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Relationship between the Osmotic Potential of Cell Sap and the Water Saturation Deficit during the Wilting of Leaf Tissue

Bohdan Slavík

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

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Závislost osmotického potenciálu buněčné šťávy na vodním deficitu během vadnutí listových pletiv

Byly zjišťovány změny osmotického potenciálu (osmotického tlaku) buněčné šťávy (vylisované z listových pletiv usmrcených při 100° C) při pasivní vodní bilanci (vadnutí) částí čepele v závislosti na zvětšujícím se vodním deficitu (na ztrátě vody). Teoreticky by totiž bylo možno předpokládat, že voda vydaná při pasivní vodní bilanci pochází rovnoměrně z veškeré vody buněčné, tedy také poměrně z podílu, obsaženého v buněčné šťávě. V tom případě by se buněčná šťáva koncentrovala úměrně vznikajícímu deficitu. V naprosté většině pozorovaných případů stoupal však osmotický tlak (klesal osmotický potenciál)strměji než teoreticky odpovídá současné ztrátě vody. Ze zjištěných rozdílů mezi zmíněným teoretickým průběhem a mezi nalezenými hodnotami byl vypočítán odhad percentuálního podílu „mobilní" vody v buňce, tj. toho podílu, kterého se vždy bezprostředně týkají změny obsahu vody v buňce. Tento podíl „mobilní" vody byl u dospělých listů kolem 70 až 80 %. Velikost podílu „mobilní" vody závisela na rychlosti vzniku vodního deficitu: Při rychlém vadnutí byl u dospělých listů zjištěn menší podíl než při vadnutí pomalém. To svědčí o tom, že „mobilní" podíl buněčné vody je vymezován podle vodní bilance buňky dynamickou rovnováhu intracelulárních difusních proudů vody podle gradientů difusního tlaku vody mezi jednotlivými podíly buněčné vody, jež jsou určeny různou vazbou („vázaná "voda) i různou lokalisací v buňce.

Summary

Changes in the osmotic potential of the cell sap in passive water balance (wilting) of parts of the leaf blade were determined in relation to increasing water saturation deficit. Theoretically it could be assumed that the water lost in passive water balance comes from the total cell water equally, hence also from an aliquot part from the water contained in the cell sap. In that case the concentration of the cell sap would increase in proportion to the growing water

* *Address:* Na cvičišti 2, Praha—Dejvice

saturation deficit. In the absolute majority of investigated cases, the osmotic pressure increased (the osmotic potential fell) more steeply than corresponded theoretically to the loss of water at the time. From the difference observed between the mentioned theoretical course and the values found, an estimate of the percentage of "mobile" water in the cell, i.e. that part which was always directly involved in changes in the water content in the cell, was calculated. This "mobile" water in adult leaves constituted about 70 to 80 per cent. The proportion of "mobile" water was dependent on the rate of development of the water saturation deficit: When the wilting was rapid the amount observed in adult leaves was smaller than in the case of slow wilting. This proved that the "mobile" proportion of the cell water is determined according to the water balance by a dynamic equilibrium of intracellular diffusion currents of water according to gradients of the potential between the different parts of cell water which are determined by different linkage ("bound" water) as well as different localization in the cell.

Introduction

In an earlier study (SLAVÍK 1955) it was concluded that with gradual wilting of cut off lea ve of sugar beet, the osmotic potential of the cell sap (determined cryoscopically) did not change in parallel with the increasing water saturation deficit, i.e. that its increase did not correspond to the theoretical values calculated from the values of the total water saturation deficit. In his experimental material the author had found a temporary decline in the osmotic potential pressure during wilting similar to that found by LAISNÉ (1939); WALTER and SCHALL (1957) repeated similar tests on the leaves of *Fraxinus excelsior* L. and *Sambuccus nigra* L. They found that the osmotic pressure of the cell sap during artificial wilting increased without any temporary decline. Since however, these authors did not carry out a quantitative comparison of the rise in the osmotic potential and simultaneous water loss, the important question still remained unanswered of whether the changes in osmotic potential (*sensu* SLATYER and TAYLOR 1960) of the cell sap in passive water balance correspond to the quantitative loss of the corresponding percentage of water content, e.g. whether a water saturation deficit of 20 per cent loss of the initial total water content corresponds to an increase of the osmotic pressure (a reduction of the osmotic pressure) by 25 per cent of the initial value. (Calculation: Let us assume the initial osmotic pressure with full water saturation to be equal to 100 per cent, then with water saturation deficit **b** the osmotic pressure should decrease to

