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Short Communication

Water Permeability and Revolving Movement in *Phaseolus vulgaris* L. Twining Shoots

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Osmotic water permeability (P_{os}) was measured in protoplasts isolated from different tissues of *Phaseolus vulgaris* twining shoot. Parenchyma protoplasts exhibited more frequently high P_{os} values than epidermis protoplasts did. Water channels could facilitate water movement between parenchyma cells whereas cell-to-cell water transport mostly occurs through plasmodesmata in epidermis.

Key words: *Phaseolus vulgaris* L. — Revolving movement — Water channel.

In French bean (*Phaseolus vulgaris* L.), twining shoots display a revolving movement, previously called circumnutation, that occurs rhythmically in the free-moving part of the shoot. Previous results (Badot 1987) indicate that revolving movement is likely driven by turgor variations in cells of the bending zone. The involvement of ion and water fluxes has been proposed (Badot et al. 1990). Recently, Caré et al. (1998) demonstrated that revolving movement was strongly related to reversible length variations in cells of the free-moving part of the shoot. Available data argue in favor of alternative swelling and shrinking occurring in the growing cells of the bending zone. Thus, water fluxes play a central role in the revolving movement mechanism. Up to date, cell-to-cell water pathways in *Phaseolus vulgaris* have been partly documented. Millet et al. (1988) found abundant plasmodesmata only between epidermal cells of the free-moving part of the shoot. Aquaporins could be an other water pathway involved in the revolving movement.

Increasing literature shows that animal and plant membranes contain such membrane intrinsic proteins (MIP) that form water specific pores. It has been shown that their expression can be related to developmental stages or to specific tissues (Johnson et al. 1989, Höfte et al. 1992, Kammerloher et al. 1994, for review, see Maurel 1997). Involvement of aquaporins has been demonstrated in response to water stress (Yamaguchi-Shinozaki et al. 1992). They also act in processes such as cell elongation or seed germination (Ludevid et al. 1992, Kaldenhoff et al. 1995, Maurel et al. 1997).

In the present study, we investigated osmotic water permeability (P_{os}) in protoplasts originating from *Phaseolus vulgaris* twining shoots. Protoplasts were isolated from different tissues of the free-moving part, both in epidermal and parenchyma cells of the convex and concave sides. P_{os} was also measured in cells from the terminal part of the shoot. Water permeability measurements were done using the technique developed by Ramahaleo et al. (1999).

Experiments were conducted with *Phaseolus vulgaris* L. "Blanc de Juillet" provided by S.P.G. (Avignon, France). Plants were grown from seed in containers filled with vermiculite and watered with nutritive solution. Controlled growth conditions were as following: $25 \pm 1°C$, 40 ± 5 W m^{-2} light and $65 \pm 5\%$ relative humidity (Millet et al. 1988). Twelve-day to fourteen-day old plants were used for water permeability investigations. The revolving movement has already started at this stage of development.

Protoplast isolation—Epidermis of the free-moving part of the shoot was manually stripped. Cell walls were enzymatically removed by a method adapted from Thomine et al. (1995). The segments were placed in a digestive solution of 1.7% (w/v) cellulase RS (Yakult Honsha, Tokyo, Japan), 1.7% (w/v) cellulysin (Calbiochem, La Jolla, CA, U.S.A.), 0.026% (w/v) pectolyase Y23 (Seishin Pharmaceuticals, Tokyo, Japan), 0.2% (w/v) bovine serum albumin (BSA), 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM MES, 0.5 mol kg^{-1} sorbitol. The pH was adjusted to 5.5 using Tris. The segments were incubated for 30 minutes at room temperature. Then, enzymatic solution was removed and partially digested epidermal segments were kept in a stock solution: 0.4 mol kg^{-1} sorbitol, 0.5% polyvinylpyrrolidone (PVP), 2 mM $CaCl_2$, 10 mM MES (buffered to pH 5.5 with Tris). Active cyclosis and spherical shape were the two criteria chosen to select protoplasts for swelling experiments.

Swelling experiments—Changes in protoplast volume

Abbreviations: BSA, bovine serum albumin; *Lp*, hydraulic conductivity; PCMB, para-chloromercurybenzoate; *Pd*, diffusional permeability; PIP, plasma membrane intrinsic protein; P_{os}, osmotic permeability; PVP, polyvinylpyrrolidone; TIP, tonoplast intrinsic protein; V_w, molar volume of water.

