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ORIGINAL PAPER

Comparison of plant cell turgor pressure measurement by pressure probe and micromanipulation

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Abstract The conventional method of measuring plant cell turgor pressure is the pressure probe but applying this method to single cells in suspension culture is technically difficult and requires puncture of the cell wall. Conversely, compression testing by micromanipulation is particularly suited to studies on single cells, and can be used to characterise cell wall mechanical properties, but has not been used to measure turgor pressure. In order to demonstrate that the micromanipulation method can do this, pressure measurements by both methods were compared on single suspension-cultured tomato (*Lycopersicon esculentum* vf36) cells and generally were in good agreement. This validates further the micromanipulation method and demonstrates its capability to measure turgor pressure during water loss. It also suggests that it might eventually be used to estimate plant cell hydraulic conductivity.

Keywords Micromanipulation · Pressure probe · Tomato · Turgor

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Introduction

The mechanical and hydraulic properties of plant cells are critically important in determining both the physiological behaviour of plants and their behaviour in food processing. In both contexts, the turgor (hydrostatic) pressure is of particular importance. Plant cells are turgid because osmotic swelling of the protoplast is resisted mechanically by the cell wall. At equilibrium, the turgor pressure balances the osmotic pressure (strictly water potential) difference across the plasmalemma and cell wall.

The pressure probe technique is used to measure turgor and study water relations of plant cells, and can provide some mechanical property measurements (Tomos 2000). Although the probe was initially developed for large single algal cells (Steudle and Zimmermann 1971), most pressure probe studies are conducted on higher plant tissues, and the method is rarely used on single suspension cells.

Another approach to characterising the mechanical properties of plant cells, particularly

their cell wall material properties, is compression testing by micromanipulation, which was initially developed for studying the mechanical properties of animal cells (Thomas et al. 2000). This technique has been applied successfully to single plant cells (Blewett et al. 2000) and mathematical modelling has been used to derive the elastic modulus of the cell wall from compression data (Wang et al. 2004).

Although compression testing seems very successful, this study was intended to validate it further by showing how it compares with the pressure probe in estimations of turgor pressure.

Materials and methods

Plant material

The tomato cell line, *Lycopersicon esculentum* vf36, was kindly provided by Unilever Research, Colworth Laboratory, Sharnbrook, UK. The cells were grown on an MS basal salts medium in a shaker at 100 rpm at 25°C in low light, and were subcultured weekly (Blewett et al. 2000). Single tomato cells were obtained from 2-week-old cultures. A 72 μm mesh sieve was used to separate single cells from any aggregates. The viability of the cells was checked by Neutral Red staining, and was always over 80%, and was usually over 90%.

Measurement of compression

The temperature of the cells during compression was 24 ± 1°C.

Details of the compression testing method can be found in Wang et al. (2004). In essence, a single tomato cell was compressed between the base of a chamber containing cells suspended in medium, and the flat end of a micromanipulation probe. This probe was connected to a force transducer with a maximum force reading of 5 gf and a claimed resolution of 100 μN, which was fixed on a micromanipulator programmed to travel a chosen distance, at 23 μm/s in this case. This compression speed was too fast for significant water losses across the cell membrane during compression (Wang et al. 2004). When such a cell had been compressed to 30% deformation, the sharpened tip of a pressure probe microcapillary was inserted to the cell either immediately or at some chosen time. The turgor pressure of the tomato cell was measured with the pressure probe by the method described in Boyer (1995), whilst the force being imposed on the cell was recorded continuously. The pressure probe measurement typically took 10–15 s.

The side microscope of the micromanipulation equipment allowed the cell to be seen clearly from the side (Fig. 1). Calculation of the turgor pressure of the tomato cells was possible by dividing the compression force by the contact area (of cell and micromanipulation probe) estimated from the side view assuming axisymmetry along the probe axis. This calculated pressure was compared with the turgor pressure found directly from the pressure probe.

Results and discussion

When 52 single tomato cells were compressed to 30% deformation, the mean turgor pressure obtained from the pressure probe (3.3 ± 0.2 bar), and calculated from the compression force and contact area by image analysis (3.2 ± 0.2 bar), were not significantly different.

Figure 2 shows when a cell was compressed and held, the force reached a nearly constant level after 10–20 s of relaxation. The introduction of

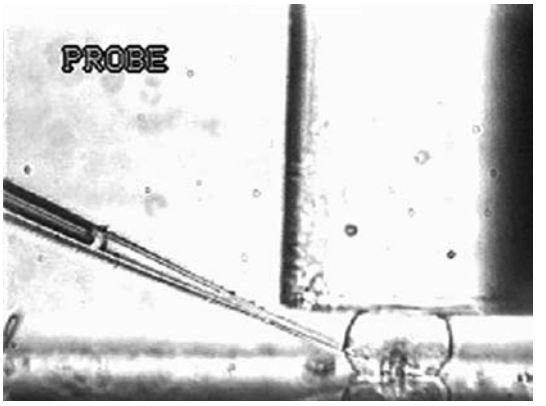

Fig. 1 Single tomato cell compressed between a micromanipulation probe and the bottom surface of a glass chamber. The probe diameter was 200 μm. The pressure probe microcapillary has been inserted into the cell from the left

