Plant Physiol. (1987) 83, 592–595
0032-0889/87/83/0592/04/$01.00/0

Pressure Probe and Isopiestic Psychrometer Measure Similar Turgor[1]

Received for publication May 5, 1986 and in revised form September 22, 1986

HIROSHI NONAMI, JOHN S. BOYER*[2], AND ERNST STEUDLE[3]
Department of Plant Biology, Department of Agronomy, and United States Department of Agriculture, Agricultural Research Service, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Turgor measured with a miniature pressure probe was compared to that measured with an isopiestic thermocouple psychrometer in mature regions of soybean (*Glycine max* [L.] Merr.) stems. The probe measured turgor directly in cells of intact stems whereas the psychrometer measured the water potential and osmotic potential of excised stem segments and turgor was calculated by difference. When care was taken to prevent dehydration when working with the pressure probe, and diffusive resistance and dilution errors with the psychrometer, both methods gave similar values of turgor whether the plants were dehydrating or rehydrating. This finding, together with the previously demonstrated similarity in turgor measured with the isopiestic psychrometer and a pressure chamber, indicates that the pressure probe provides accurate measurements of turgor despite the need to penetrate the cell. On the other hand, it suggests that as long as precautions are taken to obtain accurate values for the water potential and osmotic potential, turgor can be determined by isopiestic psychrometry in tissues not accessible to the pressure probe for physical reasons.

The accurate measurement of turgor is essential for understanding physiological processes such as osmoregulation (12, 17, 19), cell expansion (8, 18), and stomatal opening (14, 21). With the recent development of the miniature pressure probe (10, 22), it has become possible to measure turgor directly in the cells of higher plants. However, turgor can also be measured by determining the water potential (ψ_w)[4] and osmotic potential (ψ_s) of the tissue and calculating the turgor by difference (9). This method, generally based on vapor pressure measurements using thermocouple psychrometers, is presently the most widely used (3). It is not known whether the two methods give comparable values. Therefore, we made a comparison of turgor measured by both methods in the same tissue.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* [L.] Merr. cv Wayne) seedlings were grown from seeds that were disinfected in a 1% solution of NaOCl for 3 min, rinsed with flowing water for 1 h, and sown in vermiculite containing adequate water (5.0 ml of 0.1 mM $CaCl_2$/g of vermiculite, *i.e.* a ψ_w of −0.01 MPa). The seedlings were grown at 29 ± 0.5°C and 100% RH in the dark until transplanting. After 60 h, each seedling was transplanted to a 200 ml beaker containing water-deficient vermiculite (0.63 ml of 0.1 mM $CaCl_2$/g of vermiculite, *i.e.*, a ψ_w of −0.28 ± 0.02 MPa). For those seedlings to be measured with the pressure probe, the top of the beaker was covered with a plastic film to retard evaporation from the vermiculite. The seedlings were returned to the growth conditions (29 ± 0.5°C and 100% RH in the dark) until use. All seedling manipulation was carried out under a green safelight (green fluorescent bulb wrapped in green plastic sheet having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm).

Turgor Measurement. *Pressure Probe.* Prior to the measurement of cell turgor with the probe (10, 22), the seedling was coated with petrolatum and the boundary between the stem and the plastic film which covered the vermiculite surface was carefully sealed with petrolatum. The seedling was covered with wet tissue paper on the petrolatum to further minimize water loss from the surface. To insert the capillary, a small window was made in the wet paper. The seedling was mounted vertically on a holder to stabilize the stem. On the holder, the seedling grew at the same rate as it would without having been placed on the holder. For manipulation of the probe, the tissue was illuminated with green light (light emitted from a tungsten lamp was guided through a light pipe which had a green plastic filter having maximum transmission at 525 nm and negligible transmission below 475 nm and above 575 nm).

The turgor of cortical cells in the mature region of the stems (hypocotyl base) was measured by inserting the probe tip into the stem and noting when the oil in the probe was pushed back by the entry of solution from a cell. Although the tip of the probe could not be seen, the entrance of the solution was instantaneous and was diagnostic for the penetration of a cell. The cell solution entering the tip of the microcapillary during the first penetration was used to form the solution/oil boundary (meniscus) at a small distance from the stem surface so that the position of the boundary was observable under a microscope. As the probe penetrated deeper cells, the tip tended to fill with solution from intervening cells. Thus, upon penetrating a new cell, the volume of solution entering the probe was negligible and only enough to push the boundary within the microcapillary. The position of the boundary could be changed with the aid of a small motor which moved a metal rod in the oil reservoir of the probe.

