(Aus dem Botanischen Institut der Universität für Bodenkultur)

A simplified pressure-volume method for the estimation of osmotic adjustment with the pressure chamber

By S. B. Kikuta and H. Richter

(With 2 figures)

Summary

A method for the estimation with a pressure chamber of osmotic potential (Ψ_0) at known water saturation deficits in leaves is described and tested. The new approach is based on the concept of pressure-volume (PV) curve analysis and requires only one measurement of total water potential to be made at a water saturation deficit (WSD) value higher than the turgor loss point. The result may then be extrapolated to any other WSD. Advance knowledge of an average value for the x-intercept of the PV curve and of the maximum WSD of the turgor loss point in the material to be analyzed is required for this extrapolation. The method was tested on 170 PV curves from two varieties of durum wheat. Average Ψ_0 values derived from a single point and the average x-intercept were in good agreement with Ψ_0 from full curves. Osmotic adjustment in stressed leaves is easily recognized. A further test on 45 PV curves from four other durum varieties, again using the average x-intercept derived from the first set of curves, confirms the merit of the new method. It may be recommended for testing large numbers of leaves in parallel. A protocol is suggested which will permit the estimation of osmotic adjustment in previously unstudied plant material, which is for instance necessary in breeding programs.

Key-words: osmotic potential, pressure-volume (PV) curves, pressure chamber, osmotic adjustment, *Triticum durum*.

Eine vereinfachte Druck-Volumen-Methode zur Bestimmung der osmotischen Anpassung mit der Druckkammer

Zusammenfassung

Es wird eine Methode beschrieben und getestet, die es erlaubt, mit der Druckkammer osmotische Potentiale (Ψ_0) in Blättern bei einem bekannten Wassersättigungsdefizit zu bestimmen. Die neue Vorgangsweise gründet sich auf das Konzept der Analyse von Druck-Volumen-Kurven (PV-Kurven) und erfordert nur eine einzige Messung des Gesamtwasserpotentials bei einem Wassersättigungsdefizit (WSD), das höher als der Turgorverlustpunkt liegt. Das Ergebnis kann dann bis zu einem beliebigen anderen WSD extrapoliert werden. Ein durchschnittlicher Wert für den Schnittpunkt der PV-Kurven mit der x-Achse sowie der Maximalwert des Turgorverlustpunktes im Untersuchungsmaterial müssen für diese Extrapolation bekannt sein. Die Methode wurde an 170 PV-Kurven von zwei Varietäten des Durumweizens getestet. Die durchschnittlichen Ψ_0-Werte, die von einem Einzelpunkt und dem Durchschnittswert des Schnittpunktes mit der x-Achse abgeleitet wurden, stimmten gut mit dem Ψ_0-Wert der kompletten Kurven überein. Osmotische Anpassung in gestreßten Blättern ist leicht zu erkennen. Ein weiterer Test an 45 PV-Kurven von vier anderen Durum-Varietäten, wobei wieder der Durchschnittswert für den x-Abschnitt aus dem ersten Kurvensatz verwendet wurde, bestätigt die Vorteile der neuen Methode. Sie kann für die parallele Untersuchung einer großen Zahl von Blättern empfohlen werden. Es wird eine praktische Vorgangsweise beschrieben, die es erlaubt, die osmotische Anpassung in vorher nicht untersuchtem Pflanzenmaterial zu bestimmen, was etwa für Züchtungsprogramme wichtig sein kann.

Schlüsselworte: osmotisches Potential, Druck-Volumen-(PV) Kurven, Druckkammer, osmotische Anpassung, *Triticum durum*.

1. Introduction

Osmotic potential (Ψ_0) was the first parameter of plant water relations intensively investigated (de Vries 1877, Dixon and Atkins 1910, Höfler 1917, Walter 1931). In the recent past, attention of crop physiologists has focused on the phenomenon of osmotic adjustment, the decrease in osmotic potential under the impact of drought stress. A number of crop species show differences in osmotic adjustment between cultivars, and these differences are in many cases well correlated with yield differences under water stress (Blum et al. 1981, Blum and Pnuel 1990, Morgan 1983, Morgan et al. 1991, Andersen and Aremu 1991, Ludlow et al. 1990, Wullschleger and Oosterhuis 1991). Use of this trait as a selection criterion has however been limited by the lack of a rapid and reliable method for evaluating a large number of lines in parallel.