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were triggered by osmotic shocks: protoplasts were transfered from an initial solution (400 mOsm kg^{-1}) to an hypo-osmotic solution (200 mOsm kg^{-1}). Swelling experiments were performed and volume changes were recorded as described by Ramahaleo et al. (1999). Briefly summarized, a micropipette driven by a micromanipulator (Narishige Co., Tokyo, Japan) was used to catch and to transfer one single protoplast from the initial solution (100 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Tris, 0.05% BSA, with a sorbitol concentration adjusted to give a final osmolality of 400 mOsm kg^{-1} and pH adjusted to 7.2 using MES) to the hypo-osmotic solution (the same as above without sorbitol). Osmolalities of the initial solution and the hypo-osmotic solution were checked using a Wescor 5500 (Logan, UT, U.S.A.) vapor pressure osmometer. Transfer was accomplished in a few seconds by a translation of the microscope slide and changes in protoplast diameter were recorded using a camera and a video tape recorder (Fig. 1).

Osmotic permeability (P_{os}) measurements—The protoplast diameter was measured on a video monitor at 40 ms to 10 s intervals, depending on the values of the osmotic water permeability. The cell volume V was calculated assuming that the part of the protoplasts outside the pipette was spherical, as described in Ramahaleo et al. (1999). As a negative pressure was applied to the micropipette (100–150 Pa), protoplasts were sometimes sucked into the pipette and slight deformations may occur (Fig. 1). The corresponding volume was taken into account in the calculation. Micropipette tips were cylindrical and their internal diameter was measured before investigations. Protoplast volume was estimated by the following expression:

$$V=\frac{\pi\times D^3}{6}+\frac{\pi\times d^2\times L}{4}+\frac{\pi\times d^3}{24}-v(D)$$

where D was the protoplast external diameter, d the micropipette internal diameter and L the length of protoplast intrusion into the pipette. D, L and d were directly measured on video screen. v(D) is the small volume at the tip of the pipette (Fig. 1). In our conditions, v(D) variations were negligible during the measurements (Ramahaleo et al. 1999).

The changes in relative volume with time were fitted to a linear equation to obtain the initial rate of swelling, $d(V/V_0)/dt$. This value was used to calculate the osmotic water permeability, P_{os} ($\mu m\ s^{-1}$) using the following formula:

$$P_{os}=\frac{V_0\times[d(V/V_0)/dt]}{S_0\times V_w\times(Osm_1-Osm_2)}$$

with V_0, S_0: initial volume and surface of the protoplast; V_w: molar volume of water ($V_w=18.10^{-6}\ m^3\ mol^{-1}$); Osm_1, Osm_2: osmolalities in the initial solution (400 mOsm kg^{-1}) and in the hypo-osmotic solution (200 mOsm kg^{-1}).

On a methodological point of view, validity and accuracy of P_{os} measurements have been discussed in details by Ramahaleo et al. (1999). Only large variations (at least