Using this adjustment, the boundary was returned to the same position it occupied before the tip entered the cell. At this position, the boundary was moved back and forth slightly to make certain that the probe remained in hydraulic contact with the cell solution. Plugging of the tip could be readily detected

[1] Supported by National Science Foundation grant PCM79-09790 and Department of Energy grant DE-FG05-84ER13273 to J. S. B. and by a grant from the Deutsche Forschungsgemeinschraft (Ste 319/1-2) to E. S.

[2] Present address: Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

[3] This work was done while E. S. was on leave from the Institut für Radioagronomie of the Kernforschungsanlage Jülich, Postfach 1913, D-5170 Jülich, Federal Republic of Germany.

[4] Abbreviations: ψ_w, Water potential; ψ_s, osmotic potential; ψ_p, turgor.

because the boundary could not be moved and big changes in pressure occurred upon only slightly moving the metal rod.

Before penetrating the cells, the pressure probe was calibrated with a test gauge. Outputs of the transducer were linearly related to the pressure applied (69 mV/MPa) (Fig. 1). Since both ascending and decending pressure gave the same linear relation, the pressure probe did not exhibit any hysteresis. The response time was only a fraction of a second.

Psychrometer. A thermocouple chamber (Ⱡ, 5) was coated with melted and resolidified petrolatum (2) and loaded with one hypocotyl segment about 1.5 cm long from the mature region of each of four stems. The ψ_w was measured by the isopiestic technique (5). This measurement was immediately followed by a measurement of ψ_s in the same tissue using the same technique after freezing at −70°C and thawing (9). Turgor was calculated by subtracting ψ_s from ψ_w. No correction was made for dilution of the protoplast solution by water imbibed in the wall because the entire wall volume was only 3.9% of the cell volume (11), which could be neglected. The measurements of ψ_w required 3 to 4 h and those of ψ_s 2 to 3 h.

RESULTS

Figure 2 shows that ψ_w and ψ_s decreased in the mature region of the stems after transplanting the seedlings to the drier vermiculite. The ψ_w decreased until it reached −0.7 MPa after 30 h and then increased. The transient decrease of ψ_w below that of the vermiculite was due to the fact that water was drawn from the mature region into the elongating region because the zone of elongation had a much lower ψ_w than the mature region (7). After long times, the ψ_w of the tissue approached the ψ_w of the vermiculite. The ψ_s decreased more slowly than the ψ_w after transplanting. Turgor, which was the difference between ψ_w and ψ_s, decreased and then increased after 30 h. This experimental system therefore provided a range of turgor over which the comparison of the pressure probe and psychrometer could be made.

When the microcapillary tip of the pressure probe was inserted into a cell, the tendency for cell solution to enter the tip could be prevented by increasing the pressure of the oil in the probe. As the cell solution/oil boundary was returned to its original position in the tip, the required pressure should have been equal to the turgor of the cell provided that the amount of cell solution

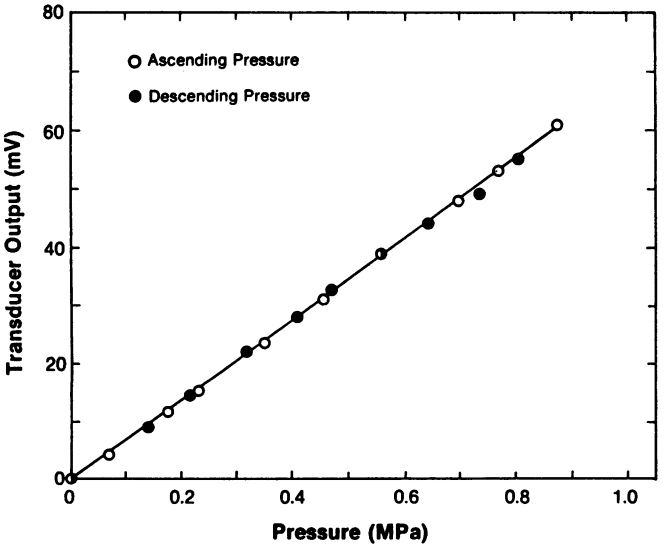

FIG. 1. Calibration of transducer output (y) of the pressure probe with a test gauge under ascending and descending pressure (x) ($y = 69x$, $r = 0.999$).

