ABSTRACT

Sucrose uptake was studied in isolated, immature pea cotyledons (Pisum sativum L. cv Marzia) in relation to their developmental stage. During the developmental period examined the water content of the cotyledons decreased from \( \approx 80\% \) "stage 1" to \( \approx 55\% \) "stage 2". When assayed in an isotonic medium (400 osmoles per cubic meter) the influx capacity per gram fresh weight for sucrose was almost constant during this developmental period. The influx could be analyzed into a saturable component \( (k_c = 9 \text{ moles per cubic meter}; V_{\text{max}} = 150 \text{ nanomoles per minute per gram fresh weight}) \) and an unsaturable component \( (k_e = 0.5 \text{ nanomoles per minute per gram fresh weight per mole per cubic meter}) \). Incubation in a hypotonic medium reduced the sucrose influx in stage 1 cotyledons, up to 80\% reduction at 0 milliosmole (medium without mannitol), but had no effect on sucrose uptake by stage 2 cotyledons. Reduced uptake in a hypotonic medium (100 osmoles per cubic meter) could be attributed to a lowering of the \( V_{\text{max}} \) from 150 to 36 nanomoles per minute per gram fresh weight. During incubation of stage 1 cotyledons and stage 2 cotyledons in a hypotonic medium (200 osmoles per cubic meter) their volume increased by 16\% and 5.6\%, respectively, while the calculated turgor pressure increased from 0.2 to 0.6 megapascal for cotyledons of both developmental stages. Reduced sucrose influx in hypotonic medium, therefore, seems to be related to cell swelling (membrane stretching) rather than to increased turgor pressure.

During the development of the pea seed large amounts of starch and protein are accumulated in the cotyledons. The assimilates, necessary for the synthesis of these storage compounds, are imported through phloem strands in the funiculus and seed coat. Because there are no protoplasmic connections between seed coat and embryo (6, 17, 23), after unloading from the phloem, assimilates have to leave the seed coat symplast before reaching the growing embryo and have to pass the plasma membrane of the cotyledonary cells.

The presence of unsaturable and saturable uptake systems for both amino acids (1, 10) and sucrose (12, 13, 22) have been demonstrated in isolated cotyledons from developing legume seeds. Saturable uptake of both sucrose and amino acids seems to be proton symport (1, 10, 13, 22). Moreover, a 62 kD protein has been identified, whose properties indicate that it is involved in the saturable sucrose uptake by developing soy bean embryos (20).

During seed development the kinetics of amino acid uptake by pea cotyledons changes. At early developmental stages amino acid uptake is unsaturable but at later stages this transport pathway is supplemented by a saturable system (10).

The aim of this study was to obtain information about sucrose uptake by maturing pea cotyledons during the period of development in which most of the starch is accumulated. Using freshly isolated cotyledons, the capacity of sucrose influx at various developmental stages was probed with \( [U-^{14}\text{C}]\)sucrose. A kinetic analysis of sucrose uptake was made at the start and toward the end of starch accumulation.

It has been suggested that the osmolarity of the solution in the seed apoplast could regulate the rate of phloem unloading, assimilate metabolism and assimilate compartmentation in developing legume seeds (18, 23). Also, effects of medium osmolarity on sugar uptake by various tissues (3, 15, 19, 24, 26) have been described. Therefore, effects of medium osmolarity on sugar influx were also investigated.

MATERIAL AND METHODS

Plant Material

Pea plants (Pisum sativum L. cv Marzia) were grown from seeds obtained from Nunhems Zaden BV, Haelen, The Netherlands. Cotyledons were collected and prepared as described: the water content of the cotyledons was taken as an index of their developmental stage (8). Usually, two developmental stages were compared: an early developmental stage, referred to as stage 1 (water content of the cotyledons: 80–75\%; fresh weight: 25–45 mg per cotyledon) and a later stage of development, referred to as stage 2 (water content of the cotyledons: 55–50\%; fresh weight: 125–140 mg per cotyledon). Cotyledons were used within 2 h after their isolation.

Water Relations

The water potential of freshly isolated cotyledons was determined from the changes in their fresh weights after incubation in media with various osmolarities (9). The osmotic potential of the cell sap of freshly isolated cotyledons was measured with a Wescor 5100 C vapor pressure osmometer. The cell sap was collected by squeezing the cotyledons after a freezing/thawing treatment.

