Spatial and Temporal Distribution of Sucrose Synthase in the Radish Hypocotyl in Relation to Thickening Growth

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Sucrose synthase (SuSy) is a key enzyme in the development of storage root of radish. Clarification of its spatial and temporal expression during the thickening growth of radish hypocotyl, which later develops into storage root, was carried out immunologically using light microscopy. Sequential harvests at 3, 7, 11 and 13 d after sowing (DAS) were performed on two radish cultivars having different sink capacity. A very low level of SuSy was observed 3 DAS for both cultivars. White Cherrish (WC; strong storage root) showed the maximum level of SuSy between 7 and 11 DAS with increased cell development (thickening), while in Kosena (K; low storage root) the level remained high after 13 d of growth. A high level of SuSy was found in companion cells, which was consistent with previous observations, but SuSy was also found in the xylem parenchyma and in some cortical cells. The level of SuSy differed according to the localization and depended highly on cell development. Both cell division and cell enlargement were stimulated in WC compared with K. The role of SuSy during thickening growth of radish hypocotyl is discussed in terms of utilizing photosynthates.

Key words: Immuno-cytochemistry — Kosena — Raphanus sativus L. — Storage root — Sucrose synthase — White Cherrish.

Abbreviations: DAS, day after sowing; K, Kosena; PBS, phosphate buffered saline; PBST, PBS with 0.1% (v/v) Tween 20; SuSy, sucrose synthase; WC, White Cherrish.

Introduction

Sink-source interaction and its regulation play important roles for plant growth and development (Roitsch 1999). In radish, the major sink is the storage root and its thickening starts from early stages of development. In a previous experiment White Cherrish (WC), a radish cultivar with a large sink of storage root, was grown at different levels of CO2 to assess the influence of sink capacity on photosynthetic activity of source leaves (Usuda and Shimogawara 1998). CO2 elevation resulted in an enhanced sink capacity that was responsible for absorbing higher level of photosynthates and for preventing over-accumulation of carbohydrates in radish leaves at dawn and also from down regulation of photosynthesis. In order to investigate the crucial factors for sink capacity, further studies were initiated with two contrasting radish cultivars. WC (Usuda et al. 1999a) and Kosena (K) (Usuda et al. 1999b), the latter having a small storage root similar to the radish wild type, showed that sink capacity was strongly related to the level and activity of sucrose synthase (SuSy) but not to the activity of invertase.

SuSy, which catalyses the reversible conversion of sucrose and uridine diphosphate (UDP) into fructose and UDP-glucose, is a key enzyme involved in carbohydrate metabolism. SuSy has a predominant role in the cleavage of sucrose in cereal endosperm and potato tuber providing substrates for starch synthesis in storage organs (Chen and Chourey 1989, Wang et al. 1994). Under normal conditions SuSy is supplying UDP-glucose for cell wall biosynthesis and energy, via respiration process, and also for unloading in the phloem (Martin et al. 1993, Nolte and Koch 1993). It has been shown that the activity of SuSy was enhanced in response to anaerobiosis, cold stress or light (Maraña et al. 1990, Ricard et al. 1991). These results indicate that SuSy plays a crucial role for sink development of certain species, although, in other species, invertase carries out this role (e.g. Sturm and Tang 1999, see Usuda et al. 1999a, Usuda et al. 1999b for further discussion).

Several studies showed that the expression of SuSy genes is spatially and temporally separated within the different compartments of the plant. SuSy was shown to be particularly associated with vascular tissues of Ricinus communis L. seedlings (Geigenberger et al. 1993) and potato leaves (Fu and Park 1995). More precisely it was specifically localized in the companion cells of maize leaves and citrus fruits (Nolte and Koch 1993). Expression of the enzyme was observed in the phloem tissues of transgenic tobacco with the use of a sucrose synthase promoter from maize (Yang and Russel 1990) and from Arabidopsis (Martin et al. 1993). However, the protein was rather generally revealed in maize roots (Koch et al. 1992) and potato tubers (Fu and Park 1995). The pattern of distribution is more likely related to the sink capacity and shows tissues and cells specific localization. Temporal and spatial changes of SuSy level have also been observed in the phloem zone of growing leaves (Nguyen-Quoc et al. 1990, Brangeon et al. 1996), indicating a role in the transport of sucrose, and in seed endosperm (Chen and Chourey 1989, Wittich and Vreugdenhil 1998), indicating a role in starch synthesis. Other works confirmed the role of SuSy in sugar transport and starch synthesis in rice
(Wang et al. 1999), barley (Guerin and Carbonero 1997), tomato fruits (Sun et al. 1992, Wang et al. 1994, N’chobo et al. 1999) and potatoes (Zrenner et al. 1995). However, one study on cotton (Ruan et al. 1997) led to a contradictory result indicating that SuSy had no apparent role in starch synthesis and the expression of SuSy in cotyledons suggested a role in protein and lipid synthesis. These results demonstrate the physiological significance of SuSy and its fundamental role in controlling cell differentiation and plant development.