Methods for the determination of osmotic potential may be divided into three groups: measurements on single living cells, measurements on killed tissues or the press saps prepared from them, and pressure-volume curve (PV) analysis. All these methods are time-consuming; moreover, most of them provide data which are either not representative for the bulk of leaf cells or of limited accuracy. Single-cell methods (de Vries 1877, Höfler 1917) are best suited for geometrically regular cells to be found only in specialized tissues such as the epidermis. Measurements on freeze-killed samples (Ehlig 1962) average over all the tissues and cell types present; however, the symplastic solution becomes diluted by apoplastic water when the selective permeability of the cell membranes breaks down on freezing, and the absolute values for Ψ_0 are thus rather unreliable (Tyree 1976). Additional errors are introduced where press saps are prepared (Kikuta and Richter, in press). Finally, PV analysis gives average potentials for whole organs and is the accepted standard of accuracy, but there is a major drawback: while the single steps in the construction of a pressure-volume curve, the paired weighings and determinations of total water potential (Ψ_t) in (typically) a pressure chamber (Scholander et al. 1965, Tyree and Hammel 1972), are less time-consuming than any of the other measurements, it takes a sequence of ten or more of them for the establishment of a complete PV curve. Even when using two or more pressure chambers, one person cannot easily process more than about ten leaves in one day, and this is sometimes not enough, especially where a statistical evaluation of comparatively small differences in Ψ_0 is called for, as might be the case when screening for osmotic adjustment. In addition, leaves of crop species with soft petioles require extreme care to avoid injury during repeated measurements.

This paper describes an effort to utilize the framework of the PV technique for determining osmotic potentials of leaves from a single Ψ_t measurement. Values for Ψ_0 derived from complete PV curves of wheat were statistically compared with estimates from one point on the curve. The results show that the single-point method which was already discussed but not evaluated by WENKERT (1980) may serve as a viable alternative for the measurement of osmotic potentials at known water saturation deficits. A suitable protocol for the estimation of osmotic adjustment is outlined.

2. Materials and Methods

2.1 Plant material

In experiments 1, 2 and 3, *Triticum durum* L. cvs. Grandur and ST 73/333 were grown in pots filled with 14 kg of black soil in a partly climate controlled glasshouse under natural light. For experiments 1 (rapid dehydration of detached leaves for about 4 hours) and 2 (7 day stress of potted plants), plants were cultivated from March to June in 1981 and 1982. For details of cultivation and stress application see KIKUTA and RICHTER (1986, 1988).

Plants used in experiment 3 were grown from March to July in 1980. Between planting and full development of leaf 4 the pots were kept well watered. 17 days after plant emergence two different irrigation regimes were imposed: half of the pots were kept at about 80 % of soil water capacity, whereas the other half were dried to approximately 33 % of soil water capacity. Pots were weighed daily and the amount of water necessary to maintain the different irrigation regimes added. Between day 71 of the stress period, when the plants were at full flowering, and day 101, when the stage of milk ripeness had been reached, flag leaves of both varieties and both treatments were severed at regular intervals for the construction of PV curves.

In experiment 4, plants of both varieties were grown in the field at the Research Station of the Institute of Plant Production, University of Agriculture, Vienna, in Großenzersdorf, Lower Austria, from March to July in 1981. During May and June, at the stages of anthesis and milk ripeness, flag leaves of the main shoots were measured by the PV curve technique.

The single-point method was further tested on 45 complete PV curves from a breeding program of the Institute of Plant Production, University of Agriculture, Vienna. Four varieties of different genetic origin, code-named A to D, had been stressed for periods of variable length. All control and stress treatments of the varieties were combined for the evaluation.

2.2 Measurement procedures

173 standard (WSD v. Ψ_t^{-1}) pressure-volume curves from durum wheat flag leaves (*Triticum durum* L. cvs. Grandur and ST 73/333) had previously been evaluated for osmotic and elastic adjustment in pot cultures (KIKUTA and RICHTER 1986), for rapid osmotic adjustment in detached leaves (RICHTER and WAGNER 1983, KIKUTA and RICHTER 1988), and for changes during development under field conditions (KIKUTA and RICHTER, unpublished). All the measurements followed "bench drying" procedures described in our previous publications.

2.3 Theoretical considerations

The PV curves served as a data pool for statistical comparisons. Values for Ψ_0 derived from complete PV curves of living leaves were compared with those of a single-point method based on the following reasoning.