Uptake Experiments

The uptake of sucrose and glucose was studied in a manner similar to that previously described for the uptake of valine (10). Briefly, four cotyledons were incubated in 4 mL of basal
medium, which contained 0.5 mol·m⁻³ CaCl₂, 10 mol·m⁻³ Mes-KOH (pH 5.5), supplied with 3.2 kBq [U⁻¹⁴C]sucrose (0.18 GBq·mmol⁻¹) or 3.8 kBq [U⁻¹⁴C]glucose (0.14 GBq·mmol⁻¹) and the desired amount of unlabeled sucrose or glucose. The osmolarity of the medium was adjusted to the desired level with mannitol. Uptake rates were computed from the uptake after 15 and 45 min of incubation and were expressed on the basis of fresh weight, which was determined before the uptake experiments. Data points are the mean values of at least four measurements. The concentration dependency of the uptake rate was evaluated by fitting various rate equations, essentially as described by Borstlap and Schuurmans (2).

**Metabolic Fate of Exogenous Supplied Sugars**

Eight cotyledons were incubated for 2.5 h in 4 mL of basal medium and the desired amount of labeled sugar, and subsequently for 10 min at 0°C in fresh medium of the same composition, but without label. After the uptake experiment the cotyledons were lyophilized and extracted three times with 80% (v/v) ethanol at 70°C for 1, 2, and 3 h, respectively. The ethanol fractions were evaporated at 60°C under nitrogen. The dry residue was dissolved in 1 mL water and 1 mL chloroform was added. After thorough mixing and centrifugation (2000g, 2 min) the water phase was replaced by a fresh one. Water fractions were pooled and lyophilized. The residue was finally dissolved in 0.5 mL water. After hydrolysis of sucrose with β-fructosidase (Boehringer Mannheim, West Germany) sugars were separated by descending paper chromatography on Whatman MM1 using water-saturated phenol as a solvent. Spots of glucose and fructose were made visible by spraying with anisidine reagent [0.4% (w/v) p-anisidine, 2.5% (w/v) H₃PO₄ in 67% (v/v) ethanol] and subsequent heating for 1 to 2 min at 120°C. The spots were cut out and their radioactivity was determined by liquid scintillation counting after adding 1 mL 80% (v/v) ethanol and 10 mL of scintillation cocktail (Lumagel; Lumac, the Netherlands).

**RESULTS**

**Water Relations of Cotyledons**

Water potentials of freshly isolated cotyledons were determined by measuring their changes in fresh weight after incubation in media of various osmolarities (9). Water potentials of stage 1 and stage 2 cotyledons were -1.1 MPa and -1.0 MPa, respectively, and the osmotic potentials of their cell sap were -1.3 MPa and -1.2 MPa, so that the calculated turgor pressure of the cotyledonary cells was equal to 0.2 MPa for both stages of development (Table I).

Cotyledons incubated in a hypotonic medium absorbed water. In basal medium supplied with 200 mol·m⁻³ mannitol ($\psi_w \approx -0.48$ MPa), their volume increased by 16% in stage 1 cotyledons and by 5.6% in stage 2 cotyledons (Table I). Accordingly, an increase in cell surface can be estimated to be 10.4 and 3.7% in stage 1 and stage 2 cotyledons, respectively. After equilibration, the water potential of the cotyledons should be equal to that of the bathing solution, viz.

**Table I. Components of the Water Potential of Freshly Isolated Cotyledons and of Cotyledons after Equilibration in a Medium Containing 200 mol·m⁻³ Mannitol ($\psi_w = -0.48$ MPa)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Developmental Stage</th>
<th>$\psi_w$</th>
<th>$\psi_z$</th>
<th>$\psi_r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshly isolated</td>
<td>Stage 1</td>
<td>-1.1 ± 0.0</td>
<td>-1.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Equilibrated in 200 mol·m⁻³ mannitol</td>
<td>Stage 2</td>
<td>-1.0 ± 0.2</td>
<td>-1.2 ± 0.0</td>
<td>0.2 ± 0.3</td>
</tr>
</tbody>
</table>

* Weight changes expressed as percentage of initial fresh weight.  
* Weight changes expressed as percentage of initial amount of tissue water.  
* Computed from $\psi_z$ of freshly isolated cotyledons, taking into account the amount of water absorbed.  
* Computed from $\psi_r = \psi_w - \psi_z$.

