

Derivation of Plant Cell Wall Water Content by Examination of the Water-Holding Capacity of Membrane-Disrupted Tissues

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Received 30 March 1989

ABSTRACT

Plant cell lumina are several orders of magnitude larger than cell wall pores. If the membranes of the plant sample are disrupted and the tissue dried out, a ψ value should be reached at which the cell lumina have drained but the cell walls remain relatively hydrated. The water content of membrane-disrupted tissues at low ψ may, therefore, serve as a good approximation of plant cell wall water content (CW).

The relationship between ψ and water content of membrane-disrupted tissues was measured for four grass species over the ψ range of 0 to -40 MPa. It was found that most water loss occurred above a ψ of -10 MPa. CW was estimated from the water content of membrane-disrupted tissues at lower ψ and it was found that there was very little intra-or intraspecific variability in CW estimates when water content was expressed as a percentage dry weight of the tissue. This is in contrast to the high variability in CW estimated from the analysis of inverse water potential-water content (IP-WC) curves measured with the thermocouple psychrometer. Applications of the membrane-disruption method of estimating CW of plant tissue are discussed.

Key words: Cell wall water, thermocouple psychrometer, membrane-disruption.

INTRODUCTION

The most common method for estimating the weight of water in the plant apoplast (AW) is by extrapolation of an inverse water potential-water content (IP-WC) curve of plant tissue to $1/\psi=0$ (Tyree and Jarvis, 1982). If the IP-WC relationship is measured with a pressure chamber, the AW estimate will include cell wall water (CW) and any water held in xylem cell lumina (Tyree and Jarvis, 1982). If the IP-WC relationship is measured with a thermocouple psychrometer, however, air will displace water in the xylem cell lumina, and extrapolation to $1/\psi=0$ will yield an estimate of CW only (Tyree, 1976). Unfortunately, AW and CW estimates extrapolated from IP-WC curves are highly variable and sometimes negative (Neufeld and Teskey, 1986; Wilson, Fisher, Schulze, Dolby, and Ludlow, 1979). Tyree and Richter (1982) hypothesized that this variability is caused by errors in IP-WC curve measurements that are magnified by the long extrapolation required to derive AW and CW estimates. It follows from this hypothesis that an estimate of AW or CW obtained from a more accurate technique could be used to improve the accuracy of other water relations parameters estimated from the IP-WC curve (Wenkert, 1980). In

particular, an estimate of the full-turgor osmotic potential of the symplast (ψ_0) could be derived from an AW (CW) estimate and only one additional IP-WC data point from non-turgid tissue (Wenkert, 1980).

Plant cell lumina are several orders of magnitude larger than cell wall pores (Carpita, Sabulase, Montezinos, and Delmer, 1979) which makes possible an alternative method for CW estimation. Small pores hold water against greater tension than large pores and, therefore, if the cell membranes of a plant sample are disrupted and the tissue dehydrated, a ψ should be reached at which the cell lumina have almost completely drained but the cell wall pores remain close to full hydration. Drying past this point should yield very little additional water until the ψ associated with cell wall pore drainage is obtained.

Several workers have measured the water holding capacity of membrane-disrupted plant tissue but did not distinguish between cell wall water and water held in the cell lumina (Gaff and Carr, 1961; Wiebe, 1966; Boyer, 1967; Teoh, Aylmore, and Quirk, 1967; Noy-Meir and Ginzburg, 1969). Only Teoh *et al.* (1967) and Noy-Meir and Ginzburg (1969) have measured the water holding

capacity of membrane-disrupted plant tissue to ψ values below -2.6 MPa, and in both of these studies the plant material was shown to lose most of its water between 0 and -10 MPa and relatively little below -10 MPa. It is hypothesized here that the water content of membrane-disrupted plant tissue below a ψ of -10 MPa is a good approximation of CW.

In this study, the water content of membrane-disrupted plant tissue of four grass species was measured over the ψ range of 0 to -40 MPa. CW estimates derived from the water content of membrane-disrupted tissues at ψ values below -10 MPa were compared to those derived from analysis of IP-WC curves measured with the thermocouple psychrometer. The objectives of this study were to compare the two methods for estimating CW and to evaluate the utility of estimating ψ_0 by extrapolation from single IP-WC data points and a CW estimate derived from membrane-disrupted plant tissue. Other applications of the membrane-disruption method for estimating CW are discussed.

