ± 0.02 a | – 0.80 ± 0.08 a | 0.57 |
| Difference     | Upper | 0.21      | 0.16     | 0.05     |
|                | Lower | 0.23      | 0.16     | 0.07     |

| Vertical roots |       |          |          |          |
| Elongating tissue | Right | – 0.51 ± 0.08 a | – 0.92 ± 0.04 a | 0.41 |
|                | Left  | – 0.49 ± 0.09 a | – 0.93 ± 0.03 a | 0.44 |
| Mature tissue  | Right | – 0.27 ± 0.10 a | – 0.79 ± 0.07 a | 0.52 |
|                | Left  | – 0.27 ± 0.07 a | – 0.79 ± 0.05 a | 0.52 |
| Difference     | Right | 0.24      | 0.15     | 0.11     |
|                | Left  | 0.22      | 0.14     | 0.08     |

1) Tissues were sampled 2 h after roots were put in the experimental box. Values represent means ± standard errors (n = 5 for bending roots and n = 4 for vertical roots). When values are followed by same letter of the alphabet, there is no significant difference between the two sides of the root (paired t-test, 5% level).

2) Turgor pressure calculated by subtracting osmotic potential from water potential.

3) The difference in each parameter between the mature and elongating tissues.

4) Each root was cut into two halves, to produce a right and a left side.

Table 4. Hydraulic conductivity of tissues of gravitropically bending roots.

| Location | Side | Hydraulic conductivity of tissue (10⁹ m s⁻¹ MPa⁻¹) | Ratio |
|----------|------|--------------------------------------------------|-------|
| 3 mm     | Upper| 2.03                                             | 2.3   |
|          | Lower| 0.89                                             | 1.0   |
| 4 mm     | Upper| 1.05                                             | 4.2   |
|          | Lower| 0.25                                             | 1.0   |

1) Hydraulic conductivity through the tissue from the mature zone to the elongating zone was calculated by dividing the rate of increase in volume per unit surface area (Table 2) by the difference in water potential (Table 3).
Densities of the bands of the 19-kD protein detected with anti-MIP and the 24-kD protein detected with anti-PIP

The densities of the bands of 19-kD and 24-kD proteins, detected with anti-MIP and anti-PIP, respectively, increased linearly with the amount of total protein in the samples loaded onto the gel for SDS–PAGE (Fig. 6). We compared the densities of bands of 19-kD protein, detected with anti-MIP, and those of 24-kD protein, detected with anti-PIP, between the upper and lower sides of roots that had bent gravitropically.

At the beginning of bending (Fig. 7, 1 h), the upper side of the root had higher densities of proteins that reacted with anti-MIP (Fig. 7A) and anti-PIP (Fig. 7B) than the lower side. However, the difference was very small. The densities of bands fluctuated even in the vertically elongating roots. When the curvature was increasing rapidly (Fig. 7, 2 h), there were no significant differences in the transverse distributions of these proteins.

Discussion

The differential elongation in gravitropism is caused by a difference in cell enlargement rate (Fig. 4 and Table 2). The cell enlargement is caused by the increase in cell volume which is mainly due to the increase in volume of vacuole. In this study, the roots were in vapor-saturated air, and the elongating root cells probably acquired water from the cotyledons, via the mature tissue. Therefore, the difference in water potential between the mature and elongating tissue was considered to be the driving force of the water flow (Table 3). As shown in Tables 3 and 4, the difference in water-uptake rate resulted from the difference in hydraulic conductivity within the elongating tissue rather than a difference in the driving force of the water flow. We could not identify the factors responsible for the difference in hydraulic conductivity within the tissue, but the hydraulic conductivity of the tissues in this study might be affected by the resistance of cell membranes (plasma membrane and tonoplast) and apoplast (outside the cells). In the hydrotropically bending root, both wall extensibility and the hydraulic conductivity of a cell (and, subsequently, the hydraulic conductivity of the tissue) contribute simultaneously to the differential elongation (Hirasawa et al., 1997; Miyamoto et al., 2002). Moreover, water must move...
across membranes at least once to enter an elongating cell, and the resistance of membranes to water flow is generally much greater than the resistance of the apoplast. Therefore, the most plausible hypothesis is that a gravitropic change occurs in the membranes.