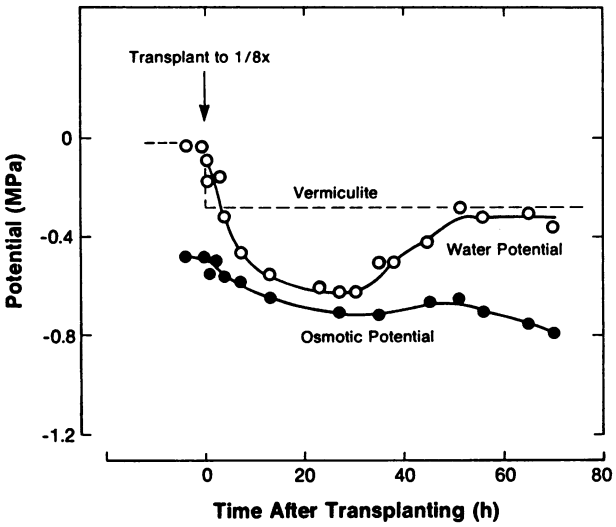

FIG. 2. ψ_w and ψ_s in the mature region of soybean stems measured with the isopiestic psychrometer before and after transplanting the seedlings from wet to relatively dry vermiculite. ψ_w of the vermiculite is shown by the dashed line.

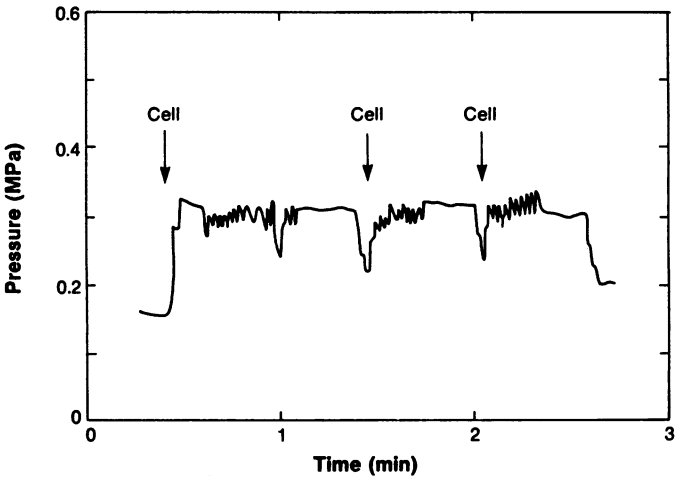

FIG. 3. Recorder tracing of the output from the pressure probe during typical turgor measurement in three cells. Probe tip enters cell at arrows. Rise in pressure occurs as operator returns cell solution to original position in the tip. Meniscus is moved slightly to assure hydraulic continuity of cell solution in probe and in cell (ripples in trace). Generally stable pressures while tip is in cell indicate no leakage occurred during the measurement. Pressure is lowered before moving to next cell to conserve oil in tip. See text for details.

removed from the cell was negligible (see "Material and Methods" and [16]). Figure 3 shows the initial rapid rise necessary to make this adjustment in a typical cortical cell from the mature region of the stem and indicates that the turgor was 0.3 MPa in the first cell. To make sure that the probe and the cell were hydraulically connected, the meniscus was moved back and forth around the original meniscus position. This is represented by ripples in the figure. If an object became lodged in the capillary tip, the solution/oil boundary would not move and the pressure would rapidly rise or fall by a very large amount. We determined the turgor, averaging the ripple-changes of pressure, in an average time of less than 30 s. After the turgor was measured, the pressure was decreased and the tip of the microcapillary was moved to the next cell. Immediately after the tip of the capillary was moved deeper, we could determine whether it had entered a cell because the meniscus would rapidly move into the probe, and the process

could be repeated.