$$c = \frac{100 \,.\, 100}{100 - b} = \frac{100}{1 - \frac{b}{100}} \text{ per cent}$$

In order to be able to answer this question a series of experiments with the wilting of cut leaves was carried out complying with the important requirement of homogeneity of the material by using comformable small parts of the leaf blades or corresponding halves of the blades.

Material and Methods

First series of experiments: Increase of the water saturation deficit in cut off parts of leaf blades.

100-day-old *Nicotiana Sanderae* hort. plants, in the stage of 10 to 12 leaves in the leaf rosette, cultivated in flower pots in a green-house were used. Fully mature leaves of medium insertion level were cut off and water saturated overnight with the leaf-stalks in water in the dark in an atmosphere saturated with water vapour (STOCKER 1929). A rectangle about 5 × 9 cm was cut out from the middle of each half of the blade (Heterogeneity of a leaf blade: SLAVÍK 1959 and 1963) and from it in turn (see Fig. 1) two sets of four minute rectangles alternating in chess-board fashion: set *a* was killed immediately by boiling at 100° C in a closed test tube, the second parallel set *b* was quickly weighed with an accuracy of 0·001 g., put loosely on a thin nylon mesh for a period of 5 or 10, 15, 20 or 30 minutes respectively at 20° C ± 1 and a relative air

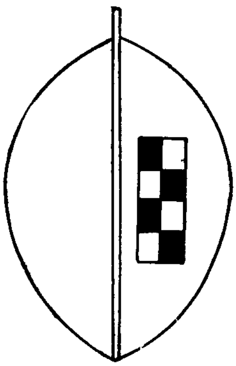

Fig. 1.

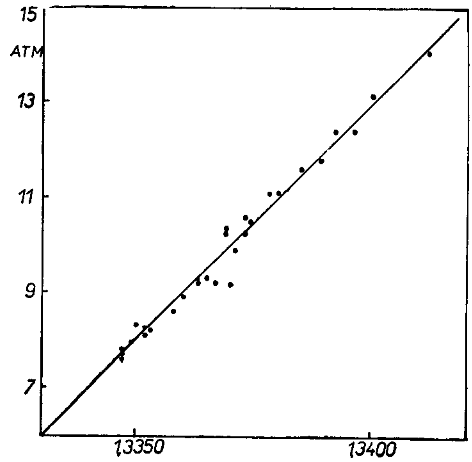

Fig. 2.

Fig. 1. Diagram of parallel pairs of samples from each half of the leaf blade. The small rectangles of the same colour (black or white) together represent one sample.

Fig. 2. The relationship between the refractive index (abscissa) and the osmotic pressure of the cell sap, determined cryoscopically (ordinate: atm.) from the leaf tissues of *Nicotiana Sanderae* hort.; material identical with the test material. Temperature: 20° C. The regression line used in the first test series for the calculation of the osmotic potential from values of the refractive index determined refractometrically.

humidity of 50 ± 3 per cent. After this exposure (wilting) they were again weighed and killed as above. In all samples prepared in this way (a total of 144 pairs) the refractive index was determined in the exuded sap. The water content in fully water saturated leaves was determined in an adequate number of parallel samples (n = 40) by the described method. The average (93·24 ± ± 0·32 per cent) of water in fresh weight was then used for the calculation of the percentage of water loss during wilting, i.e. for the calculation of the water saturation deficit as a percentage of the initial water content. This procedure was necessary since in samples in which the refractive index of the cell sap was determined, it was not possible to determine the water content gravimetrically and hence also the deficit. For the indirect determination of the osmotic pressure of the cell sap by means of the refractive index, which was necessary with reference to the negligible size of the samples, a regression line between the refractive index and the cryoscopically determined osmotic potential was used, which was constructed (Fig. 2) using the determination of the two values in the 40 samples of leaf tissues collected from identical material and treated identically (killed at 100° C and pressed with a hand-press). For the applicability of this method see SLAVÍK 1959.