MATERIALS AND METHODS

Plant material

Eragrostis lehmanniana (Erle), *E. intermedia* (Erin), *Digitaria californica* (Dica), and *Cenchrus ciliaris* (Ceci) seedlings were grown in 0.8 dm³ plastic tubes containing a Commodo sandy loam soil. After eight months the seedlings were transplanted into 3.8 dm³ pots containing the same soil. Seedlings were kept in the greenhouse throughout and were sampled when 10 months old.

Water-holding capacity of membrane-disrupted tissue

The water-holding capacity of membrane-disrupted tissue was measured in both a dehydration and hydration phase to determine the magnitude of hysteresis effects. Sixteen to 32 leaves were cut at the ligule from each of 16 plants of each species. The leaves were shortened to 6.0 cm in length, dried at 65°C for 24 h to disrupt cell membranes, and separated into two lots.

Water content during tissue dehydration

Leaf samples from one lot were broken up by hand into segments less than 3.0 mm long, mixed with 50 cm³ of distilled water, shaken, and stored in the refrigerator for 24 h. Individual samples were poured on to filter paper under suction in a Büchner funnel and washed with an additional 500 cm³ of distilled water. Excess water was suctioned off and the wet cellular residue stored in a sealed vial in the refrigerator overnight. A 0.2 g sample was placed in a tared sample cup and ψ measured with a thermocouple psychrometer (Decagon Devices SC-10a, Pullman WA)¹ that had been pre-calibrated with standard salt solutions (Lang, 1967; Greenspan, 1977). Sample weight was recorded, air blown over the cup to dehydrate the sample by 3% to 10% of the initial weight, and another psychrometric and weight measurement taken. Vapour equilibration of membrane-disrupted tissue samples required less than 1 h. This procedure was continued until ψ of the sample dropped to below -40 MPa. The sample was then dried for 24 h at 65°C

and weighed. Water content was calculated as percentage dry weight (%D_w) according to the formula:

$$\%D_w = 100(W - D_w)/D_w \quad (1)$$

where W is sample weight and D_w sample dry weight. The data for 16 plants of each species were aggregated for interspecific comparison of tissue water-holding capacity. A plot of water content (%D_w) against ψ revealed that for all species most tissue water loss occurred at ψ values above -10 MPa, but the relationship remained curvilinear below -10 MPa. The data, however, appeared linear when transformed to a log-log plot of water content against $-\psi$. Linear regression lines for CW estimation were, therefore, calculated from the log-transformed data for points below a ψ of -10 MPa. Since the water content of membrane-disrupted tissue was not constant below -10 MPa, five separate estimates of CW were made corresponding to the water content of membrane-disrupted tissues at -10 (CW₁₀), -15 (CW₁₅), -20 (CW₂₀), -30 (CW₃₀), and -40 (CW₄₀) MPa ψ .

Water content during tissue hydration

The water-holding capacity of membrane-disrupted tissue in the second lot was determined in exactly the same way as the first lot with the following exceptions. After the initial rehydration and suctioning of membrane-disrupted tissue, 0.2 g samples were dried at 65°C for 24 h, brought to a water content of 10% (D_w) with distilled water, and kept in sealed vials in the refrigerator overnight. The samples were placed in a tared sample cup, ψ measured, and weight recorded. Instead of drying the samples, however, water was then added with a hypodermic syringe to raise the water content of the samples by 3% to 10% of the initial weight. ψ and weight were re-measured and the process repeated until ψ of the samples rose above -1.0 MPa. Approximately 1.5 h were allowed for equilibration between water addition and re-measurement of ψ . CW estimates were also obtained from these data by examination of regression lines calculated for log-log plots of water content against $-\psi$. The regression lines for dehydration and hydration treatments were tested for equality of slope and intercept ($P \leq 0.05$). Comparisons were made both between species and between hydration and dehydration treatments within a species.