In order to evaluate the importance of SuSy in sink capacity, the present study investigated the spatial and temporal distribution of SuSy during the thickening growth of radish hypocotyl, which later develops into storage root. Immuno-histological localization was carried out with a SuSy primary antibody produced from mung bean SuSy, revealed by gold-silver staining and visualized with light microscopy. Sequential harvests at 3, 7, 11 and 13 DAS were performed on two radish cultivars having different sink capacity. WC with a strong storage root ability and K with a lower storage root, being similar to the wild radish, were used. The level and localization of SuSy are discussed according to physiological and developmental changes in the hypocotyl.

**Materials and Methods**

**Preparation of plant material**

Seeds of *R. sativus* L. (cv White Cherrish and cv Kosena) were germinated by incubation on moist filter paper in darkness for 3 d. Seedlings were then transferred into hydroponic culture and submitted to a 14-h light/10-h dark cycle with a day/night temperature of 25/20°C. For detailed growth conditions see Usuda et al. (1999a). At 3, 7, 11 and 13 DAS the upper parts (1 cm from the base of the shoots) of the hypocotyl were harvested. Samples were immediately fixed in 3.7 % (w/v) formaldehyde solution for 4 h at room temperature, dehydrated and embedded in melted paraffin wax (Paraplast, Oxford Labware, St Louis, U.S.A.) following the method described in Demura and Fukuda (1996). Samples were cut into 15 μm thick sections, with a rotary microtome (Yamato, RV-240, Asaka, Japan), which were attached on microscopy slides by drying on a hot plate at 45°C. Sections were dewaxed in xylene, rehydrated and rinsed in water prior to immunological treatment or staining.

**Immu-no-cytochemistry**

After washing in PBS and blocking in a goat serum (Histofine SAB-PO kit, Nichirei, Tokyo, Japan) the sections were incubated overnight at 4°C with a SuSy primary antibody raised in rabbit against the SuSy protein isolated from mung bean or with a pre-immune serum as control. Slides were washed with PBST and then incubated with a secondary antibody (AuroProbe™ Amersham International plc, Little Chalfont, U.K.) at room temperature for 1 h. The specific immune reaction was revealed using the IntenSET™ M Silver enhancement kit (Amersham International plc, Little Chalfont, U.K.). The slides were prepared for microscopical observations by dehydration and xylene treatment before mounting in Canada Balsam oil. Sections were examined in light microscopy (Nikon, Eclipse E800, Tokyo, Japan) using a Normarsky filter. Images acquisition was performed with a SPOT RT camera (Diagnostic Instruments inc., Burroughs, MN, U.S.A.) and printed on a Fujix Pictrography 3000 (Fuji Film, Tokyo, Japan).

**Stainings**

Sections from each hypocotyl (5 mm from the base of the shoots) were stained with 0.1% (w/v) toluidine blue in 0.01 M Na2CO3, and viewed in bright-field optics to show general cell anatomy and development. For visualization of the phloem differentiation the sections, originating from the same region as those used for immunocytochemistry, were stained with 0.05% aniline blue in 67 mM potassium-phosphate buffer, pH 8.6, for 2 h. In order to reduce the background of wall autofluorescence, sections were previously stained in a Schiff’s reagent for 4 min and rinsed with tap water. Sections were mounted in fresh aniline blue and viewed with UV fluorescence optics (Nikon, Eclipse E800, Tokyo, Japan).

**Image analysis and measurements**

Images from the transverse sections stained with toluidine blue and acquired with the SPOT RT digital camera were used for determining cell size and cell number. Sections of three samples of each cultivar were chosen in order to be approximately 5 mm from the base of the shoots. Images of the 7 and 13 DAS were reconstructed by adjustment of the different optical fields of each section and saved as a unique image. Further processing was performed on each reconstructed image, using the tool options of an image analysis program (Corel Photo Paint), in order to clarify the developmental changes in the radish hypocotyl. On the 7 DAS sections of WC and K, the replication of different tissues was divided into four types: (1) cortex, including epidermis, cortex parenchyma and endodermis, (2) other parenchyma, including pericycle, phloem parenchyma, cambium and xylem parenchyma, (3) xylem vessels, and (4) pith. In the case of the 13 DAS sections, parenchyma was further divided into three types: (1) pericycle, phloem parenchyma and cambium, (2) ray, and (3) xylem parenchyma.