The data sets indicate that our wheat leaves lose turgor (Ψ_P) between WSD = 5 % and WSD = 23 %, depending on variety and treatment. Any Ψ_t value obtained from a leaf with a WSD of more than 23 % must therefore represent a value for Ψ_0 as well. This results from the basic equation

$$(-)\Psi_t = (-)\Psi_0 + (+)\Psi_P \tag{1}$$

Such isolated Ψ_0 values cannot be directly compared, however, unless they are standardized to a uniform water saturation deficit. Using data points scattered over a range of WSD values, we may extrapolate each one to an arbitrarily set, uniform water saturation deficit. This extrapolation (fig. 1) will give a Ψ_0 value identical with the one derived from the complete PV curve only when two conditions are met:

a) The data point used must be situated directly on the straight regression line for all the data points after turgor loss, and
b) the line used for a graphical or calculatory extrapolation must start at the intercept of the straight-line portion of the full PV curve with the x-axis.

The straight "osmotic regression line" of a PV curve from a single leaf has a correlation coefficient typically higher than 0.996; therefore, using an arbitrary single data point for the extrapolation rather than the complete regression line will neither lead to systematic errors nor to large data scatter. Condition b), however, would require advance information about the intercept of each individual PV curve with the x-axis, which is clearly impossible to obtain. It is therefore necessary to assume an average value for the intercept, which may be derived from the analysis of a sufficient number of complete PV curves. It remains to be seen how much error is introduced by such a procedure.

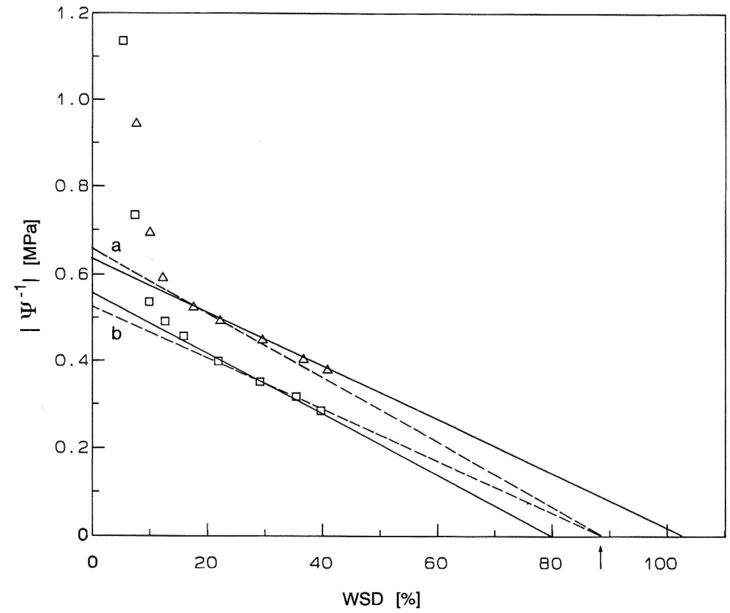

Fig. 1: Extrapolation from an average x-intercept at 88.49 % WSD (↑) results in values for $\Psi_{0\,(sat)}$ a) more positive or b) more negative than those derived from a full curve, depending on the x-intercept of the individual curve. Deviations at 20 % WSD are smaller than at full saturation

Our evaluation has to start with two simple consequences of PV curve geometry (cf. fig. 1).

1. When we extrapolate along an incorrectly estimated regression line, the deviation between the estimated and the true Ψ_0 value for a given WSD will become larger with the distance of extrapolation beyond the single point on the PV curve.
2. There is a lower WSD limit for suitable data points, the value at the turgor loss point, as well as an upper limit, which is set by dehydration injury to the leaf cells resulting in deviations from the straight line (KYRIAKOPOULOS and RICHTER 1981).

From these facts the following recommendations are suggested:

i) In order to minimize errors, we should compare osmotic adjustment at a value in the range of the individual data points or close to it, instead of extrapolating to WSD = 0 %, the value for full saturation. In the case of wheat, we set this value at WSD = 20 %; values for Ψ_0 at full saturation are included to show the effects of such a comparatively long extrapolation.

ii) We should only use data points from a narrow range of WSD values in a region securely greater than the turgor loss point but lower than the severe dehydration leading to injuries; for wheat, we arbitrarily accepted values between 23 and 30 %.

2.4 Statistical analysis

The Lotus 1-2-3 program was used to calculate PV curve parameters. Means and standard errors were estimated with standard statistical functions of the Statgraph program. Since the data were not distributed normally, significances of differences were tested with the non-parametric Mann-Whitney two-sample statistic (U-test) and the Wilcoxon matched-pairs signed rank test. The large data sets made even small differences significant in most cases.

