Response of the Succulent Leaves of *Peperomia magnoliaefolia* to Dehydration

**WATER RELATIONS AND SOLUTE MOVEMENT IN CHLORENCHYMA AND HYDRENCHYMA**

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**ABSTRACT**

Relative water content, solute concentrations, and osmolality were determined in the water storage tissue (hydrenchyma) and the assimilatory tissue (chlorenchyma) of the succulent leaves of *Peperomia magnoliaefolia* (Jac) (Piperaceae) during slow desiccation. Relative water loss was significantly greater for the hydrenchyma than for the chlorenchyma. When whole leaves had lost 50% of their initial water content, the concomitant decrease of the relative water content of the hydrenchyma was 75 to 85%, but of the chlorenchyma only 15 to 25%. In spite of this differential water loss, the osmolality in both tissues increased to the same extent, indicating solute flow from the hydrenchyma to the chlorenchyma during desiccation. Solute translocation appeared to be unspecific, probably reflecting symplastic mass flow from one tissue to the other. The observed volume preservation of the chlorenchyma stabilized photosynthesis of *Peperomia magnoliaefolia* (Jac) leaves, which was less inhibited by a given decrease of the relative water content of the whole leaves than in nonsucculent leaves.

**MATERIALS AND METHODS**

**Plant Material.** The plants were propagated by cuttings from a single *Peperomia magnoliaefolia* (Jac) plant and grown in the greenhouse at an average temperature of 25 to 35°C during the 14 h light period (humidity 55–65%) and 20°C during the dark period (humidity about 40%). The bifacial leaves of *P. magnoliaefolia* (Jac) possess at their adaxial side several layers of large, chloroplast-free cells with large central vacuoles. The radial cell walls in this multiple epidermis (9) typically show very regular folding during drought-induced shrinkage (13). Adjacent to the inner cell layer of the hydrenchyma, there is a layer of small, extremely chloroplast-rich chlorenchyma cells, followed by several layers of somewhat larger cells with decreasing chloroplast content down to the lower epidermis. Chlorenchyma and hydrenchyma of *P. magnoliaefolia* (Jac) can be separated with reasonable accuracy by simple cutting with a razor blade.

**Fresh Weight, Dry Weight, and RWC.** Thirty-three to 35 leaf discs of 6.5 mm diameter were obtained from detached leaves with a cork borer prior to wilting the plants and during the desiccation period. After weighing the leaf discs, the hydrenchyma and chlorenchyma tissues were separated. After determining the fresh weight of the tissues, the samples were dried at 90°C until no further weight change occurred. The percent relative water content (% RWC) was defined as

\[
\frac{(FW_s - DW_s) \times 100}{(FW_s - DW)}
\]

with *FW*<sub>s</sub> and *DW*<sub>s</sub> being fresh weight or dry weight after desiccation, *FW*, and *DW*, being initial fresh weight and dry weight of the leaves harvested from well-watered plants before desiccation.

**Osmolality.** Dried tissue samples were extracted by boiling in 1 ml of distilled H<sub>2</sub>O for about 10 min. After removal of debris by centrifugation for 20 min at 15,000g, the osmolality of the tissue sap was determined by freezing-point-depression measurement with a Knauer semimicro osmometer (Knauer K.G., Oberursel, FRG). The osmolalities were also determined in cold aqueous extracts of fresh tissue samples.

**Ion Content.** After suitable dilution of the water extracts, cations (sodium, potassium, and magnesium) were determined by atomic absorption spectroscopy (Beckmann Instruments), and anions (chloride, phosphate, nitrate, malate, and sulfate) by isocratic anion chromatography (Biotronik, Maintal, FRG).

**Contents of Soluble Sugars and Amino Acids.** After suitable dilution of cold aqueous extracts from fresh tissue samples we used the anthrone-reaction after Morris (10) to determine the content of reducing sugars. In the same extracts we determined the amino acid content using the ninhydrin-reagent.