Measurements of the surface areas for the total section and each tissue type were carried out, after printing, with a leaf area meter (AM 100, ADC, Hoddesdon, U.K.), and calibrations for area calculations were adjusted to the magnification of the images. Determination of the cell size and cell number was made with a sub-sample of the tissue considered. Surface areas were measured by the method described above and cells were counted individually on the computer screen by using the zoom option of the image analysis program. The average count of cells for each sub-sampled tissue was ranging from 150 to 400 cells, except for xylem vessel and the pith where all cells were counted for each section. Mean area of transverse section of the cells was taken as cell size and was calculated with the area and the cell number was adjusted to the magnification of the images. Determination of the surface area and the cell number of a sub-sample. Total cell number was estimated with the total surface of the tissue type. Data in Table 1 are the mean values of three samples±standard deviation, and significance is the result of a *t*-test comparing WC and K (*p* < 0.05, **p** < 0.01).

**Results**

**Distribution of sucrose synthase**

Differential distribution of SuSy protein was monitored during the two first weeks of development in radish hypocotyl of W and K. Temporal variations in the level of SuSy were determined by immunogold silver-staining reactions and are presented in Fig. 1. Observations of the labeled transverse sections showed that the level of SuSy was low at 3 DAS (Fig. 1A, E) and was increased from 7 DAS for the two cultivars of WC and K (Fig. 1B, C and Fig. 1F, G). After 13 d of growth the amount of SuSy was reduced for WC (Fig. 1D) while it remained high for K (Fig. 1H). Sections have been taken approximately at the same location within the hypocotyl, i.e.
Sucrose synthase distribution between 1 and 3 mm from the shoots. Their diameter measured at 3, 7, 11 and 13 DAS were 1.1, 1.4, 3.3 and 5.1 mm for WC, and 1.2, 1.5, 2.3, and 3.5 mm for K, respectively. The distribution of SuSy protein also showed a spatial differentiation (example for WC at 7 DAS presented in Fig. 2), immunogold stain was mainly observed in the phloem zone (Fig. 2C) but also in the xylem parenchyma (Fig. 2D) and in some cortical cells (Fig. 2A). Localization of the SuSy in the cortex appears to be related to the cytoplasmic content of particular cells (Fig. 2B).

Fig. 1 Light micrographs of transverse sections in radish hypocotyl harvested at 3 d (A, E), 7 d (B, F), 11 d (C, G) and 13 d (D, H) after sowing for White Cherrish (A to D) and Kosena (E to H) cultivars. Immunogold label (representative dark precipitate are marked with arrowheads) show the localization of sucrose synthase. Sections are originating from a region located around 2 mm from the base of the shoots. Bars = 200 μm.
In order to be more precise on the localization of SuSy in the phloem zone a complementary staining with aniline blue was performed on the sections (Fig. 3, 4). Aniline blue specifically stains for vascular vessels of the phloem and particularly for callose (Fig. 3A, D). Comparison of the sections of hypocotyl stained with aniline blue and immunogold indicated clearly that the companion cells of the phloem were the specific localization for SuSy (Fig. 3B, E) for both WC and K cultivar. Longitudinal sections of the hypocotyl on which similar stainings were performed confirmed the specific localization of SuSy in companion cells (Fig. 4A, B, D, E).

Structural changes in the thickening of the hypocotyl

Morphological and anatomical changes in different tissues of radish hypocotyl between 7 and 13 DAS are given in Table 1. Measurements, including surface area, cell size and cell number were performed on transverse sections of WC and K stained with toluidine blue (see Fig. 5). Distribution of the different zones (cortex, parenchyma, xylem vessels and pith) are expressed in percent of the total surface while mean cell sizes and mean cell numbers were estimated from a sub-sample area of the sections. At 7 DAS transverse sections of WC and K had similar surface area. Development of hypocotyl during the second week of growth was such that transverse area increased significantly ($p < 0.05$) for WC (18-fold) and was larger than for K (4-fold) resulting in a significant ($p < 0.01$) difference in size at 13 DAS (Table 1 and Fig. 5). In the present estimation of the transverse surface area of the cortex, epidermis and endodermis have been included, while in the determination of cell size and cell number only the cortical cells were considered. Parenchyma includes pericycle, phloem parenchyma, cambium, xylem parenchyma and ray. Percentage of cell area occupied by the pith is the mean average of three samples but observations showed its presence only for one sample of both cultivars. Pith developed mainly at the upper most part of the hypocotyls, and is often absent at 5 mm from the base of the shoots.