We measured the turgor in cortical cells located 100 to 500 μm below the surface of the stems. The length and width of these cells were 300 to 370 μm and 55 to 70 μm, respectively. The volume was 700 to 1450 pl (the vacuole occupied more than 80% of the total volume) and the diameter of the capillary tip was about 2 to 4 μm. When the tip was located 20 to 40 μm inside a cell, the volume of glass introduced into the cell was estimated to be only 0.4 to 1.7 pl. Because the ripples in pressure were made with only 5 to 10 pl of volume change, it is safe to assume that manipulation of the probe did not affect the cell environment significantly (16). Leakage of cell solution caused by penetration of the cell could be readily detected because turgor decreased instead of remaining constant. Leakage occurred only rarely. The location of the capillary tip in the cell should not have influenced the turgor measurement because pressure should have been identical throughout the protoplasm and would be detected whenever the tip penetrated the plasmalemma. The turgor of neighboring cells usually was similar, as shown in Figure 3. However, turgor sometimes differed randomly between cortical cells.

Both the psychrometer and the pressure probe gave similar values of turgor in the stems throughout the experiment (Fig. 4). As turgor decreased during the first 20 h (Fig. 4A) after transplanting to vermiculite having low ψ_w (Fig. 2), the pressure probe

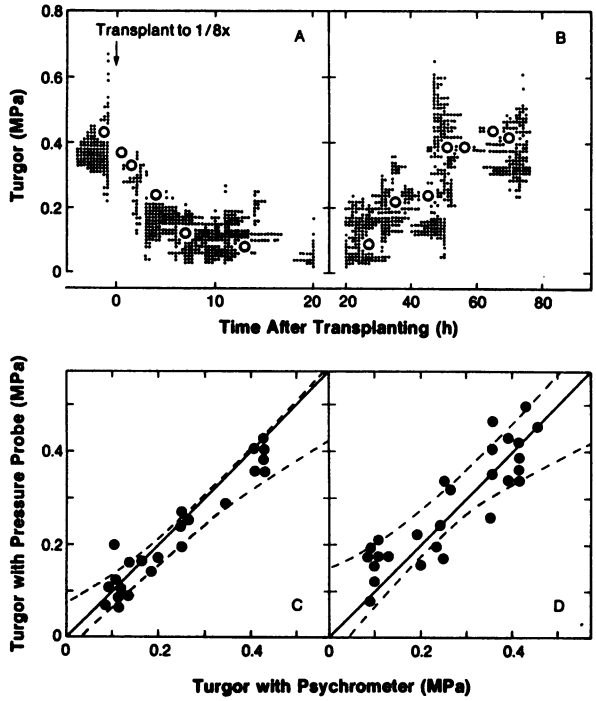

FIG. 4. ψ_p measured with the pressure probe and isopiestic psychrometer in the mature region of soybean stems. Individual pressure probe and psychrometer measurements are shown during dehydration in the first 20 h after transplanting (A) and rehydration during the next 60 h (B), as in Figure 2. Small dots indicate turgor of individual cells measured with the pressure probe, and open circles indicate turgor of excised tissues from four seedlings measured with the isopiestic psychrometer. In (C) and (D), the average turgor was calculated for single seedlings with the pressure probe (10–60 cells per seedling) using the data in (A) and (B) and plotted with the turgor measured with the isopiestic psychrometer. The regression equations were $y = 0.84\,x + 0.02$ ($r = 0.954$) for the data in Figure 4C and $y = 0.86\,x + 0.05$ ($r = 0.881$) for the data in Figure 4D. Dotted lines indicate the hyperbolic confidence band around the regression equations at $p \leq 0.01$. The isopotential position is shown by the diagonal line.

readings, which were for individual cells, were scattered about the psychrometer measurements, which gave volume-averaged turgor for the tissue as a whole (4, 20). A similar behavior was observed as turgor recovered between 20 and 80 h after transplanting (Fig. 4B). The turgor in control seedlings transplanted to vermiculite having high ψ_w did not change (data not shown).

To make the pressure probe readings more comparable to the psychrometer measurements, we averaged the turgor of the individual cells (10–60 cells for each seedling) and plotted the averaged turgor of individual plants with corresponding psychrometer measurements (Fig. 4, C and D, using data from Fig. 4, A and B, respectively). A regression equation and the hyperbolic confidence band around the regression equation were calculated for $P \leq 0.01$ using the Working-Hottelling formula (13) which assumes a normal distribution of the data measured with both techniques. Figure 4, C and D, shows that both the regression and the isopotential line are within the calculated confidence band.