**Table II. Metabolic Fate of Exogenously Supplied Sugars**

The amount of label present in fructose was expressed as percentage of the total amount of label present in glucose and fructose. Radioactivity was determined after hydrolysis of sucrose with β-fructosidase. Cotyledons were incubated in 4 mL of basal medium with 0.5 mol·m⁻³ fructose, 0.5 mol·m⁻³ glucose, and 21.8 kBq [U⁻¹⁴C]glucose (0.14 GBq·mmol⁻¹) or with 0.5 mol·m⁻³ unlabeled sucrose and 61.3 kBq [fructosyl⁻¹⁴H]sucrose (370 GBq·mmol⁻¹). Stage 1 cotyledons, water content 75.2%; stage 2 cotyledons, water content 54.8%.

<table>
<thead>
<tr>
<th>Uptake of [¹⁴C]Glucose: Radioactivity Present in Fructose</th>
<th>0 mosM</th>
<th>400 mosM</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total extracted</td>
<td>0.0%</td>
<td>36.0 ± 0.2</td>
</tr>
<tr>
<td>Stage 1 cotyledons</td>
<td>ND</td>
<td>57.3 ± 2.5</td>
</tr>
<tr>
<td>Stage 2 cotyledons</td>
<td>ND</td>
<td>57.3 ± 2.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Uptake of [¹⁴H]fructosyl-Sucrose:</th>
<th>0 mosM</th>
<th>400 mosM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 cotyledons</td>
<td>99.5 ± 0.5</td>
<td>99.0 ± 0.6</td>
</tr>
<tr>
<td>Stage 2 cotyledons</td>
<td>97.5 ± 0.3</td>
<td>98.8 ± 0.9</td>
</tr>
</tbody>
</table>

* Not determined.
—0.48 MPa. The osmotic potentials then may be calculated from the dilution of the internal osmotics as a result of water absorption. Under this condition the calculated turgor pressure of the cotyledonary cells equals 0.6 MPa for both stages of development (Table I).

**Uptake of Sucrose without Prior Hydrolysis**

Exogenously supplied glucose is easily converted into fructose, presumably by phosphoglucose-isomerase (Table II). Therefore, it might be expected that conversion of fructose into glucose can also occur. After uptake of \([fructosyl-1^3H]\) sucrose, the label was highly preserved in the fructosyl-moiety (Table II). This indicates that sucrose was not hydrolyzed by an external invertase before uptake, because otherwise conversion of labeled fructose into labeled glucose would have occurred. Therefore, these results indicate that the sucrose molecule enters the symplasm intact by a sucrose carrier.

This was further borne out by experiments in which the effect of glucose and fructose on sucrose uptake was assayed (Table III). Neither of the hexoses had any appreciable effect on the uptake rate of sucrose. Table III also shows that glucose could be taken up by the cotyledons, especially in the later developmental stages. Sucrose had no effect on glucose uptake in stage 1 cotyledons, but enhanced the uptake rate of glucose in stage 2 cotyledons by 45%.

**Time Course of Sucrose Uptake**

The uptake of labeled sucrose at an initial concentration of 5.7 \( \times 10^{-3} \) mol \( \cdot m^{-3} \) was linear with time in both stage 1 and stage 2 cotyledons (Fig. 1A). After 2 h of incubation \(^4\)C-label had accumulated 2.6-fold in stage 1 cotyledons and 4.3-fold in stage 2 cotyledons. At a high external sucrose concentration (100 mol \( \cdot m^{-3} \)) uptake deviated from linearity during the 2 h incubation period (Fig. 1B), but it was approximately linear with time between 15 and 45 min of incubation. For both stage 1 and stage 2 cotyledons the lines through the first four points in time made distinct intercepts with the ordinate, which can be attributed to a free space volume of 15 to 20 \( \mu L \cdot g \) fresh weight\(^{-1} \).