Leaf or stem succulence allows plants adapted to dry habitats to survive much longer during a drought before a critical relative water content is reached. When the water storage tissue (hydrenchyma) and the assimilatory tissue (chlorenchyma) can be anatomically distinguished, the former often suffers more loss of water during drought than the latter (3, 12, 13). Apparently, chlorenchyma cells shrink less than the cells of the hydrenchyma and prevent an excessive reduction of photosynthetic capacity, which depends on RWC<sup>1</sup> under water stress (4). Reasons for differential tissue shrinkage include differences in osmoregulation (or 'volume regulation') and differences in tissue elasticity. For example, both solutes and water move from hydrenchyma to chlorenchyma in cacti during prolonged desiccation (2).

The purpose of the present study was to analyze water relations, solute concentrations, and physiological activities in the hydrenchyma and the chlorenchyma of the succulent leaves of *Peperomia magnoliaefolia* (Jac) during drought. For both tissues, we determined osmolality and concentrations of anions and cations as a function of the relative water content of whole leaf tissue. The response of photosynthetic capacity to dehydration was measured and compared with the response of nonsucculent spinach leaves.

<sup>1</sup>Abbreviation: RWC, relative water content.
Table 1. Summary of Fully Turgent Leaf Characteristics

Data are presented as average of six samples ± sd. Inorganic solutes were measured in aqueous extracts of dried and ground samples. Sugars and amino acids were measured in separate aqueous extracts of a fresh leaf homogenate. Osmolarities were measured both in fresh leaf cell sap and in extracts from dried and ground samples.

<table>
<thead>
<tr>
<th></th>
<th>Hydrenchyma</th>
<th>Chlorenchyma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue thickness (mm)</td>
<td>1.2 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Fresh wt (g dm⁻²)</td>
<td>10.7 ± 4.0</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>Dry wt (g dm⁻²)</td>
<td>0.5 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Solutes (mmol L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>8.0 ± 2.0</td>
<td>8.0 ± 1.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>42.0 ± 10.0</td>
<td>33.0 ± 9.0</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>46.0 ± 15.0</td>
<td>34.0 ± 10.0</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>21.0 ± 5.0</td>
<td>22.0 ± 5.0</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>1.0 ± 0.5</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>15.0 ± 5.0</td>
<td>4.4 ± 2.0</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>10.5 ± 3.0</td>
<td>18.2 ± 6.0</td>
</tr>
<tr>
<td>Malate⁻</td>
<td>4.6 ± 2.0</td>
<td>12.5 ± 3.0</td>
</tr>
<tr>
<td>Sum of ions</td>
<td>148.1</td>
<td>132.7</td>
</tr>
<tr>
<td>Soluble sugars</td>
<td>12.5 ± 2.8</td>
<td>40.1 ± 9.0</td>
</tr>
<tr>
<td>Amino acids</td>
<td>3.4 ± 0.6</td>
<td>7.0 ± 4.8</td>
</tr>
<tr>
<td>Sum of all solutes</td>
<td>164.0</td>
<td>179.8</td>
</tr>
</tbody>
</table>

Measured osmolality (mosmol kg⁻¹)
- in extracts from fresh samples: 170.0 ± 27.0
- in extracts from dried samples: 180.0 ± 21.0

Photosynthetic Capacity. O₂-evolution from leaf discs with 3.5 cm diameter was measured with a Hansatech O₂-electrode (Bachofen, Reutlingen, FRG) under the following conditions: temperature 22°C, irradiance 280 W m⁻². In the cuvette, air space CO₂-concentrations of 5% or higher were generated by adding either carbonic anhydrase (5 IU/ml) or small amounts of 0.1 M H₂SO₄ to 0.3 ml of 1 M KHCO₃ (see "Results"). Chl was determined according to the method of Arnon (1).