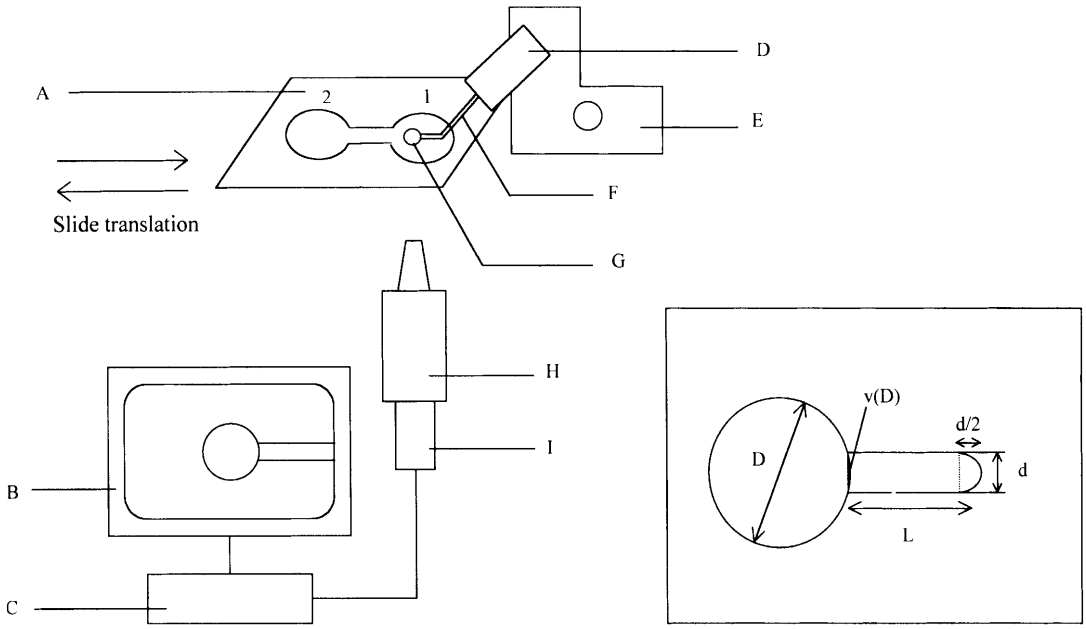

Fig. 1 Schematic representation of the experimental set up used to measure osmotic water permeability (P_{os}) in bean shoot protoplast. A, microscope slide; B, video monitor; C, video-tape recorder; D, micropipette holder and manometer; E, micromanipulator; F, micropipette; G, protoplast; H, microscope; I, video camera; 1, initial solution; 2, hypo-osmotic solution. Inset: parameters measured to calculate the protoplast volume. D, protoplast diameter; d, micropipette internal diameter; L, length of the protoplast intrusion into the pipette; v(D), protoplast volume at the tip of the pipette. (after Ramahaleo et al. 1999).

Table 1 Osmotic water permeability (P_{os}, $\mu m\ s^{-1}$) and initial diameter (μm) of protoplasts originating from various tissues

Origin of shoot protoplasts	n	D (μm) (mean ± SD)	P_{os} ($\mu m\ s^{-1}$) median
Bending zone epidermis	29	23.5 ± 4.0	40
Terminal part epidermis	8	22.5 ± 2.5	36
Bending zone parenchyma	31	27.5 ± 4.0	62
Concave part epidermis and parenchyma	28	25.5 ± 5.0	46
Convex part epidermis and parenchyma	32	25.0 ± 4.0	51

n, number of measurements.

a 50% change in P_{os}) have been taken into account for physiological interpretation.

Osmotic permeability (P_{os}) in bean cells—The diameters of the protoplasts used in our experiments ranged between 18.5 and 35.5 μm in the initial solution (400 mOsm kg^{-1}). These variations in protoplast diameter were related to in situ variations in cell size. Table 1 gives the initial diameter (mean ± SD) and P_{os} medians for protoplasts originating from various twining shoot tissues. The mean diameter of the protoplasts obtained from epidermal strips taken from the bending zone was 23.5 ± 4.0 μm (n = 29). After an osmotic shock from 400 to 200 mOsm kg^{-1}, P_{os} values of these protoplasts ranged between 5 and 300 $\mu m\ s^{-1}$, the median P_{os} value being 40 $\mu m\ s^{-1}$ (Table 1). Protoplasts isolated from terminal part epidermis had initial diameter (22.5 ± 2.5 μm) and P_{os} values (median = 36 $\mu m\ s^{-1}$) very closed to those measured in bending zone epidermis.

In the literature, there are only few results dealing with P_{os} measurements in epidermis and available data were obtained using different methods: pressure probe in *Elodea densa* leaf epidermis (19 ± 3 $\mu m\ s^{-1}$, Steudle et al. 1982) and in *Pisum sativum* epicotyl epidermis (3–30 $\mu m\ s^{-1}$, Cosgrove and Steudle 1981), and deplasmolysis in *Allium cepa* bulb inner epidermis (6–8 $\mu m\ s^{-1}$, Url 1971). By comparison, P_{os} values in bean cells, displayed a high variability. This P_{os} variability did not originate from differences from shoot-to-shoot differences because similar dispersions were obtained from protoplasts originating from a single shoot. Such a dispersion in P_{os} values was also found in protoplasts from root cells (Ramahaleo et al. 1999). As suggested by these authors, it may correspond to differences in aquaporin expression or activity. Most of the epidermal protoplasts (90%) exhibited osmotic water permeabilities lower than 100 $\mu m\ s^{-1}$ (Fig. 2) as reported in all other studied plant materials. Furthermore, it appeared that P_{os} measured in bean cells were not dependent on the protoplast diameter.