After 1 week of growth, proportion of the transverse surface area within the different tissue types was similar in WC and K in cell size and cell number. The area of cortex, paren-
Sucrose synthase distribution

Chyma, xylem vessels and pith were ca. 90%, ca. 8%, ca. 1%, and ca. 1%, respectively (Table 1). During the second week of growth, morphological and anatomical changes between WC and K were observed. The number of cortical cells (as well as cell layers, data not shown) stayed constant for all the observed samples at 7 or 13 DAS. Cortex cell size was significantly ($p < 0.01$) but differently increased for WC (10-fold) and K (3-fold). Despite that strong cell enlargement in the cortex its proportion in total surface area was reduced. Significantly higher ($p < 0.05$) reduction of the proportion of cortex in WC indicates that other type of tissues were stimulated in their development. Xylem vessels were increased in size and number in the same

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**Fig. 3** Transverse sections of radish hypocotyl harvested at 7 d after sowing. Light-fluorescence micrographs of sections stained with aniline blue (callose from the sieve element are bright blue) in White Cherrish (A) and Kosena (D) cultivars. Light immunogold localization showing sucrose synthase in companion cell (arrows) to the proximity of sieve element (arrowheads) for White Cherrish (B) and Kosena (E). Control sections treated with pre-immune serum are presented in (C) for White Cherrish and (F) for Kosena. Sections are originating from a region located around 2 mm from the base of the shoots. Bars = 50 μm.
order of magnitude for both cultivars. The proportion of surface area occupied by the xylem vessels was significantly ($p < 0.01$) increased in K while it was decreased (not significantly) in the hypocotyl of WC. Between 7 and 13 DAS the most significant changes occurred in the development of parenchyma. Surface area and its proportion of the total surface, cell size and cell number were strongly increased (Table 1). Total parenchyma representing more than 50% of the total surface was increased approximately six times in WC while it occupied only 22% of the total surface for a three-times increase in K.

After 2 weeks of growth the enlargement of the hypocotyl was the result of growth in the stelar portion. The epidermis

Fig. 4 Longitudinal sections of radish hypocotyl harvested at 7 d after sowing. Light-fluorescence micrographs of sections stained with aniline blue (callose from the sieve element are bright blue) in White Cherrish (A) and Kosena (D) cultivars. Light immunogold localization showing sucrose synthase in companion cell (arrows) in White Cherrish (B) and Kosena (E). Control sections treated with pre-immune serum are presented in (C) for White Cherrish and (F) for Kosena. Images were taken in the region where transverse observations were made, around 2 mm from the base of the shoots. Bars = 50 μm.
Sucrose synthase distribution

The cambium appeared in the fundamental parenchyma lying between the metaxylem and the primary phloem. As growth continued there were lateral extensions in cambium evolving into pericyclic parenchyma. The secondary phloem consisted of sieve tubes, companion cells and some parenchyma while the xylem was made up of large reticulate vessels surrounded by parenchyma. The secondary xylem vessels were arranged in approximate radial rows separated tangentially by rays of parenchyma (Fig. 5). Within the stelar portion, parenchyma was divided into three different groups. The first group included pericycle, phloem parenchyma and cambium, and the second and third groups included ray and xylem parenchyma, respectively (Table 2). At this stage of thickening growth, development of the different types of parenchyma was distinct for the two cultivars (Table 2 and Fig. 5). The proportion of pericycle, cambium and phloem parenchyma was significantly ($p < 0.05$) lower for WC than for K but because of increased cell size and cell number the total transverse surface of those tissues was more than seven times larger in WC hypocotyl at 13 DAS (Table 2). Cell differentiation into ray parenchyma

![Fig. 5](image_url)
was greater for WC than for K. Both cell size and cell number were significantly ($p < 0.05$ and $p < 0.01$, respectively) increased and resulted in a significantly ($p < 0.01$) greater proportion of that tissue in the sections of WC after 2 weeks of growth. The proportion of ray parenchyma in percent of the total parenchyma was double in WC compared to K (42% and 21%, respectively) but its surface area was 17 times higher for WC than for K at 13 DAS (Table 2). Cell size and cell number of the xylem parenchyma were significantly ($p < 0.05$) larger for WC but the proportion of this tissue in the total parenchyma was lower than for K (Table 2). However, the total surface area of xylem parenchyma was almost five times greater for WC than K at that stage of development. Xylem vessels showed the trend observed for xylem parenchyma. These observations with WC were essentially similar to those of Hayward (1938).