Second experimental series: Wilting of the cut-off halves of leaf blades.

Sound mature leaves of medium insertion level were selected from 100-day-old leaf rosettes of *Nicotiana Sanderae* hort., artificially water saturated according to Stocker's method (STOCKER 1929) (24 hours in the dark in an atmosphere saturated with water vapour with the stalks submerged in water.) From each leaf one half of the blade (alternately left and right half) was immediately killed in a closed vessel at 100° C and in the pressed out cell sap the osmotic potential determined cryoscopically, the other half was immediately weighed with an accuracy of 0·01 g. and then left to wilt freely on a thin nylon mesh at 20 — 0·5° C with a relative air humidity of 60 ± 3 per cent for 10, 20, 30, 40, 50, 60, 80, 100, 120 or 200 minutes, respectively. After these intervals the second halves of leaf blades were again weighed, killed in the same way and the osmotic potential in the cell sap determined cryoscopically. A total of 60 pairs of samples were treated by this method.

Acknowledgement

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Results and Discussion

In the first experimental series the values of the water saturation deficit and the values of osmotic pressure of the cell sap calculated from the refractive index according to an empirically found regression line (Fig. 2) were determined in 140 pairs of samples (before and after wilting). Fig. 3 gives the different values of the relative increase in osmotic pressure (decrease in osmotic potential) compared with the initial value before wilting in relation to the water deficit reached, and the line constructed using their so-called moving averages of water saturation deficit. In addition, two theoretical curves are plotted in this graph. The first (100) is based on the assumption that the cell sap is concentrated during wilting in proportion to the losses of the total water content in thetissue (curve — 100 per cent), i.e. that the transpiration water lost during wilting comes equally from all parts of water present in the cell. The fractions of cell water with different linkage and different localization in the cell organs are considered.

There is an evident difference between this theoretical curve (100 %) and the empirical line constructed according to the so-called moving averages. From this difference is follows that during wilting of the sections of the leaf blades the osmotic pressure increases more rapidly than would correspond to the simultaneous water loss. If during wilting water were taken equally from all the parts of water in an "average" cell of the test tissue (average values of osmotic potential of the cell sap are obtained from the whole tissue), then the part of cell water which we get after killing the leaf as the so-called cell sap,

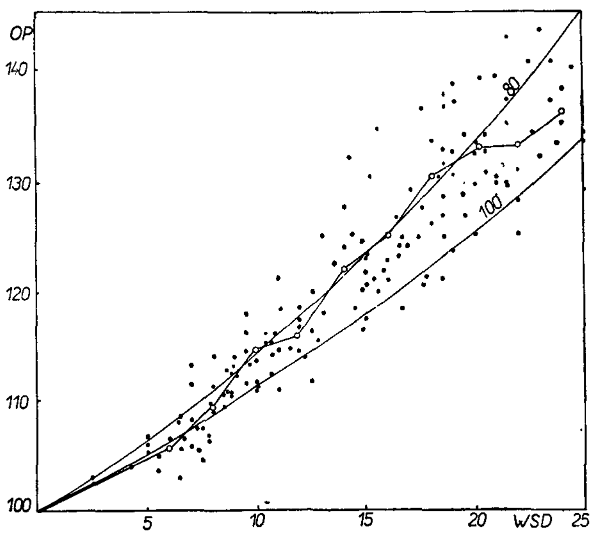

Fig. 3. Results of the first test series. Wilting of the sections of leaf blades of *Nicotiana Sanderae* hort. Relationship between the rising water saturation deficit (W.S.D.) (abscissa: in percentage of the initial water content at saturation) and the osmotic pressure determined cryoscopically (ordinate: in per cent of the initial value). The empirical line was constructed according to the averages in classes of two per cent of W.S.D. The theoretical curves 80 and 100 see text.