RESULTS

Relative Water Content (% RWC) and Osmolality. Wilting experiments were carried out both with intact plants and detached leaves. The latter have the advantage that they are unable to import or export solutes during wilting. Therefore, changes in the concentration of mineral ions in different parts of the leaf must be the result of solute movements within the leaf. In addition, the fresh weight of each individual leaf can be determined prior to the wilting period. With whole plants, the initial leaf fresh weight was obtained on a leaf area basis as the average of a large number of samples.

As expected for succulent leaves, the relative water content (% RWC) decreased slowly when water was withheld. The decrease was much faster in detached leaves than in leaves attached to the plant during wilting; 50% RWC was reached after 10 and 20 d, respectively. With nonsucculent, mesophytic leaves (spinach), 50% RWC was reached after 10 to 12 h under comparable conditions.

In leaves of well-watered P. magnolifolia (Jacq) plants, average leaf thickness was about 2 to 3 mm, depending on leaf age. On a volume basis, about 60% of the total leaf tissue was hydrenchymatic tissue (Table 1; Fig. 1). However, the hydrenchyma had a much lower dry weight per unit leaf area than the chlorenchyma (Table 1). The difference is explained by a lower cell wall mass per leaf volume and a lower protein and starch content (data not shown). During wilting, the fresh weight of the hydrenchyma decreased significantly faster than that of the chlorenchyma (Fig. 1). In both types of tissue there was only a small decrease of dry weight per leaf area during water loss (Fig. 1).

The RWC of both tissues was calculated from the fresh and dry weight and plotted as a function of the RWC of the whole leaf tissue (Fig. 2). It is evident that RWC of the hydrenchyma decreased much faster than that of the chlorenchyma. When detached leaves had lost 50% of their water content, RWC of the hydrenchyma had decreased by about 75%, whereas the chlorenchyma had lost only 25% of its saturating water content. Results were similar with detached and attached leaves. Since RWC can be used as an indicator of relative cell volume (5), the data show that the chlorenchyma cell volume is preserved at the expense of the hydrenchyma cell volume.

The osmolalities of hydrenchyma and chlorenchyma from detached leaves were separately determined and plotted as a function of the RWC of whole leaves during wilting (Fig. 3). In extracts of dried material from leaves of well-watered plants, the osmolality was around 250 mosmol kg⁻¹ in the chlorenchyma, but only about 180 mosmol kg⁻¹ in the hydrenchyma. In extracts from fresh tissue the corresponding values were only 200 and
170 mosmol kg⁻¹. This discrepancy is presumably due to partial starch hydrolysis in dried samples. We therefore assume that values from fresh tissues are closer to reality. Within the relatively thin leaves (2 mm) water potential gradients are presumably negligible. In any case, the osmolarity difference between chlorenchyma and hydrenchyma of well-watered plants indicates a turgor difference of about 0.7 bar (but see "Discussion"). When water loss proceeds and turgor decreases, the osmolarities of both chlorenchyma and hydrenchyma increase and become very similar (Fig. 3); after the whole leaf tissue has reached 40% RWC, the osmolarities of both tissues are identical and both increase with decreasing RWC to the same extent, irrespective of the fact that the shrinkage is much stronger in the hydrenchyma than in the chlorenchyma.

It has to be mentioned that the increase in total leaf sap osmolality as a function of the decrease in RWC agreed with theory in experiments with detached leaves. However, with leaves attached to the plants the increase in leaf sap osmolality was significantly smaller than expected from changes in RWC (not shown). Thus, additional solute movements from leaves to other organs might occur in whole plants.

Ion Content in Chlorenchyma and Hydrenchyma. To find out whether solute movement from the hydrenchyma to the chlorenchyma during dehydration is specific, we analyzed all major osmotically relevant inorganic anions and cations in the same aqueous extracts which were used for the determination of osmolarities. Sugars and amino acids were determined separately in aqueous extracts of freshly harvested plant material.

In leaves from well-watered plants, various solutes were unequally distributed between hydrenchyma and chlorenchyma, e.g. phosphate concentrations were 3-fold higher in the hydrenchyma, but sugar concentrations were 3-fold higher in the chlorenchyma (Table I).