Effect of membrane-disruption technique on tissue water-holding capacity

Tissue membranes were disrupted by four different methods to determine if disruption technique affects tissue water holding capacity and derived CW estimates. Twenty-five to 30 g of fresh leaf tissue were gathered from sample plants of each species, divided into four treatments and cut into lengths of less than 3.0 mm. Treatment 1 was dried at 105°C , treatment 2 at 65°C and treatment 3 at room temperature. Treatment 4 was frozen in liquid nitrogen and stored in the freezer. Each treatment was divided into five subsamples and water-holding capacity of the tissues determined exactly as previously described with stepwise dehydration and ψ measurement. The data were transformed to log-log plots of water content against $-\psi$ and regression lines calculated for the points below -10 MPa. Regression lines for different treatments within a species were tested for equality of slope and intercept ($P \leq 0.05$).

¹ Mention of a trademark name or proprietary product does not constitute endorsement by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Inverse water potential-water content curves for living tissue

Analysis using all data points: Fourteen plants of each species were watered and enclosed in large plastic bags to hydrate overnight. These plants had been interspersed with those used for the membrane-disruption experiments. Eight leaves from each plant were excised at the ligule, shortened to 6.0 cm length and immediately placed between damp paper towels. It was assumed that these leaves were at full turgor when excised because a separate sample did not gain weight when the cut ends were immersed in water for up to 24 h. It had been previously determined that the leaves of these plants lost turgor at a water content between 80% and 85% of the full turgor weight. Initial leaf weight was measured and the leaves dried at room temperature so that the water contents of leaves from a given plant covered the range between 60% and 80% of the full turgor weight. Leaves were weighed and their ψ measured with thermocouple psychrometers (JRD Merrill, Logan UT)¹ that had been pre-calibrated with standard salt solutions (Lang, 1967). Psychrometer output was read every 30 min for 8 h but vapour equilibrium generally occurred within 3–4 h. After ψ measurement the samples were dried for 24 h at 65 °C and weighed. ψ and weight of each leaf was measured only once and the data from each set of eight leaves were aggregated to determine one IP–WC curve for each plant. $1/\psi$ was plotted against water content (%Dw) and a regression line was calculated for the data points from each plant. CW and ψ_0 were estimated by extrapolation of the IP–WC curve to $1/\psi = 0$ and to the average full turgor water content of the leaves, respectively (Wilson *et al.*, 1979). Only lines with a coefficient of determination greater than 0.6 were used for derivation of water relations parameters.

Analysis using single data points and a CW estimate: An estimate of ψ_0 was also determined for each plant by extrapolating the line connecting a CW estimate, from the membrane-disruption experiment, and single IP–WC data points, to the full turgor water content of each leaf (Wenkert, 1980). Five CW estimates, corresponding to the membrane-disrupted tissue water contents for each species at –10, –15, –20, –30, and –40 MPa, were tested. Mean values of ψ_0 derived by extrapolation from standard IP–WC curves using all data points (Wilson *et al.*, 1979) were compared to those derived by extrapolation from CW estimates and single IP–WC data points (Wenkert, 1980) using a *t*-test ($P \leq 0.05$). A comparison was also made between ψ_0 estimates derived by the latter method using the different values of CW.

RESULTS

The water-holding capacity of membrane-disrupted tissue followed the same pattern for all species. Upon dehydration, most water was lost above a ψ of –10 MPa and relatively little below –10 MPa (Fig. 1). Tissue water content was not constant, however, below this ψ but continued to decline over the entire range measured (Fig. 1). The water content of membrane-disrupted tissues was also found to be lower during rehydration than during dehydration (Fig. 1). The hysteresis effect was more marked at ψ values greater than –15 MPa where there was also less scatter about the rehydration curve (Fig. 1). Between –15 and –40 MPa, the average change in water content across all species and both hydration treatments was approximately 8.5% (Dw).

E. intermedia tissue held more water than the other species over the ψ range of –10 to –40 MPa, but only by a few per cent (Table 1). Tissue water content differences between any species or treatment at a given ψ did not exceed 5% Dw for ψ values below –15 MPa and within-species differences between hydration treatments were always less than 3% (Dw; $P \leq 0.05$; Table 1).