would be concentrated proportionally to the whole water loss. This means that the osmotic potential in Fig. 3 would increase according to the theoretical curve 100. When the gradient of the osmotic pressure increase is steeper, it certainly denotes that the fraction of cell water, whose osmotic pressure is determined in the cell sap, contributes to the water loss by transpiration to a greater extent than the other fractions and at the same time that these other fractions loose water from the cells in passive water balance less readily or more slowly. This may be caused not only by their different linkage, but (with relative slow diffusion) also by their differing localization in the cell. From this it also follows that the part of cell water whose osmotic concentration is determined in the cell sap, is the part which is most readily accessible for the loss of water during passive water balance. It can be also designated as the "mobile" part of the cell water (SLAVÍK 1955).

From the difference in the theoretical curve 100 and the actually found decrease in osmotic potencial during the occurrence of the water deficit, it can easily be calculated how large this "mobile" part of cell water is. We can plot other theoretical curves under the assumption that generally **a** per cent of the total water content in the cell is formed by this easily accessible "mobile" part of water in the cell.

(Simple calculation: The osmotic pressure rises from 100 per cent at full water saturation at the beginning of wilting to c per cent with a water saturation deficit of b per cent of the initial water content.

Then $$c = \frac{100 \,.\, 100}{100 - \frac{100b}{a}} = \frac{100}{1 - \frac{b}{a}} \text{ from which } a = \frac{b}{1 - \frac{100}{c}}$$)

In Fig. 3 also the second theoretical curve constructed under the assumption that **a** = 80 is shown. This theoretical curve is also in good agreement with the empirically found relationship.

In the second experimental series, pairs of halves of mature leaf blades, one half fully water saturated and the other wilting, were used for the cryoscopic determination of the osmotic potential of cell sap. The results from 60 pairs of samples are shown in Fig. 4, where also the theoretical curves are plotted for the part of mobile water **a** = 100, **a** = 80 and **a** = 50 per cent, respectively, from

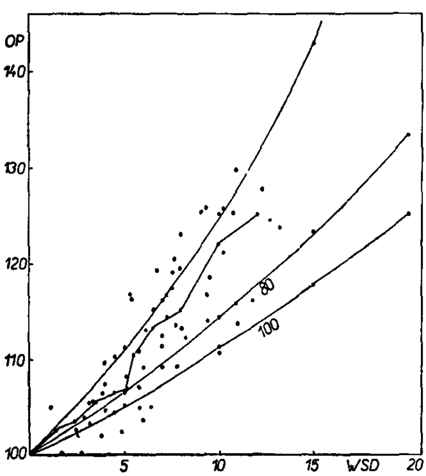

Fig. 4. Results of the second test series. Wilting of cut-off halves of leaf blades of *Nicotiana Sanderae* hort. Relationship between the rising water saturation deficit (W.S.D.) (abscissa) and the osmotic pressure of the cell sap (determined cryoscopically) (ordinate: in per cent of the initial value). The empirical line constructed according to the averages in variation classes of 1 per cent W.S.D. each. The theoretical lines 50 (upper), 80 and 100 see text.

the total cell water. 78 per cent of all values are higher than the theoretical line **a** = 80. The empirical line constructed by using moving averages from the single values corresponds approximately to the theoretical relationship in which the part of the mobile water amounts to about 60 per cent of the total water present in the cell.

From the two experimental series it, therefore, follows that during an increase in wate saturation deficit, osmotic potential (osmotic pressure) of the cell sap does not decrease (increase) proportionally to the total water loss, but more steeply. From this it further follows that that part of the cell water whose osmotic potential is determined in the cell sap pressed out of the killed tissue does not correspond to the total cell water but only to a small part of it, which we called the "mobile" part. In some of our experiments this part represented only 50 per cent of the total water content. From this mobile part comes, therefore, primarily the main part of the transpiration water, the loss of which did not result in the concentration of the total cell water proportionally, but mainly of its mobile part.