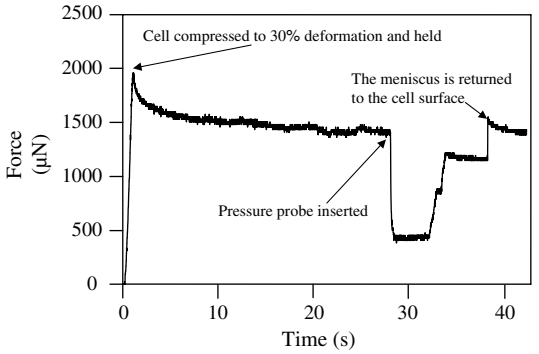

Fig. 2 Force data during a compression-relaxation experiment on a single tomato cell, showing the force response during a turgor pressure determination using the pressure probe

the probe resulted in a decrease of the force as turgor decreased with the entry of cytoplasm into the probe. As the pressure in the pressure probe was increased to drive the meniscus towards the cell, the turgor pressure rose, and the force increased.

Figure 3 shows good agreement between turgor pressure measured with the pressure probe and that calculated from the micromanipulation method, when the pressure in a chosen cell was dropping over 75 s. It is believed that this drop was due to water loss across the membrane due to the increased turgor pressure, but there could also have been a contribution from leakage around the point of entry of the pressure probe into the cell.

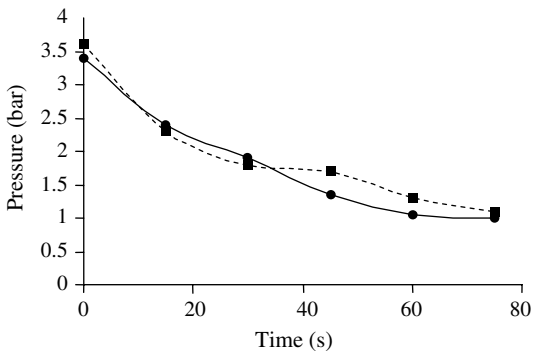

Fig. 3 Series of turgor pressure determinations by the pressure probe as a tomato cell relaxes, with the corresponding estimates from micromanipulation. It was presumed the relaxation was due to water loss from the cell. Typical 95% confidence limits in both cases are ±0.2 bar

In addition, examination of 52 cells at various times gave a good correlation between the methods (Fig. 4), although the fit suggests the micromanipulation method gave slightly lower values than the pressure probe.

Given the problems of using the pressure probe on a single cell, especially in the adverse circumstances of this work (where access to the cell is restricted by the micromanipulation probe and chamber), it is not clear that the pressure probe measurements are necessarily superior. In any case, the generally good correlation validates the micromanipulation method.

Conclusion

It appears that the micromanipulation and pressure probe methods measure similar turgor pressures, and this is further evidence of the validity of the micromanipulation method, at least for cells like *L. esculentum* vf36, which are mostly close to spherical. It is now intended to use a high-speed camera and image analysis to gather large amounts of volume, contact area and non-contact area data during cell relaxation, in the expectation that hydraulic conductivity might also be estimated.

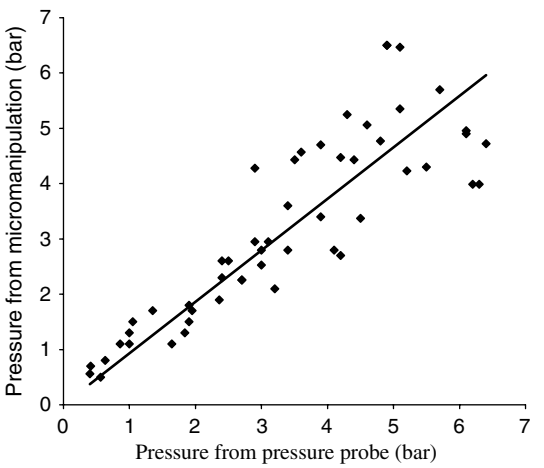

Fig. 4 Correlation of turgor pressure measured by pressure probe and micromanipulation. 52 cells were characterised at various times during relaxation following a rapid compression. The least squares relationship, forced through the origin, was $P_m = 0.93\ P_p$ where P_p is the pressure probe measurement and P_m that of micromanipulation. The correlation coefficient was 0.86

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References

Blewett J, Burrows K, Thomas C (2000) A micromanipulation method to measure the mechanical properties of single tomato suspension cells. Biotechnol Lett 22:1877–1883

Boyer JS (1995) Measuring the water status of plants and soils. Academic Press Inc., San Diego, pp. 103–106. ISBN 0-12-122260-8

Steudle E, Zimmermann U (1971) Hydraulic conductivity of *Valonia utricularis*. Z Naturforsch 26b:1302–1311

Thomas CR, Zhang Z, Cowen C (2000) Micromanipulation measurements on biological materials. Biotechnol Lett 22:531–537

Tomos D (2000) The plant cell pressure probe. Biotechnol Lett 22:437–442

Wang CX, Wang L, Thomas CR (2004) Modelling the mechanical properties of single suspension-cultured tomato cells. Ann Bot 93:443–453