In Figures 4 and 5 the relative distribution of each ion (as percentage of total ion content) between hydrenchyma and chlorenchyma is plotted as a function of the RWC of whole leaf tissue during dehydration. The percentage of all anions and cations increased in the chlorenchyma during wilting, indicating that solute translocation from hydrenchyma to chlorenchyma was unspecific. The increase in total ion content as a function of tissue RWC gives higher values than expected from the theoretical curve for the chlorenchyma, but lower values for the hydrenchyma, thus also indicating solute movement to the chlorenchyma during slow desiccation (Fig. 6). Some stressed plants were rewatered after the leaves had reached 25% RWC. Six d after rewatering the distribution of solutes between the tissues was the same as prior to the wilting period (not shown).

Photosynthetic Capacity of P. magnoliaefolia (Jac) Leaves during Water Stress. We have recently reported that many plant species respond similarly to dehydration, when changes in photosynthetic capacity of the mesophyll (CO₂ and light saturating) are measured as a function of the cell volume (4) or of the RWC (5). Solute and water flow from hydrenchyma to chlorenchyma decrease water loss and shrinkage of the assimilatory tissue during drought. Indeed, photosynthesis of leaves from P. magnoliaefolia (Jac) was found to be less sensitive to dehydration than that of other species (data not shown). Half-maximal inhibition of photosynthetic capacity was not observed until RWC of whole P. magnoliaefolia (Jac) leaves had reached...
evolution rates were often low (about 25 μmol mg Chl⁻¹ h⁻¹), traces were nonlinear, and decreased to zero a few minutes after the light was switched on (not shown). However, when the CO₂-concentration in the cuvette was drastically increased by addition of 0.1 ml of 0.1 N H2SO₄ to 0.3 ml of 1 M KHCO₃ the resulting CO₂-concentration of about 11% was high enough to give reproducible rates of O₂-evolution (75 μmol mg Chl⁻¹ h⁻¹), which were linear for at least 15 min.

When the photosynthetic capacity of *P. magnoliifolia* (Jac) leaves at such extremely high CO₂-concentrations was plotted as a function of the RWC in the chlorenchyma, the sensitivity of the leaves came much closer to that reported for spinach or other xerophytic leaves (4), although it was still slightly less sensitive than expected (not shown; see "Discussion").

**DISCUSSION**

The relative insensitivity of the photosynthetic capacity of *P. magnoliifolia* (Jac) leaves to dehydration is a consequence of the maintenance of the chlorenchyma volume at the expense of the hydrenchyma. This was also found by other authors (12). It is important to understand how preferential water loss from the hydrenchyma can occur.

We have always observed a difference in osmolality of about 30 mosmol kg⁻¹ between extracts from hydrenchyma and chlorenchyma of leaves from well-watered plants. At first sight, this suggests that the turgor pressure in the chlorenchyma is about 1 bar higher than in the hydrenchyma. Such a turgor difference could be responsible for a differential cell shrinkage during the initial phase of dehydration. It is, however, for several reasons difficult to accept the existence of a significant turgor difference between hydrenchyma and chlorenchyma. Usually, epidermal tissues such as the hydrenchyma (9) are symplastically connected with the mesophyll. Water loss from the leaves is so slow that hydraulic resistance cannot lead to significant turgor differences. An analysis of several solutes showed that the difference in the osmolality between both tissues was mainly due to the higher sugar content of the chlorenchyma (cf. Table 1). In leaf tissues, sugars are the predominant solutes in the sieve tube-companion cell complex of the veins, which are known to have a significantly higher turgor pressure than the mesophyll. In *P. magnoliifolia* (Jac) leaves, the veins are embedded in the chlorenchyma layers adjacent to the inner hydrenchyma cells, not in

![Fig. 6](image)

**Fig. 6.** The sum of all measured ion concentrations (in mmol L⁻¹) in hydrenchyma (above; open symbols) and chlorenchyma (below; closed symbols) as a function of the RWC of the tissues. The dashed lines indicate theoretical values as calculated from the decrease in RWC. Each datum point represents the average of three samples.