**Effects of Medium Osmolarity on Sucrose Influx at Different Stages of Development**

In stage 2 cotyledons, the sucrose influx was only slightly affected by the osmolarity of the incubation medium. It was

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**Table III. Effects of Hexoses on the Uptake of Sucrose, and of Sucrose on the Uptake of Glucose**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Addition</th>
<th>Stage 1 cotyledons</th>
<th>Stage 2 cotyledons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (0.5 mol ( \cdot m^{-3} ))</td>
<td>None</td>
<td>8.6 ± 0.7</td>
<td>11.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Glucose (5 mol ( \cdot m^{-3} ))</td>
<td>9.8 ± 0.7</td>
<td>10.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>Fructose (5 mol ( \cdot m^{-3} ))</td>
<td>9.7 ± 1.0</td>
<td>11.0 ± 0.2</td>
</tr>
<tr>
<td>Glucose (0.5 mol ( \cdot m^{-3} ))</td>
<td>None</td>
<td>1.9 ± 0.1</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Sucrose (5 mol ( \cdot m^{-3} ))</td>
<td>1.8 ± 0.1</td>
<td>11.3 ± 0.2</td>
</tr>
</tbody>
</table>

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**Figure 1.** Time course of sucrose uptake in stage 1 cotyledons, water content 79.0% (C) and in stage 2 cotyledons, water content 54.1% (C). The incubation medium (4 mL) contained 4.1 kBq \([U-14C]\) sucrose and an initial concentration of sucrose of 5.7 \( \times 10^{-3} \) mol \( \cdot m^{-3} \) (A) or 100 mol \( \cdot m^{-3} \) (B). Medium osmolarity was 400 mosM. Data are the means ± se of three experiments.
SUCROSE UPTAKE BY IMMATURE PEA COTYLEDONS

Figure 2. Effect of medium osmolarity on the uptake rate of sucrose in stage 1 cotyledons, water content 77.1% (○) and in stage 2 cotyledons, water content 54.0% (□). Cotyledons were incubated in medium with an initial sucrose concentration of 0.5 mol·m⁻³. Medium osmolarity was varied with mannitol. Data are the means ± se of six experiments.

almost constant (8.0 nmol·min⁻¹·g fresh weight⁻¹) between 0 and 400 mosM and then decreased by ≈30% when medium osmolarity was further raised to 800 mosM (Fig. 2).

A slight reduction (≈12%) of sucrose influx at higher osmolarities was also observed with stage 1 cotyledons. In isotonic medium, the influx in stage 1 cotyledons was almost the same as in stage 2 cotyledons, approximately 7 nmol·min⁻¹·g fresh weight⁻¹. But in contrast to stage 2 cotyledons, sucrose influx in stage 1 cotyledons was strongly reduced in hypotonic medium, up to 70% reduction in a medium without mannitol (Fig. 2).

Obviously, the hypotonic condition reduces sucrose influx at earlier stages of development, but not in later stages. More detailed information is presented in Figure 3. Measurements of sucrose influx in cotyledons of various developmental stages were performed in isotonic as well as in hypotonic medium, so that information was obtained about the influx capacity during development and its sensitivity to the hypotonic condition. When assayed in an isotonic medium (400 mosM) influx capacity was almost constant (6–7 nmol·min⁻¹·g fresh weight⁻¹) during the developmental period examined (Fig. 3). There is some indication of the influx capacity being lower at the earliest developmental stage. The reduction of the influx in hypotonic medium was largest in the earlier developmental stages studied (80% reduction in cotyledons with a water content of 78%), but the reduction became gradually less. At the latest developmental stages (water content less than 58%) equal sucrose influxes were measured in medium with or without 400 mol·m⁻³ mannitol.

Concentration-Dependency of Sucrose Influx

The influx of sucrose into cotyledons of two developmental stages was measured in the concentration range 10⁻² mol·m⁻³ to 100 mol·m⁻³ in medium with an osmolarity of 100

Figure 3. Uptake rates of sucrose in isolated pea cotyledons of various developmental stages. Cotyledons were incubated in medium with an initial sucrose concentration of 0.5 mol·m⁻³ in the absence (○) or presence (□) of 400 mol·m⁻³ mannitol. Data are the means ± se of at least three experiments.