As several authors reported tissue-specific expression of aquaporins (Melroy and Herman 1991, Opperman et al. 1994, Hollenbach and Dietz 1995, Yamada et al. 1995), we investigated P_{os} in parenchyma cells of *Phaseolus vulgaris* twining shoots. In the bending zone parenchyma, P_{os} was measured in 31 protoplasts (mean diameter ± SD, 27.5 ± 4.0 μm). The median P_{os} value was 62 $\mu m\ s^{-1}$ (Table 1). Thus, parenchyma exhibited higher P_{os} values than epidermis: 39% of measured P_{os} were higher than 100 $\mu m\ s^{-1}$ (Fig. 2). A similar pattern in P_{os} values had been found in growing pea epicotyl from osmotic Lp (hydraulic conductivity) measurements (Cosgrove and Steudle 1981): $Lp = 0.2$ to 2.10^{-6} cm s^{-1} bar^{-1} in epidermis and $Lp = 0.4$ to 9.10^{-5} cm s^{-1} bar^{-1} in cortex, so $P_{os} = 3$ to 30 $\mu m\ s^{-1}$ in epidermis and $P_{os} = 50$ to 1,200 $\mu m\ s^{-1}$ in cortex. Even if the threshold between "high" and "low" P_{os} values is difficult to estimate (Chrispeels and Agre 1994, Haines 1994), P_{os} values lower than 100 $\mu m\ s^{-1}$ are usually associated with diffusional permeability (Pd) whereas values higher than 100 $\mu m\ s^{-1}$ are considered to argue for water channel activity. Thus, we think that a greater number of bean parenchyma cells had functional aquaporins while most epidermal cells would not display water channel activity.

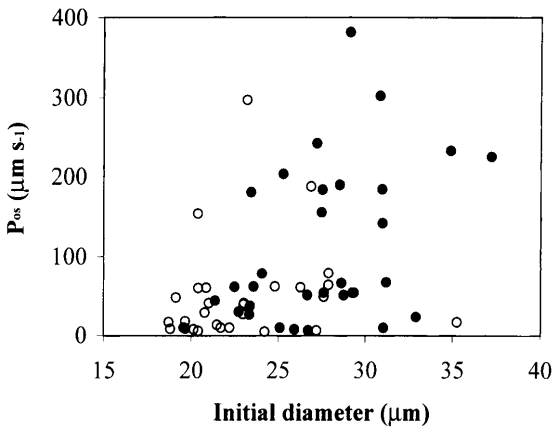

Fig. 2 Distribution of P_{os} values for epidermis (○) and parenchyma (●) protoplasts from bending zone of *Phaseolus* shoot versus protoplast initial diameter.

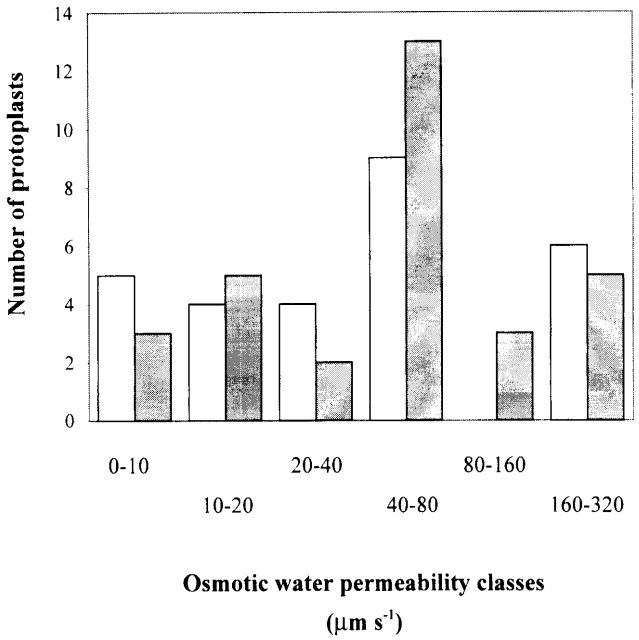

Fig. 3 Histogram for P_{os} values measured in protoplasts originating from convex (□) or concave (■) part of the twining shoot. A logarithmic scale was used for the P_{os} axis. n, number of protoplast in each class.