The specificities of the antibodies that we used were confirmed in an analysis of membrane fractions from the root tissues of *Pisum sativum* L. (Fig. 5). We detected intense bands of 19-kD, 24-kD, and 55-kD proteins with the antibodies. The intensities of the bands of 24-kD and 55-kD were higher in fraction III (the membrane fraction with the highest density) than in fraction I (the membrane fraction with the lowest density). The 19-kD protein was detected in only fraction I. Plasma membranes are found in a membrane fraction at a higher density than in the tonoplast fraction (Quail, 1979). From our results, we inferred that the 24-kD and 55-kD proteins should be plasma-membrane-specific aquaporins (PIPs) and the 19-kD protein should be a tonoplast-specific aquaporin (TIP). Contamination by plasma membrane of fraction I might increase the detectable intensity of putative PIP in fraction I. Because aquaporins are 26 to 35 kD proteins (Santoni et al., 2003), the 55-kD protein might be a dimer. A tetramer has been found in spinach (Karlsson et al., 2000). We compared the amino acid sequences of the synthetic oligopeptides used as antigens with those of proteins in the database by a BLAST search. All the sequences, with high homology to our synthetic oligopeptides were aquaporins. Moreover, in a preliminary experiment with membrane fractions from rice, radish and tobacco, anti-PIP reacted with some proteins in the plasma membrane fraction but not in the tonoplast fraction, but anti-MIP reacted with some proteins in both fractions (data not shown). Therefore, these antibodies are apparently specific to putative aquaporins although other membrane proteins might react with them, particularly with anti-MIP. It is likely that the 19-kD protein detected with anti-MIP was a TIP and the 24-kD protein detected with anti-PIP as a
We confirmed that the antibodies could detect a 1.5-fold difference in the levels of the proteins of interest (Fig. 6). The hydraulic conductivity on the upper side of the root was more than three times greater than that on the lower side. However, there was no significant difference in the levels of putative aquaporins between the two sides of the root (Fig. 7). There are several possible explanations for this absence of any difference. The difference in hydraulic conductivity of the samples used for protein analysis might not be as big as that shown in Table 4 because the difference in hydraulic conductivity was calculated from measurements on cortical cells, where the difference in the elongation rate was the biggest, while the upper and lower halves of the root were used for the protein analysis. However, the hydraulic conductivity of the samples used for analysis of proteins was 1.5 times bigger on the upper side of the root, than that on the lower side, on the average. Alternatively, the detected bands of protein might contain the various putative aquaporins. Levels of proteins with higher activity in water flow might increase on the upper side so as to increase the hydraulic conductivity on the upper side. Finally, the activity of existing proteins might change in response to the gravi-stimulus. Such regulation would be very logical because plants could economize the cost of synthesizing new aquaporins after exposure to the gravi-stimulation. Further experiments are needed to determine whether changes in the activities of existing water channels are sufficient to cause the difference in hydraulic conductivity that results in differential elongation.

Candidate molecules that might regulate the activity of aquaporins are the calcium-dependent protein kinases. Phosphorylation of the serine residues of aquaporins by these kinases increases the activity of the aquaporins (Johansson et al., 1996; Johansson et al., 1998) and calcium is essential for signaling in gravitropism (Sinclair and Trewavas, 1997; Fasane et al., 2002). Calcium-dependent phosphorylation of aquaporins is involved in drought tolerance (Kjellbom et al., 1999) and flower opening in tulip (Azad et al., 2004). The acidification of cell walls might also be involved. A decrease in the pH of the cell wall on the upper side of the root could induce an increase in the pH of the cytosol, after which the activity of the plasma-membrane-specific water channels would increase. This hypothesis is supported by the finding that the activity of plasma-membrane-specific water channels decreases when the pH of the cytosol decreases (Gerbeau et al., 2002; Tournaire-Roux et al., 2003). The movement of protons from the cytosol to the apoplast induces the loosening of the cell wall and results in gravitropic differential elongation (Muday, 2001). In addition, the expression and regulation of expansins (Darley et al., 2001) and yieldins (Okamoto et al., 2001), which are cell-wall proteins, might also be involved at the molecular level in the pH-dependent cell-wall extensibility associated with cell-wall loosening.

Finally, water uptake by cells must be affected by the accumulation of solutes, as well as by other parameters, on which we did not focus in the present study. For example, a reduction in the rate of accumulation of solutes lowers the cell-elongation rate of barley leaves under salt stress (Fricke and Peters, 2002). In gravitropically bending roots, the osmotic potential of elongating tissue was the same on both sides of the roots (Table 3). Thus, cells with a higher elongation rate accumulate solutes more rapidly than the cells on the other side. Therefore, a difference in the rate of uptake of solutes by elongating cells or the rate of generation and degradation of compounds within the cells might be essential in maintaining differences in cell enlargement rates among the cells. The increase in volume caused by hydraulic conductivity, solute accumulation (this study; Miyamoto et al., 2002), and the extensibility of the cell wall (Hirasawa et al., 1997; Hoson et al., 2001) must involve many other processes, such as the synthesis of cell walls and cell components. For a comprehensive understanding of the mechanisms of cell growth, further physiological and molecular biological research is obviously necessary.

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