![Fig. 7](image)

**Fig. 7.** Change in the relative photosynthetic capacity of *P. magnoliifolia* (Jac) leaf discs (open symbols) compared to spinach leaf discs (closed symbols) during desiccation when measured at high CO₂-concentrations (about 11%). Dashed line: O₂ evolution of *P. magnoliifolia* (Jac) leaf discs at about 5% CO₂. Each datum point represents the value from one sample.

values as low as 20 to 10%. In spinach, for comparison, the same inhibition occurred at a RWC of about 40 to 50% (Fig. 7). It is worth mentioning that extremely high CO₂-concentrations were needed to observe the striking insensitivity of *P. magnoliifolia* (Jac) photosynthesis to dehydration. In the leaf O₂ electrode used by us and many other laboratories (Hanselach), the CO₂-concentration in equilibrium with a 1 M KHCO₃ solution (about 5%) is usually considered sufficient to overcome high stomatal resistances and to saturate photosynthesis even under water stress. We found this assumption to be incorrect for *P. magnoliifolia* (Jac) leaves and for leaves of other xerophytic species (data not shown).

At about 5% CO₂, half maximal inhibition of photosynthesis of *P. magnoliifolia* (Jac) was already observed when only 10% water was lost (dashed line, Fig. 7). In addition, at 5% CO₂, O₂...
the hydrenchyma itself. Thus, chlorenchyma extracts are always a mixture of mesophyll and phloem extracts, and this is thought to be the main reason for the observed difference in osmolality. If this conclusion is correct, the osmolalities in assimilatory cells and in hydrenchyma cells are similar, and in fact the sum of all measured anions and cations is nearly identical in both tissues. However, direct turgor measurements are needed before final conclusions can be drawn.

According to these considerations we presently favor the hypothesis that the chlorenchyma volume maintenance at the expense of the hydrenchyma volume is mainly due to solute and water flow from hydrenchyma to chlorenchyma. This flow is thought to be unidirectional and presumably symplastic. In a simplistic view, the two tissues can be considered as two compartments connected by tubings (Fig. 8). Compartment (H) contains no chloroplasts and has cell walls which can strongly fold without damage (13). The other, chloroplast containing compartment (C), has rigid cell walls. Upon dehydration, only compartment (H) will shrink. Due to the symplastic connections, osmolality, water potentials, and hydrostatic pressure should be the same in both compartments at any stage of dehydration. Thus, the differential volume change of both tissues at a similar pressure change has to be due to a different cell wall elasticity.

In an attempt to explain water stress effects on photosynthesis we have recently pointed out that direct inhibition of photosynthesis by dehydration at the level of the mesophyll cells includes two phases (6). Effects of fast and moderate dehydration are often quickly reversible, and these effects are thought to be mediated by enzyme inhibition due to increased intracellular solute concentrations (7). After more severe dehydration, effects become more or less irreversible, depending on plant species (4). This part of inhibition is thought to be caused by mechanical (or chemical) membrane damage. Volume preservation may prevent mechanical membrane damage. It will, however, not prevent an increase of the internal solute concentration as has been pointed out above. Thus, in order to avoid enzyme inhibition, incompatible solutes coming from the hydrenchyma have to be sequestered in the chlorenchyma vacuoles as has been shown for NaCl in plants under salinity (8, 11). Quite in contrast to faster dehydration during wilting of detached mesophytic leaves or even more during osmotic dehydration, the extremely slow wilting of succulent leaves might facilitate sequestration of incompatible solutes and osmotic compensation in chloroplast and cytosol. To fully understand the volume preservation system of succulent leaves, a solute distribution analysis for chloroplasts and extrachloroplasm space under dehydration is needed.

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LITERATURE CITED