The method of cell membrane disruption did not greatly affect tissue water holding capacity below a ψ of –15 MPa. The water content range associated with any ψ lower than –15 MPa was less than 4% Dw for any membrane-disruption treatment within a species (Table 2). There was a large difference between treatments, however, in the level to which the tissue rehydrated in distilled water after membrane disruption. For all species, the order of water retention after tissue rehydration and suctioning, from lowest to highest, was as follows: drying at 105 °C, drying at 65 °C, freezing, and air drying (Table 3).

CW estimates derived by extrapolation of IP–WC curves were highly variable (Table 4) compared to variability in the water content of membrane-disrupted tissues

TABLE 1. Regression information for water-holding capacity of membrane-disrupted tissue derived from a log–log plot of water content against negative water potential for the points below –10 MPa

(DH), dehydration treatment; (RH), rehydration treatment; (CW₁₅), CW estimated from the regression curve at $\psi = -15$ MPa; (CW₃₀), CW estimated from the regression curve at $\psi = -30$ MPa.

Species	Technique		Slope	Intercept	r^2	CW ₁₅ (%Dw)	CW ₃₀ (%Dw)
Ceci	DH	a [*]	–0.447	1.85	0.93	21.1	15.5
Dica	DH	a	–0.500	1.93	0.83	22.0	15.5
Erin	DH	b	–0.437	1.87	0.91	22.8	16.9
Erle	DH	a	–0.426	1.83	0.86	21.3	15.9
Ceci	RH	c	–0.323	1.65	0.79	18.6	14.9
Dica	RH	d	–0.405	1.77	0.81	19.4	14.7
Erin	RH	e	–0.367	1.73	0.80	19.9	15.4
Erle	RH	cd	–0.370	1.72	0.79	19.1	14.8

* The regression lines for those treatments followed by the same letter could not be distinguished ($P \leq 0.05$).

TABLE 2. Regression information for water-holding capacity of membrane-disrupted tissue derived from a log-log plot of water content against negative water potential for the points below -10 MPa using four membrane-disruption techniques; drying at 105°C , drying at 65°C , air drying at room temperature, and freezing in liquid nitrogen

(CW_{15}) CW estimated from the regression curve at $\psi = -15$ MPa. (CW_{30}) CW estimated from the regression curve at $\psi = -30$ MPa.

Species	Disruption technique		Slope	Intercept	r^2	CW_{15} (%Dw)	CW_{30} (%Dw)
Ceci	105°C	a ^a	-0.386	1.77	0.97	20.5	15.7
Ceci	65°C	a	-0.425	1.83	0.93	21.2	15.8
Ceci	air	b	-0.557	1.97	0.89	20.4	13.9
Ceci	freeze	c	-0.451	1.88	0.94	22.3	16.3
Dica	105°C	d	-0.486	1.88	0.95	20.2	14.4
Dica	65°C	e	-0.429	1.82	0.91	20.7	15.4
Dica	air	d	-0.527	1.94	0.95	20.8	14.4
Dica	freeze	f	-0.470	1.83	0.85	18.7	13.5
Erin	105°C	g	-0.385	1.79	0.95	21.8	16.7
Erin	65°C	g	-0.437	1.87	0.92	22.7	16.7
Erin	air	h	-0.495	1.97	0.98	24.4	17.3
Erin	freeze	i	-0.409	1.88	0.97	25.0	18.8
Erle	105°C	j	-0.427	1.80	0.96	20.1	15.0
Erle	65°C	j	-0.442	1.82	0.97	20.0	14.7
Erle	air	k	-0.442	1.85	0.98	21.4	15.7
Erle	freeze	l	-0.472	1.89	0.99	21.6	15.6

^a The within-species regression lines for those treatments followed by the same letter could not be distinguished ($P \leq 0.05$).

TABLE 3. Means and standard errors (in parentheses) of membrane-disrupted tissue water content (%Dw) after overnight hydration followed by leaching under suction in a Büchner funnel

Membrane-disruption was achieved by either drying at 105°C , drying at 65°C , air drying at room temperature or freezing in liquid nitrogen. Within-species water contents could all be distinguished ($P \leq 0.05$).