3. Results

3.1 x-intercepts

The average WSD value for the x-intercept, derived from 173 complete PV curves from flag leaves of Grandur and strain 73/333 (controls, stress treatments, field material), was 88.49 %. This value was used as the starting point for the extrapolations of single data points on PV curves. For 17 (= 10 %) of the PV

Table 1

X-intercepts of PV curves from flag leaves of varieties Grandur and ST 73/333. Plants from control/stress treatments and field material. % WSD = water saturation deficit in percent; n = number of PV curves

Treatment, Variety	Experiment	Mean ± SE (% WSD)	Range (% WSD)	n
Total (All treatments, Grandur & ST 73/333)	1, 2, 3, 4	88.49 ± 0.60	69.56 – 119.40	173
All treatments, Grandur	1, 2, 3, 4	87.35 ± 0.82	69.56 – 119.40	92
All treatments, ST 73/333	1, 2, 3, 4	89.79 ± 0.85	73.38 – 108.98	81
Control, Grandur	1, 2, 3	86.88 ± 1.10	69.56 – 105.95	42
Control, ST 73/333	1, 2, 3	88.63 ± 1.22	77.54 – 105.78	35
Stress, Grandur	1, 2, 3	89.46 ± 1.51	75.91 – 119.40	35
Stress, ST 73/333	1, 2, 3	93.58 ± 1.32	76.25 – 108.98	31
Field, Grandur	4	83.71 ± 1.54	71.22 – 92.87	15
Field, ST 73/333	4	84.66 ± 1.43	73.38 – 91.97	15

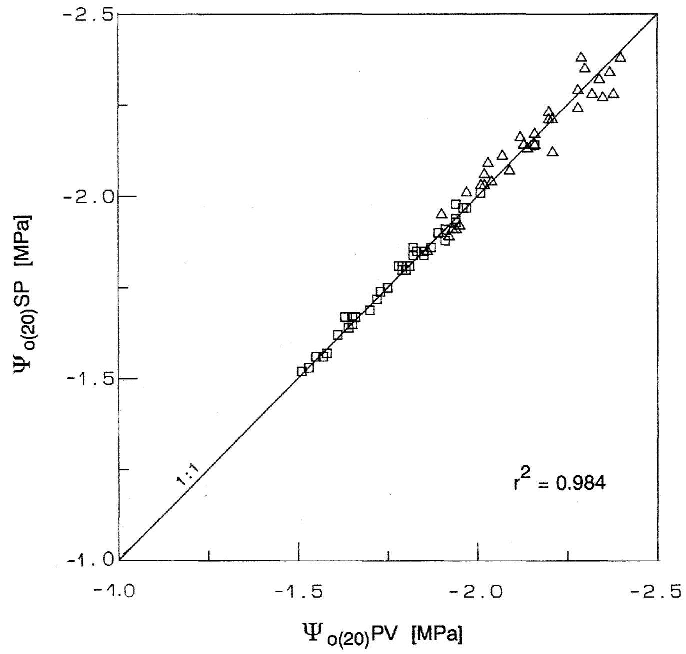

Fig. 2: Comparison of osmotic potentials at 20 % WSD from complete PV curves ($\Psi_{0\,(20)}$ PV) *and from the single-point method* ($\Psi_{0\,(20)}$ SP). □ *control leaves,* △ *stressed leaves of experiment 1 of varieties Grandur and ST 73/333*

curves analyzed, the x-intercept was found above a WSD of 100 %. A significant deviation from the average WSD value was observed in two subsets: the stress variant of ST 73/333 had intercepts at higher WSD values, the field variant of Grandur deviated towards lower values. Details of the analysis for the complete set of curves and for subsets are given in table 1.

3.2 Osmotic potentials

170 of the 173 curves had at least one data point in the preset range between 23 % and 30 % WSD. In cases where more than one point was available in this range, the one closest to the turgor loss point was used for extrapolations.

3.2.1 The single-point osmotic potential at 20 % WSD

Figure 2 shows a plot of PV curve $\Psi_{0\,(20)}$ vs. single-point $\Psi_{0\,(20)}$ indicating that the values are close to the 1:1 line. The correlation coefficients for the linear regression were better than 0.98 for the total and for all subsets.

The average value for the difference D_{20} (= PV curve $\Psi_{0\,(20)}$ minus single point $\Psi_{0\,(20)}$) was +0.009 MPa (table 2). It was positive in 108 cases and negative in 62 cases. The range of D_{20} over all the 170 PV curves did not exceed 0.23 MPa. The field variants of both varieties showed the highest average deviation from the full PV curves. The highest single D_{20}, found for a leaf of ST 73/333 grown in the field, was +0.13 MPa.