Figure 4. Concentration dependency of the uptake rate of sucrose in stage 1 cotyledons (A) and in stage 2 cotyledons (B) from medium with an osmolarity of 100 mosM (○) or 400 mosM (□). The curves drawn were computed from the parameters given in Table IV. Data are the mean values (± se) of at least six experiments.
or 400 mosM (Fig. 4). Analysis by curve-fitting showed that the concentration dependency of sucrose uptake conformed to a rate equation consisting of one Michaelis-Menten term and a linear term. Table IV shows the values of the various parameters. If the osmolarity of the incubation medium was 400 mosM, very similar values were found for $K_m$ ($\approx 9$ mol·m$^{-3}$) and $V_{\text{max}}$ ($\approx 150$ nmol·min$^{-1}$·g fresh weight$^{-1}$) at both stages of development. The proportionality constant of the linear component diminished 1.7-fold during development, from 0.67 to 0.40 nmol·min$^{-1}$·g fresh weight$^{-1}$·(mol·m$^{-3}$)$^{-1}$ (Table IV). In stage 1 cotyledons the inhibition of the uptake in medium with a low osmolarity resulted from a reduction of the $V_{\text{max}}$ to 36 nmol·min$^{-1}$·g fresh weight$^{-1}$ (Table IV). The osmolarity of the medium also affected the other parameters. Using a medium with an osmolarity of 100 mosM, the calculated $K_m$ values were 1.7-fold smaller and the proportionality constant of the linear uptake was approximately 1.7-fold higher than in experiments where cotyledons were incubated in a medium with an osmolarity of 400 mosM (Table IV).

**Effects of pH on Sucrose Influx**

In medium with 400 mol·m$^{-3}$ mannitol sucrose uptake by both stage 1 and stage 2 cotyledons depended on the pH of the incubation medium. From a maximum uptake rate of 6.8 and 10.2 nmol·min$^{-1}$·g fresh weight$^{-1}$ at pH 4 uptake rates decreased to 1.8 and 4.2 at pH 8 for stage 1 and stage 2 cotyledons, respectively (Fig. 5). In medium without mannitol, sucrose influx by stage 1 cotyledons was greatly reduced. The remaining influx, which can be attributed to the linear component, was essentially independent of the external pH (Fig. 5).

**DISCUSSION**

**Mechanism of Sucrose Uptake**

The water potential of freshly isolated immature legume embryos is approximately $-1.0$ MPa (Table I) (9, 21, 25). This water potential should be equal to the water potential in the surrounding apoplast, if in situ equilibrium between the water potentials of the embryos and their apoplast is assumed. Based on the amounts of solutes, originating from the apoplast of *Phaseolus* seed coats and embryos, Patrick (18) has estimated a similar apoplastic water potential. It is likely, therefore, that the influx capacity measured at 400 mosM reflects that capacity in developing pea cotyledons in situ. Hence, sucrose influx is inhibited by low medium osmolarity rather than stimulated by high medium osmolarity.

During the whole developmental period studied the capacity of sucrose influx (measured at an external concentration of 0.5 mol·m$^{-3}$ and in an isotonic medium [a medium osmolarity of 400 mosM]) was roughly constant (Fig. 3). Also, no major changes could be observed in the kinetics of sucrose uptake during development (Table IV). In both stage 1 and stage 2 cotyledons uptake of sucrose was mediated by two components: a saturable and an unsaturable one (Fig. 4, Table IV). Estimations of the in situ apoplastic sucrose concentration in *Glycine max* and *Phaseolus vulgaris* embryos vary from 35 to 200 mol·m$^{-3}$ (5, 12, 18). If the apoplastic sucrose concentration in pea seeds had similar values, then the con-
tribution of the saturable system to the total uptake rate will be 53 and 64% (at 200 mol·m⁻³ sucrose) for stage 1 and stage 2 cotyledons, respectively. Apparently, the saturable system plays an important role in the uptake of sucrose by the embryo during the whole developmental period examined. This contrasts with amino acid (L-valine) uptake by immature pea cotyledons, which is exclusively taken up by an unsaturable component at the earlier stages of development. A saturable amino acid uptake system was only detectable after the water content of the cotyledons had decreased to 65% (10).