Disruption technique	% Dw			
	Ceci	Dica	Erin	Erle
105°C	211.7 (2.7)	223.2 (7.4)	173.1 (2.7)	146.4 (2.2)
65°C	391.8 (9.9)	358.0 (13.1)	231.1 (2.5)	233.1 (2.7)
air	673.0 (41.9)	694.4 (28.9)	304.2 (5.9)	299.1 (7.1)
freeze	618.2 (17.1)	642.7 (17.8)	280.5 (7.4)	268.7 (3.8)

at any single ψ below -15 MPa (Fig. 1, Tables 1, 2). In contrast, estimates of ψ_0 derived from the same curves showed relatively low variability (ψ_0^1 , Table 4). Variability in ψ_0 was further reduced when this parameter was estimated by extrapolating from a line connecting individual IP-WC data points and CW estimates determined from membrane-disrupted tissues (ψ_0^2 , Table 4). ψ_0 estimates derived by the two methods, however, could not be distinguished ($P \leq 0.05$) regardless of the value of CW chosen for the latter method. Only ψ_0 values determined for the latter method using CW_{15} are shown in Table 4.

DISCUSSION

We hypothesized that CW could be estimated from the water content of membrane-disrupted plant tissue at ψ values below -10 MPa. Two phenomena were detected

that modify this hypothesis. There was hysteresis in the water-holding capacity curve, and the water content of membrane-disrupted plant tissue was not constant between -10 and -40 MPa (Fig. 1). Regardless of the ψ chosen, however, there was relatively little inter- or intraspecific variability in CW estimates derived from the water content of membrane-disrupted tissues for any specific ψ below -15 MPa (Fig. 1; Table 1). In contrast, CW estimates derived from IP-WC curve analyses are highly variable (Wilson *et al.*, 1979; Table 4). The data, therefore, support the hypothesis advanced by Tyree and Richter (1982) that high variability in CW and AW estimates extrapolated from IP-WC curves may be an artifact of the measurement technique.

Figure 1 shows that hysteresis is more pronounced above a ψ of -15 MPa. As the bulk of hysteresis effects in a porous matrix are associated with macropores (Hillel, 1980), it is possible that water content changes above -15 MPa are primarily associated with water in the cell lumina and that water content changes below -15 MPa are primarily associated with water in the cell wall. Carpita *et al.* (1979) estimated plant cell wall pores to be approximately 4–5 nm in diameter and pores of this size would not be expected to lose water from air entry until the pressure component of ψ was well below -40 MPa. The most likely reason for cell wall water loss between -15 and -40 MPa may, therefore, be cell wall shrinkage resulting from the large negative pressures. Tyree and Jarvis (1982) have argued that cell wall shrinkage is negligible over the ψ range normally associated with plant

TABLE 4. Water relations parameters means and standard errors (in parentheses) derived from IP-WC curve analysis

(ψ_0^1) osmotic potential at full turgor extrapolated from all IP-WC data points (ψ_0^2), average osmotic potential at full turgor extrapolated from single IP-WC data points and CW_{15} . (W_0), average full turgor water content of sample leaves. (CW), cell wall water content estimate derived by extrapolation from IP-WC curves. (N), number of plants sampled.

	Ceci	Dica	Erin	Erle
ψ_0^1 (MPa)	-1.16 (0.093) a ^a	-1.08 (1.22) abc	-0.99 (0.144) c	-0.79 (0.118) d
ψ_0^2 (MPa)	-1.13 (0.051) ac	-1.05 (0.73) bc	-1.03 (1.129) ce	-0.81 (0.085) d
W_0 (%DW)	311.9 (17.2) f	335.5 (23.0) g	172.6 (13.4) h	198.7 (19.2) i
CW (%DW)	9.5 (26.0) j	4.0 (31.3) j	28.1 (11.4) k	23.8 (13.7) jk
N	8	8	13	10

^a Values for the same parameter followed by the same letter could not be distinguished ($P \leq 0.05$).