The small differences in D_{20} between controls and stress treatments (pooled data of experiments 1, 2 and 3 of both varieties) were significant at the 0.1 % level when tested with the U-test. In control leaves (n = 75) the average difference was +0.009 MPa, while it was +0.001 MPa in stressed leaves (n = 65). The range of the D_{20} values was 0.11 MPa in the controls and 0.19 MPa in the stress variants.

Table 2

Mean values $\pm$ SE of D_{20} (= difference between PV curve Ψ_0 and single-point Ψ_0 at a WSD of 20 %) and D_{sat} (= difference between PV curve Ψ_0 and single-point Ψ_0 at full saturation) for flag leaves of varieties Grandur and ST 73/333. Plants from control/stress treatments and field material; n = number of PV curves

Treatment, Variety	Experi-ment	$D_{20} \pm$ SE (MPa)	Range (MPa)	$D_{sat} \pm$ SE (MPa)	Range (MPa)	n
Total (All treatments, Grandur & ST 73/333)	1, 2, 3, 4	0.009 ± 0.002	0.23	0.009 ± 0.005	0.38	170
All treatments, Grandur	1, 2, 3, 4	0.011 ± 0.003	0.19	0.016 ± 0.006	0.34	90
All treatments, ST 73/333	1, 2, 3, 4	0.007 ± 0.003	0.21	0.001 ± 0.006	0.38	80
Control, Grandur	1, 2, 3	0.010 ± 0.003	0.11	0.018 ± 0.008	0.25	41
Control, ST 73/333	1, 2, 3	0.008 ± 0.003	0.07	0.007 ± 0.007	0.17	34
Stress, Grandur	1, 2, 3	0.005 ± 0.007	0.19	0.000 ± 0.012	0.28	34
Stress, ST 73/333	1, 2, 3	−0.004 ± 0.005	0.12	−0.026 ± 0.010	0.27	31
Field, Grandur	4	0.025 ± 0.008	0.12	0.048 ± 0.015	0.17	15
Field, ST 73/333	4	0.027 ± 0.010	0.14	0.045 ± 0.025	0.24	15

The average value of D_{20} amounted to only 3 % of total osmotic adjustment at 20 % WSD in experiment 2 and was as small as 0.8 % in experiment 1 and 0.7 % in experiment 3 (table 3).

3.2.2 The single-point osmotic potential at full saturation

The average D_{sat} (= PV curve $\Psi_{0\,(sat)}$ minus single-point $\Psi_{0\,(sat)}$) was also +0.009 MPa. The range of deviations was 0.38 MPa. The highest average differences were again observed in the field variants of both varieties; the highest single difference (+0.22 MPa) was found in ST 73/333, field variant, for the same leaf that showed the maximum D_{20}.

D_{sat} values of the controls (pooled data of experiments 1, 2, and 3 of both varieties) were again significantly different from the stress treatments at the 0.1 % level. In control leaves the average difference was +0.013 MPa, in stressed leaves −0.012 MPa. The range of the deviation was more extended than for D_{20}, with 0.26 MPa for controls and 0.29 MPa for stress variants.

Average D_{sat} amounted to 12 % of total osmotic adjustment at full saturation in experiment 2. In experiment 1, D_{sat} was 4 %, and in experiment 3, 1 % of osmotic adjustment (table 3).

Table 3

Comparison of the extent of osmotic adjustment (= difference of osmotic potentials between controls and stress treatments) of flag leaves of varieties Grandur and ST 73/333. Osmotic adjustment is either derived from complete PV curves (PV_{sat} at full saturation, PV_{20} at a WSD of 20 %) or calculated by the single-point method (SP_{sat} at full saturation, SP_{20} at a WSD of 20 %). Values are given as means; n = number of PV curves

Experiment	Osmotic adjustment (MPa)						n
	PV_{20}	SP_{20}	Difference $PV_{20}-SP_{20}$	PV_{sat}	SP_{sat}	Difference $PV_{sat}-SP_{sat}$	
1	0.357	0.354	0.003	0.290	0.271	0.019	60
2	0.297	0.287	0.010	0.250	0.222	0.028	48
3	0.445	0.448	−0.003	0.348	0.347	0.001	20

3.3 Testing the single-point method on leaves of different wheat varieties

Results of the determination of osmotic potentials at 20 % WSD and at full saturation in leaves of the test varieties A to D are presented in table 4. The x-intercept was again assumed to be at 88.49 %, and the single PV data point was taken from the range between 23 and 30 % WSD.