Maintenance of the asymmetry of label in the sucrose molecule after uptake (Table II), no inhibition of sucrose uptake by glucose and fructose (Table III), and the pH-dependency of the sucrose influx in an isotonic medium (Fig. 5) are in accordance with the results obtained in the studies on sucrose uptake by developing soybean embryos (13, 22). It is likely, therefore, that the saturable uptake of sucrose by developing pea cotyledons, as in soybean embryos, is mediated by a proton-symport sucrose carrier (12, 13, 20, 22).

The Michaelis-constant (Kₘ) (Table IV) compares favorably with those for sucrose uptake by soybean embryos (13, 22). The V_max of sucrose uptake by pea cotyledons (Table IV) is also similar to the V_max (100 nmol·min⁻¹·g fresh weight⁻¹) reported by Thorne (22) for soybean cotyledons of a developmental stage (fresh weight: 300–400 mg·embryo⁻¹) roughly comparable to our stage 2 cotyledons. However, a much lower V_max (=10 nmol·min⁻¹·g fresh weight⁻¹) can be estimated from data given by Lichtner and Spanswick (13). The reason for this, most probably, is that Lichtner and Spanswick measured sucrose influx in cotyledons of an early developmental stage (fresh weight: 100–150 mg·seed⁻¹) using a medium with a low osmolarity.

Effect of Medium Osmolarity on Sucrose Influx

As sugar uptake in other plant tissues (3, 19, 24), the reduction of sucrose uptake by a hypotonic condition can be attributed to a reduced V_max of the saturable component (Fig. 4, Table IV). Following a suggestion by Reinhold et al. (19), several authors have assumed that reduced sucrose uptake in a hypotonic medium is caused by inhibition of the plasma membrane H⁺-ATPase at increased turgor. Observations of depolarizations of the trans-plasma membrane potential (7, 11) and reduced “proton extrusion” in hypotonic medium (19, 24) seem to support this suggestion. Conversely, stimulation of the activity of the plasma membrane H⁺-ATPase by incubation in hypertonic medium has been suggested in a study on the osmoregulatory mechanism of the alga Dunaliella salina (16).

In contrast to sucrose uptake, the uptake of L-valine by developing pea cotyledons was not affected by the osmolarity of the incubation medium, or only slightly so (10). The reason for this, most likely, is that at earlier developmental stages L-valine uptake is exclusively by an unsaturable transport pathway and is not a proton symport (10). At the developmental stage where a saturable amino acid uptake systems comes in play, the osmosensitivity of saturable sucrose uptake had already decreased considerably.

Osmosensitivity of Sucrose Uptake

In both stage 1 and stage 2 cotyledons turgor attained the same value, when incubated in a hypotonic medium (Table I). This observation implies that an increase in turgor per se cannot be the reason for the reduced sucrose uptake by developing pea cotyledons in a hypotonic medium. Incubation of stage 1 and stage 2 cotyledons in a hypotonic medium resulted in increase of cell volumes to 116 and 106%, respectively (Table I). Accordingly, plasma membrane area in stage 1 and stage 2 cotyledonary cells should have increased by 10.4 and 3.7%, respectively. It is possible, then, that the osmosensitivity is related to stretching of the plasmalemma. Indeed, Edwards and Pickard (4) have suggested that membrane stretching may be a way of perceiving an increase in turgor. However, it remains questionable if a smaller increase in membrane stretch in stage 2 cotyledons explains the vanished osmosensitivity of the H⁺-ATPase and sucrose uptake.

Two morphological changes in the cotyledonary cells during development contribute to the smaller volume increase of stage 2 cotyledons after incubation in a hypotonic medium: (a) During development cotyledonary cell walls thicken, resulting in a greater elastic modulus of those cell walls. (b) During development an increasing part of the cotyledonary cell volume is occupied by nonsomatic volume because starch grains accumulate and because vacuoles fragmentate and are transformed into protein bodies (14). These gradually changing morphological features may explain the gradual disappearance of the osmosensitivity of sucrose uptake during development.

ACKNOWLEDGMENT

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LITERATURE CITED

Effects of medium osmolarity on the release of amino acids from isolated cotyledons of developing pea seeds. Evidence for vacuolar amino-acid release at increased turgor. Planta 181: 566–573