To evaluate intrinsic errors of each technique, error propagation in the measurement of the average ψ_p with the isopiestic psychrometer was also calculated from the standard deviations of ψ_w and ψ_s (16) and was compared with the standard deviation obtained for the tissue ψ_p from measurements of individual cells using the pressure probe. In plants grown for 70 to 75 h after transplanting, the $\psi_w = -0.31 \pm 0.04$ and $\psi_s = -0.77 \pm 0.03$ MPa (average ± SD; $n = 12$ measurements on four seedlings at each measurement) with the psychrometer. The ψ_p calculated from the difference was 0.46 ± 0.05 MPa (average ± propagated error). In corresponding seedlings, the pressure probe gave a $\psi_p = 0.39 \pm 0.07$ MPa (average ± SD; $n = 155$ cells from four seedlings). Thus, the accuracy of the average ψ_p determination was similar with both techniques. It should be noted that the SD values incorporate the biological variation between different seedlings and are, thus, an upper limit. The use of different seedlings for the comparison between the techniques was necessary because the probe measurements damaged the tissue so that the seedlings could not be used afterwards in the psychrometer.

DISCUSSION

The close correspondence between turgor readings obtained with the psychrometer and pressure probe should be viewed within the context of the way the measurements were made. The most common errors in psychrometric determinations are caused by diffusive resistances to water vapor transport between the thermocouple and tissue, which result in ψ_w that are too high (5), and by dilution of the cell solution with the liquid in the cell wall after freezing and thawing, which causes ψ_s to be too high (6). Both these sources of error were minimized in this study. Isopiestic psychrometry was used for all measurements because it is not subject to diffusion errors (5). The tissue chosen for measurement had a small cell wall volume, allowing us to ignore dilution errors.

For the pressure probe, the most common problems are that the cells must be viewed under bright light and must be punctured, leading to possible disruption of the true turgor of the cells. This effect was minimized by using large cells (300–370 μm long and 55–70 μm wide) in which the probe tip would be comparatively small and the manipulations necessary to maintain hydraulic continuity would be the least disturbing. Care was also taken to prevent the tissue from dehydrating under the light during manipulation. The coating with petrolatum was especially important because the seedlings had a rather thin cuticle which was fairly permeable to water (15). When the coating was not present, probe readings were more variable and significantly below the corresponding measurements with the psychrometer, despite covering the seedlings with wet tissue paper and maintaining high humidities. We attribute the dehydration of the cells

in the absence of a coating to the need to work under bright light with the pressure probe. Only with the coating did we observe a 1:1 correspondence between the methods.

We grew the tissue and made the turgor measurements at saturating humidities to avoid the effects of transpiration on the water status of the tissue. In addition, the comparisons were made with mature tissue where complications caused by growth would not be present. In mature hypocotyl tissue, there is no effect of excision on tissue water status (4). Therefore, the measurements made with the psychrometer on excised tissue could be compared validly with those of the pressure probe in intact tissue.

Our measurements involved turgor less than 0.6 MPa. It would be desirable to make a comparison over a wider range of turgor before the strict comparability of the methods is known with certainty. However, in principle, we see no reason why the comparability should change at higher turgor.

Boyer and Potter (6) compared ψ_w, ψ_s, and turgor measured with the pressure chamber and the isopiestic psychrometer in mature sunflower leaves, and obtained virtually the same values with both methods. With the psychrometer, ψ_s had to be corrected for dilution by the apoplast solution because a significant fraction of the water in the tissue was located there (about 10% of the tissue volume). Because neither method required the penetration of living cells in order to determine turgor, the correspondence of the probe data with the psychrometer data in the present study indicates that penetration of the cells by the probe does not significantly alter the turgor of large cells. Because the three methods rely on different principles of measurement, it is unlikely that all would be subject to inherent but undetected inaccuracies that would cause turgor to deviate by the same amount from the true turgor in the cells. Therefore, the agreement between the methods suggests that accurate measurements of turgor are possible with each one.