Table 4

PV curve Ψ_0 minus single-point Ψ_0 at a WSD of 20 % (= D_{20}) and PV curve Ψ_0 minus single-point Ψ_0 at full saturation (= D_{sat}) for flag leaves of four Triticum varieties, code-named A to D. Leaves from control and stressed plants, n = number of PV curves

Variety	D_{20} Mean ± SE (MPa)	D_{20} Range (MPa)	D_{sat} Mean ± SE (MPa)	D_{sat} Range (MPa)	n
A	−0.030 ± 0.009	0.129	−0.075 ± 0.013	0.182	15
B	−0.002 ± 0.013	0.144	−0.042 ± 0.022	0.218	10
C	−0.017 ± 0.008	0.087	−0.036 ± 0.012	0.098	10
D	−0.023 ± 0.008	0.082	−0.063 ± 0.010	0.108	10

The average D_{20} over all 45 PV curves tested was −0.019 MPa. Depending on the subset, the average values for D_{20} ranged from −0.002 MPa (variety B) to −0.030 MPa (variety A). The range of D_{20} did not exceed 0.21 MPa, with the minimum at −0.128 MPa and the maximum at +0.075 MPa. The range of D_{20} values was smallest in variety D (0.082 MPa) and highest in variety B (0.144 MPa). A plot of single-point $\Psi_{0\,(20)}$ vs. PV curve $\Psi_{0\,(20)}$ had a high correlation coefficient ($r^2 > 0.98$).

The average D_{sat} over all PV curves was −0.056 MPa. Subsets had average values from −0.036 MPa (variety C) to −0.075 MPa (variety A).

Full PV curves of the test varieties showed amounts of osmotic adjustment at 20 % WSD from 0.28 to 0.66 MPa (table 5). $\Psi_{0\,(20)}$ determined by the single-point method demonstrated the adjustment response clearly, and the varieties did not change their rank in the direction of decreasing response (B, A, D, C). The difference between the values from full PV curves and single-point extrapolations did not exceed 0.035 MPa.

For $\Psi_{0\,(sat)}$ at full saturation the differences between the two methods did not exceed 0.05 MPa, which is acceptable, since the rank order of the varieties remained the same.

Table 5

Extent of osmotic adjustment (= difference of osmotic potentials between control and stressed leaves) of four Triticum varieties, code-named A to D. Osmotic adjustment is either derived from complete PV curves (PV_{sat} at full saturation, PV_{20} at a WSD of 20 %) or calculated by the single-point method (SP_{sat} at full saturation, SP_{20} at a WSD of 20 %). Values are given as means; n = number of PV curves

Variety	Osmotic adjustment (MPa) PV_{20}	SP_{20}	Difference $PV_{20}-SP_{20}$	PV_{sat}	SP_{sat}	Difference $PV_{sat}-SP_{sat}$	n
A	0.580	0.558	0.022	0.483	0.432	0.052	15
B	0.656	0.659	−0.003	0.510	0.510	0.000	10
C	0.281	0.246	0.035	0.215	0.191	0.025	10
D	0.385	0.395	−0.010	0.311	0.306	0.005	10

4. Discussion

The x-intercepts of our PV curves from two wheat varieties occupy a wide range of WSD values (table 1). Intercepts at values higher than 100 % WSD are frequent in all treatments of potted plants, but do not occur in field material. The exact causes for an intercept above 100 %, and indeed for the wide WSD range of the intercepts in genetically homogeneous material treated in the same way, remain obscure. However, these results indicate that the x-intercepts are rather variable and cannot serve as a reliable measure for the proportion of water contained in the apoplast, as often suggested. An earlier analysis of the experimental errors involved in PV curve measurements (Tyree and Richter 1982) led to the same conclusion.

Therefore, we have to regard the x-intercept as a "black box" parameter not at present open for a detailed biological interpretation. For our purposes it is reassuring that the mean values are rather constant in wheat: only for two of the data subsets is there a significant deviation from the grand total determined over all the 173 curves. We decided to use only this mean value of the x-intercept (88.49 % WSD) in the present "test run" for our simplified approach to the determination of osmotic potentials, in order to examine the reliability of results when subsets show a moderate deviation of the intercept from the mean value. Whenever, in other experiments, subsets of intercept values corresponding to different species, varieties, developmental stages or treatments give statistically significant differences, they could and should be used separately. Examples for statistically significant deviations between stressed and control leaves were for instance found in *Carex hirta* (15 % WSD), *Syringa vulgaris* (8 % WSD) and *Sorghum halepense* (7 % WSD); average intercepts for leaves of different species range from about 60 % WSD for *Euonymus europaea* to around 100 % for *Senecio fuchsii* (Kikuta and Richter, unpublished). Wenkert (1980) assumed a less extended range (75 ± 15 %) even for the most extreme curves from single leaves.