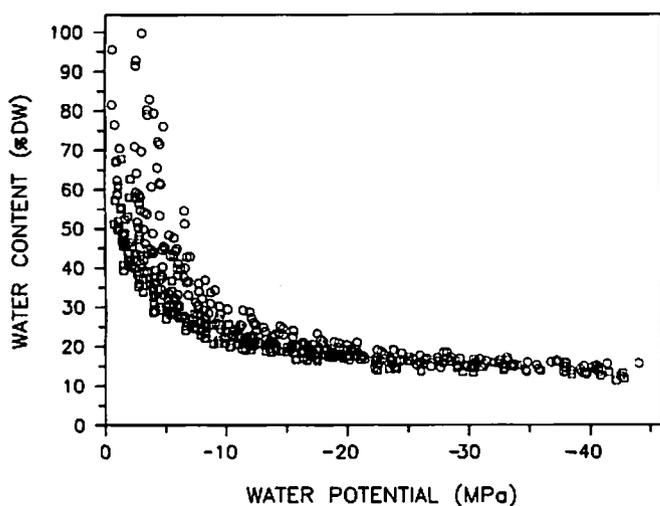


FIG. 1. Combined data for all species showing the relationship between water content and water potential of membrane-disrupted leaf tissue during dehydration (\circ) and hydration (\square) phases. One-half of the data points for each hydration treatment were randomly deleted for figure clarity.

growth because the bulk compressive modulus of cell wall material is very large. Over the 40 MPa range of ψ measured here, however, water loss from cell wall shrinkage may be significant. If the bulk of water content changes are caused by cell wall shrinkage, CW estimates from membrane-disrupted tissue will underestimate actual CW in the range of ψ values experienced by living plants. The interstices between tissue fragments and cytoplasmic remnants in the cell lumina may also have contributed to tissue water content changes at low ψ . The tissue fragments were about the same size as a coarse sand, however, and materials of this type are known to hold very little interstitial water below a ψ of -1.5 MPa (Hillel, 1980). It should be noted that total ψ was measured here, not the pressure component of ψ , and that it is the latter which affects cell wall and lumina pore drainage. Cytoplasmic remnants too large to pass through the cell wall may contribute an osmotic component to the ψ of the system.

A more vigorous technique for extracting cytoplasmic remnants (Gaff and Carr, 1961) was not used because we did not want to change cell wall water-holding capacity by altering the ratio of cell wall constituents (Tyree, 1976). Water held by cytoplasmic remnants that were not leached from the tissues would cause over-estimation of CW, but the magnitude of this effect is unknown.

In order to use CW estimates from membrane-disrupted tissues one must assume that the membrane-disruption treatment did not affect cell wall water holding capacity. Teoh *et al.* (1967) found that heating to 105°C lowered the sorptive capacity of plant material. In this study, tissue drying significantly lowered the level to which membrane-disrupted tissues rehydrated in distilled water (Table 3) and the magnitude of this effect was shown to be larger as drying temperature increased (Table 3). Oven drying, however, did not consistently raise or lower tissue water-holding capacity at low ψ (Table 2). This suggests that oven drying lowered the water-holding capacity of the cell lumina but did not affect cell wall pores. It is hypothesized that oven drying transformed cytoplasmic remnants into hydrophobic areas on the cell wall surface, inhibiting rehydration of the cell lumina after membrane disruption.

For a given species, ψ_0 values derived from CW estimates and individual IP-WC data points could not be distinguished from each other or from ψ_0 values derived by the standard IP-WC analysis (Table 4) for any CW chosen between CW_{10} and CW_{40} . This supports the hypothesis of Wenkert (1980) that ψ_0 estimates derived from a single IP-WC data point and CW estimate should be relatively insensitive to the value chosen for CW. Since variability in ψ_0 estimates was also reduced with the abbreviated IP-WC method, it may be the procedure of choice if ψ_0 is the only water relations parameter of interest.

CW estimates from membrane-disrupted tissues might also benefit two other areas of plant water relations research. The ratio of CW to total plant water is controlled by cell size and cell wall thickness and may,

therefore, be a useful index of drought tolerance (Cutler, Rains, and Loomis, 1977). CW estimates from membrane-disrupted tissues may also provide a useful approximation of the apoplasmic dilution error in frozen plant samples measured with the thermocouple psychrometer (Markhart, Sionit, and Seidow, 1981; Tyree, 1976).

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