Single quantitative results indicate that during a rapid rise in the water saturation deficit (in the case of rapid wilting), the decrease of osmotic potential is steeper than when this rise proceeds slowly. These findings are in agreement with the assumption that the mobile part of the water is always supplemented with a certain time delay from the other parts of cell water. For this reason this water volume can also be of different size and the boundary between this fraction and the other fractions of cell water is given by the equilibrium which is established according to the existence of intracellular gradients of diffusion pressure of water (water potential). The gradient changes according to the water balance. With rapid increase of the water deficit the gradient between the mobile part of cell water and its other parts, probably with differing linkages (bound water) as well as various localization in the cell, is greater than in the case of slow wilting.

The main feature of mobile water in the cell is that it is involved in the main changes in the water balance of the cell. Therefore, it is possible to indicate the changes in the water potential of this mobile part of cell water as changes of osmotic potential of the cell sap.

References

LAISNÉ, G.: Les variations de la pression osmotique du suc de tissue de *Fraxinus excelsior* L. et de quelques autres plantes au cours de la fanaison artificielle. — C. R. Acad. Sci. Paris **208** : 299—301, 1939.

SLAVÍK, B.: K dynamice vodního deficitu rostlin. [The dynamics in water saturation deficit.] — Preslia **27** : 124—153, 1955.

SLAVÍK, B.: Gradients of osmotic pressure of cell sap in the area of one leaf blade. — Biol. Plant. **1** : 39—47, 1959a.

SLAVÍK, B.: The relation of the refractive index of plant cell sap to its osmotic pressure. — Biol. Plant. **1** : 48—53, 1959b.

SLAVÍK, B.: The distribution pattern of transpiration rate, water saturation deficit, stomata number and size, photosynthetic and respiration rate in the area of the tobacco leaf blade. — Biol. Plant. **5** : 000, 1963.

STOCKER, O.: Das Wasserdefizit von Gefässpflanzen in verschiedenen Klimazonen. — Planta **7** : 382—387, 1929.

WALTER, H. und SCHALL, K.: Das Verhalten des osmotischen Wertes beim Welken abgeschnittener Blätter. — Ztschr. f. Bot. **45** : 263—272, 1957.

SLATYER, R. O., TAYLOR, S. A.: Terminology in plant—soil water relations. — Nature **187** : 922—924, 1960.

Б. Славик (Институт экспериментальной ботаники, ЧСАН, Прага)

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Зависимость осмотического давления клеточного сока на водном дефиците в течение завядания тканей листа

Изучались изменения осмотического давления клеточного сока (отжатого из тканей листа убитых при 100 гр. Ц) при пассивной водной билланции (завядании) частей пластинки листа в зависимости от возрастающего водного дефицита (потери воды). В подавляющем числе наблюдаемых случаев возрастало осмотическое давление более круто чем это теоретически отвечает осмотической потере воды. Теоретически возможно предполагать, что вода, выданная при пассивной водной билланции происходит равномерно из всей клеточной воды, следовательно, тоже в соответствующем количестве из той части, которая содержится в клеточном соке, который концентрируется соответственно возникающему дефициту. Из установленных различий между этим теоретическим ходом и между найденными величинами высчитано определение процентной части «мобильной» воды в клетке, т. е. той части, которой всегда непосредственно касаются изменения содержания воды в клетке. Эта часть «мобильной» воды в клетке у зрелых листьев составляет около 70—80%. Величина доли «мобильной» воды зависела от скорости возникновения водного дефицита: при быстром завядании у зрелых листьев установлена меньшая доля по сравнению с медленным завяданием. Это свидетельствует о том, что «мобильная» доля клеточной воды определяется по водной билланции клетки динамическим равновесием интрацеллюлярных диффузных течений воды по градиентам диффузного давления воды среди отдельными долями клеточной воды, которые определяются разной связью и разной локализацией в клетке.