Tables 2 and 4 show that it is possible to estimate the osmotic potentials of wheat at 20 % WSD and at full saturation with a high degree of accuracy from just a single point on the PV curve. The agreement with the values determined from a full curve is especially close for $\Psi_{0\,(20)}$, a fact resulting from the geometry of the PV curve. Table 3 demonstrates an outcome of great interest for the practical application of the method: the average differences between osmotic adjustments estimated from single-point vs complete PV measurements are small and do not obscure real osmotic adjustment. The ratio is especially good when we compare adjustment and deviations at 20 % WSD, where the numerical value of osmotic adjustment is large due to sap concentration and the deviations are small. It will thus be possible to use the single-point method in rapid screening of plant material for the occurrence and magnitude of osmotic adjustment, which may be its most important benefit, considering the use of osmotically adjusting strains for the breeding of drought resistant crop plants.

Finally, a test run on mixed leaf material from a number of different varieties demonstrates that even a rather crude approach (use of a single intercept derived from other durum varieties on heterogeneous and differently treated material) will lead to values which are acceptably close to those from full PV curves. Osmotic adjustment is easily recognized, and even the rank of varieties in the numerical amount of adjustment corresponds well.

How, then, could we apply the simplified single-point PV method to the determination of osmotic adjustment in an unknown object? The following steps might be taken:

1. Measurement of complete PV curves on a few leaves from control and stressed material for information on the turgor loss point and the intercept with the x-axis.
2. Calculation of a factor, $f = DW/SW$, from dry weights and saturated weights of these leaves. The averages of this factor are rather close for different treatments, whereas the range is quite extended. In durum wheat, the average was 0.218, the range 0.069 to 0.277. When we used the lowest and highest values of the range for calculating the water saturation deficits of all the 170 PV curves, they differed on average by +1.5 % and −2.1 %, respectively, from the WSD values calculated from the correct dry weights.
3. Resaturation of a number of leaves, determination of the saturated weights and "bench-drying" by free transpiration. Weight losses are followed, and WSD values calculated from saturated weights, intermediate fresh weights and the average of the factor f according to the equation

$$WSD = [(SW - FW) / SW - f \cdot SW)] \cdot 100\ \% \qquad (2)$$

4. Measurement of each leaf in the pressure chamber as soon as step 3 indicates that the WSD has reached a value in the preselected range after the turgor loss point. This range will be comparatively easy to define in a well-investigated species such as wheat, where extreme values for the turgor loss point have been published, but it may require preliminary investigations and the use of an ample safety distance from the turgor loss point in less-known plant material.
5. Determination of the true values for dry weights by the usual procedure of drying to constant weight at 100° C.
6. Insertion of DW instead of ($f \cdot SW$) in equation (2) for recalculation of the WSD at which the pressure chamber measurement was performed. Even in wheat leaves with an extreme ratio of DW/SW, the true WSD will be not more than 2 or 3 % off the value calculated with an average f. Thus, most of the measured leaves should have their WSD in the preselected range; a few may have to be eliminated from the final evaluation.
7. Numerical or graphical determination of osmotic potential at a selected WSD value, by drawing a line in a PV diagram from the average x-intercept through the point determined in the single pressure chamber measurement and reading the potential at the WSD value desired.

Modifications of this procedure will be possible. Other ways than weighing may be found for ascertaining that a drying leaf has passed the WSD of the turgor loss point. For instance, the turgor loss point in many species is near the WSD where stomatal closing reactions begin (Hinckley et al. 1980); porometer measurements could then be used to detect the correct moment for a weighing and a pressure chamber measurement, while both saturated and dry weights of the leaf are determined only later. Leaf rolling could also serve as a suitable indicator of turgor loss in some species (Turner et al. 1986, Bittman and Simpson 1989). Such modifications may prove valuable in cases where a change in osmotic potentials would seem possible in a detached leaf during resaturation.

In summary, we trust that the general approach outlined before will prove flexible enough to become a useful tool for the exploration of changes in osmotic potentials and their role in the drought adaptation of crop plants.

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References

Andersen, M. N. and J. A. Aremu, 1991: Drought sensitivity, root development and osmotic adjustment in field grown peas. Irrigation Sci. *12,* 45—51.

Bittman, S. and G. M. Simpson, 1989: Drought effect on leaf conductance and leaf rolling in forage grasses. Crop Sci. *29,* 338—344.