Permeability differences between cells originating from concave and convex part of the twining shoot were also investigated. Measurements were conducted on protoplasts originating from epidermis and underlying tissue. Tissues sampled from the concave part of the shoot did not display any significant difference in P_{os} compared to those originating from the convex part (Fig. 3). Protoplasts originating from the concave side had a mean diameter ranged from 19.0 to 37.5 μm (25.5$\pm$5 μm, n=28) and P_{os} ranged from 5 to 300 μm s^{-1} (median=46 μm s^{-1}). Diameters of protoplasts originating from the convex side were between 18.5 and 31.0 μm (25.0$\pm$4 μm) and these cells had P_{os} values in the range of 6 to 380 μm s^{-1} (median=51 μm s^{-1}).

The question of the physiological significance of P_{os} differences in bean cells has to be addressed. A main question to be answered is whether high P_{os} values measured in some protoplasts really reflect aquaporin presence. Existence of a facilitated water transport through water channels is the most tempting hypothesis to explain values higher than 100 μm s^{-1}. Mercury ion experiments would have partially answered the question. This ion was usually used to inhibit water channel activity in *Xenopus* oocytes expressing heterologous aquaporins (Preston et al. 1992, Maurel et al. 1993) and in plant cells (Henzler and Steudle 1995, Maggio and Joly 1995, Carvajal et al. 1996, Tazawa et al. 1996, Ramahaleo et al. 1999). Thus, we studied the effects of $HgCl_2$ 50 μM and 10 μM. When protoplasts originating from parenchyma were incubated with 50 μM $HgCl_2$, cyclosis was stopped and protoplasts were too fragile for manipulation. At 10 μM, mercury ion had no effect on cyclosis and water permeability was unchanged. P_{os} was also measured in the presence of the sulfhydryl reagent para-chloromercurybenzoate (PCMB) at 1 mM. This compound displayed no effect on cyclosis and P_{os}. With PCMB, some protoplasts still had P_{os} values higher than 100 μm s^{-1}. Ramahaleo et al. (1999) obtained similar results using rape root protoplasts. Thus, $HgCl_2$ and PCMB experiments did not bring additionnal evidence that high P_{os} values measured in parenchyma protoplasts correspond to the presence of aquaporins in the cell membrane. This result is not completely surprising since mercury insensitive plasma membrane aquaporins (RD28) have been recognized (Daniels et al. 1994).

An other question to be answered is whether the lack of water channel activity observed in numerous cells of twining shoot epidermis and parenchyma is consecutive to aquaporin absence or to a lack of activity of expressed water channels. Maurel et al. (1995) and Johansson et al. (1998) have recently shown that water channel activity can be regulated by phosphorylation in plants as in animals (Kuwahara et al. 1995). It is now clear that other tonoplast intrinsic proteins (TIP) and plasma membrane intrinsic proteins (PIP) have phosphorylation sites but their involvement in regulating water channel activities is still unknown. The abundance and/or the activity of aquaporins can be used by plants to regulate water flow. Interestingly, bean epidermal cells present high plasmodesmata density compared to underlying cells (Millet et al. 1988). Aquaporins would be one of the main pathways for water flow in parenchyma whereas plasmodesmata play this role in epidermal cells. It is now assumed that aquaporins are involved in plant cell turgor and volume regulation (Maurel et al. 1995, 1997). However, turgor variations are not the only mechanisms involved in bean movement. We have recently shown that revolving movement mechanism was strongly related to partly reversible variations in cell length (Caré et al. 1998). The revolving movement is markedly dependent on high growth rate in bean (Millet et al. 1988) but also in other species (Claire 1974, Melin 1975). Interestingly, water channel and active growth may be associated as reported in the elongating cells of *Arabidopsis* root and epicotyl where the plasma membrane aquaporin PIP1b was preferentially expressed (Kaldenhoff et al. 1995). Although we could not correlate a variation in P_{os} with a concave/convex origin of the protoplasts, our results suggest that cell-to-cell water transport in epidermis mostly occurs by plasmodesmata whereas water channels would facilitate water movement in the underlying tissues: water fluxes required by active growth—from xylem vessels to growing cells—would preferentially occur through aquaporins.

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