Although the pressure probe can measure turgor directly, it is not necessarily convenient to use for all plants and tissues because cell size, location in the tissue, and wall characteristics can limit the probe for physical reasons. Moreover, in tissues like soybean hypocotyls, there is the practical necessity to make large numbers of measurements in order to obtain a representative average for the tissue. Psychrometers may be used for a wider range of plant material and include large numbers of cells in each determination. However, psychrometers provide average values of water status and may miss differences within a tissue (*e.g.* between epidermis and cortex of soybean hypocotyls; [15]). This means that the differences in the measured quantities within the tissue have to be taken into account when psychrometer and probe data are compared. The comparability between the isopiestic psychrometer and the probe in soybean stems suggests that accurate turgor measurements are possible in tissues not accessible to the probe, providing that precautions are taken to minimize diffusion and dilution errors.

LITERATURE CITED

1. Boyer JS 1966 Isopiestic technique: measurement of accurate leaf water potentials. Science 154: 1459–1460
2. Boyer JS 1967 Leaf water potentials measured with a pressure chamber. Plant Physiol 42: 133–137
3. Boyer JS 1969 Measurement of the water status of plants. Annu Rev Plant Physiol 20: 351–364
4. Boyer JS, AJ Cavalieri, E-D Schulze 1985 Control of the rate of cell enlargement: excision, wall relaxation, and growth-induced water potentials. Planta 163: 527–543
5. Boyer JS, EB Knipling 1965 Isopiestic technique for measuring leaf water potentials with a thermocouple psychrometer. Proc Natl Acad Sci USA 54: 1044–1051
6. Boyer JS, JR Potter 1973 Chloroplast response to low leaf water potentials I. Role of turgor. Plant Physiol 51: 989–992
7. Cavalieri AJ, JS Boyer 1982 Water potentials induced by growth in soybean hypocotyls. Plant Physiol 69: 492–496
8. Cleland RE 1977 The control of cell enlargement. Symp Soc Exp Biol 31: 101–115
9. Ehlig CF 1962 Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol 37: 288–290
10. Hüsken D, E Steudle, U Zimmermann 1978 Pressure probe technique for measuring water relations of cells in higher plants. Plant Physiol 61: 158–163
11. Molz FJ, JS Boyer 1978 Growth-induced water potentials in plant cells and tissues. Plant Physiol 62: 423–429
12. Morgan JM 1984 Osmoregulation and water stress in higher plants. Annu Rev Plant Physiol 35: 299–319
13. Neter J, W Wasserman, MH Kutner 1983 Applied Linear Regression Models. Richard D. Irwin, Inc., Homewood, IL, pp 147–158
14. Raschke K 1975 Stomatal action. Annu Rev Plant Physiol 26: 309–340
15. Steudle E, JS Boyer 1985 Hydraulic resistance to radial water flow in growing hypocotyl of soybean measured by a new pressure-perfusion technique. Planta 164: 189–200
16. Steudle E, Jac Smith, U Lüttge 1980 Water relation parameters of individual mesophyll cells of the CAM plant *Kalanchoë daigremontiana*. Plant Physiol 66: 1155–1163
17. Steudle E, U Zimmermann 1984 Water relations of plant cells: further development of the pressure probe and of techniques for measuring pressure-dependent transport. *In* WJ Cram, K Janacek, R Rybova, K Sigler, eds, Membrane Transport in Plants. Academia, Prague, pp 73–82
18. Taiz L 1984 Plant cell expansion: regulation of cell wall mechanical properties. Annu Rev Plant Physiol 35: 585–657
19. Turner NC, MM Jones 1980 Turgor maintenance by osmotic adjustment: a review and evaluation. *In* NC Turner, PJ Kramer, eds, Adaptation of Plants to Water and High Temperature Stress. John Wiley & Sons, Inc., New York, pp 87–103
20. Tyree MT, HT Hammel 1972 The measurement of the turgor pressure and the water relations of plants by the pressure bomb technique. J Exp Bot 23: 267–282
21. Zeiger E 1983 The biology of stomatal guard cells Annu Rev Plant Physiol 34: 441–475
22. Zimmermann U, E Steudle 1978 Physical aspects of water relations of plant cells. Adv Bot Res 6: 45–117