Blum, A., G. Gozlan and J. Mayer, 1981: The manifestation of dehydration avoidance in wheat. Crop Sci. *21,* 495—499.

Blum, A. and Y. Pnuel, 1990: Physiological attributes associated with drought resistance of wheat cultivars in a Mediterranean environment. Aust. J. Agric. Res. *41,* 799—810.

De Vries, H., 1877: Untersuchungen über die mechanischen Ursachen der Zellstreckung, ausgehend von der Einwirkung von Salzlösungen auf den Turgor wachsender Pflanzenzellen. 120 pp. W. Engelmann, Leipzig.

Dixon, H. H. and W. R. G. Atkins, 1910: On osmotic pressure in plants and on a thermo-electric method of determining freezing points. Sci. Proc. Roy. Dublin Soc. (NS) *12,* 275—311.

Ehlig, C. F., 1962: Measurement of energy status of water in plants with a thermocouple psychrometer. Plant Physiol. *37,* 288—290.

Hinckley, T. M., F. Duhme, A. R. Hinckley and H. Richter, 1980: Water relations of drought hardy shrubs: osmotic potential and stomatal reactivity. Plant, Cell and Environm. *3,* 131—140.

Höfler, K., 1917: Die plasmolytisch-volumetrische Methode und ihre Anwendbarkeit zur Messung des osmotischen Wertes lebender Pflanzenzellen. Ber. deut. bot. Ges. *35,* 706—726.

Kikuta, S. B. and H. Richter, 1986: Graphical evaluation and partitioning of turgor responses to drought in leaves of durum wheat. Planta *168,* 36—42.

Kikuta, S. B. and H. Richter, 1988: Rapid osmotic adjustment in detached wheat leaves. Ann. Bot. *62,* 167—172.

Kikuta, S. B. and H. Richter, in press: Leaf disks or press saps? A comparison of techniques for the determination of osmotic potentials in freeze-thawed leaf material. J. Exp. Bot.

Kyriakopoulos, E. and H. Richter, 1981. Pressure-volume curves and drought injury. Physiol. Plant. *52,* 124—128.

Ludlow, M. M., J. M. Santamaria and S. Fukai, 1990: Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water-limited conditions. II. Water stress after anthesis. Aust. J. Agric. Res. *41,* 67—78.

Morgan, J. M., 1983: Osmoregulation as a selection criterion for drought tolerance in wheat. Aust. J. Agric. Res. *34,* 607—614.

Morgan, J. M., B. Rodriguez-Maribona and E. J. Knight, 1991: Adaptation to water deficit in chickpea breeding lines by osmoregulation. Relationship to grain yields in the field. Field Crops Res. *27,* 61—70.

Richter, H. and S. B. Wagner, 1983: Water stress resistance of photosynthesis: some aspects of osmotic relations. pp. 45—53. *In:* R. Marcelle *et al.*, eds., Effects of Stress on Photosynthesis. M. Nijhoff, Dr. W. Junk Publishers, The Hague, Boston, London.

Scholander, P. F., H. T. Hammel, E. D. Bradstreet and E. A. Hemmingsen, 1965: Sap pressure in vascular plants. Science *148,* 339—346.

Turner, N. C., J. C. O'Toole, R. T. Cruz, O. S. Namuco and S. Ahmad, 1986: Responses of seven diverse rice cultivars to water deficits. I. Stress development, canopy temperature, leaf rolling and growth. Field Crops Res. *13,* 257—271.

Tyree, M. T., 1976: Negative turgor pressure in plant cells: fact or fallacy? Can. J. Bot. *54,* 2738—2746.

Tyree, M. T. and H. T. 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.

Tyree, M. T. and H. Richter, 1982: Alternate methods of analysing water potential isotherms: some cautions and clarifications. II. Curvilinearity in water potential isotherms. Can. J. Bot. *60,* 911—916.

Walter, H., 1931: Die Hydratur der Pflanze und ihre physiologisch-ökologische Bedeutung. 174 pp. G. Fischer, Jena.
Wenkert, W., 1980: Measurement of tissue osmotic pressure. Plant Physiol. *65*, 614—617.
Wullschleger, S. D. and D. M. Oosterhuis, 1991: Osmotic adjustment and the growth response of 7 vegetable crops following water-deficit stress. Hort Science *26*, 1210—1212.

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Anschrift der Verfasser:

Dr. Silvia B. Kikuta und a. o. Prof. Dr. Hanno Richter, Botanisches Institut der Universität für Bodenkultur, Gregor-Mendel-Straße 33, A-1180 Wien