**Discussion**

Sucrose is the main carbohydrate form that is transported between plant organs and the supply for growing sinks (Farrar 1996). The sucrose metabolizing enzymes expressed in the sink tissues are likely to play a significant role in sink function. There is strong correlation between the level and activity of SuSy and the sink capacity of two contrasting radish cultivars (cv White Cherrish and cv Kosena) (Usuda et al. 1999a, Usuda et al. 1999b). It was hypothesized that high levels of enzymes involved in the breakdown of sucrose would increase sink capacity by lowering the local concentration of sucrose, thereby generating a gradient that allows further unloading of sucrose from the phloem (Wardlaw 1968).

Temporal distribution of SuSy showed that the enzyme was induced earlier in WC than in K and that the level was reduced for WC at 13 DAS while it remained high for K after the same period of growth. These results are in agreement with previous observations made after determining the protein level in the hypocotyl of both cultivars. The relative amount of enzyme in WC increased remarkably (75-fold), reached a maximum at 17 DAS and decreased till the end of the experiment (21 DAS) (Usuda et al. 1999a). The increase in K was relatively low (30-fold) and stayed to the maximum level from 13 to 21 DAS (Usuda et al. 1999b). These changes in SuSy level

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**Table 1** Morphological and anatomical changes in White Cherrish and Kosena between 7 and 13 DAS

<table>
<thead>
<tr>
<th>Tissue</th>
<th>White Cherrish</th>
<th>Kosena</th>
<th>White Cherrish</th>
<th>Kosena</th>
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<tbody>
<tr>
<td><strong>7 DAS</strong></td>
<td></td>
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<tr>
<td>Total surface (mm²)</td>
<td>1.38±0.17</td>
<td>1.76±0.29</td>
<td>24.92±3.66</td>
<td>6.92±0.98 ** (4.1) *</td>
</tr>
<tr>
<td>Cortex %</td>
<td>88.6±1.6</td>
<td>90.0±2.5</td>
<td>43.6±7.8</td>
<td>74.9±3.5 * (0.8) *</td>
</tr>
<tr>
<td>Cell size (μm²)</td>
<td>1.876±166</td>
<td>2.655±789</td>
<td>18.172±5.485</td>
<td>7.274±708 (2.7) *</td>
</tr>
<tr>
<td>Cell number</td>
<td>620±90</td>
<td>585±77</td>
<td>563±115</td>
<td>666±157 (1.1) *</td>
</tr>
<tr>
<td>Parenchyma %</td>
<td>9.4±0.7</td>
<td>8.0±1.0</td>
<td>55.6±7.8</td>
<td>22.5±3.6 ** (2.8) *</td>
</tr>
<tr>
<td>Cell size (μm²)</td>
<td>75±17</td>
<td>89±44</td>
<td>805±161</td>
<td>344±93 * (3.9)</td>
</tr>
<tr>
<td>Cell number</td>
<td>1,706±417</td>
<td>1,562±491</td>
<td>17,329±2,006</td>
<td>4,593±143 ** (2.9) **</td>
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<tr>
<td><strong>13 DAS</strong></td>
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<td></td>
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<tr>
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<td></td>
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</tr>
</tbody>
</table>

Transverse surface areas of the different tissues are expressed in % of the total surface. Mean cell sizes and cell numbers were determined from sections stained with toluidine blue. Sections are originating from a region located at 5 mm from the base of the shoots. Numbers in parenthesis show the ratio of values obtained at 13 DAS with the values obtained at 7 DAS. Values are ($n = 3$) mean±standard deviation. Significance are the result of a-test comparing White Cherrish and Kosena ($* p < 0.05$, **$p < 0.01$).

* Epidermis and endodermis are included for determination of cortex surface area but excluded for determination of cell size and cell number.
* Parenchyma includes pericycle, phloem parenchyma, cambium, xylem parenchyma and ray.
* % of total surface is the mean value of three samples while cell size and cell number is the results of one observation.
Sucrose synthase distribution during early stage of growth are highly related to sink capacity. The small temporal discrepancy, between the previous (17 DAS, Usuda et al. 1999a) and present (7 to 11 DAS) results, found in the peak of SuSy level in WC may be derived from the following reasons. Previous determination of the amount of SuSy protein was carried out on the basis of total soluble protein (Usuda et al. 1999a, Usuda et al. 1999b). During the thickening growth (up to 21 DAS), the soluble protein content per dry weight of hypocotyl decreased after 13 DAS in WC but stayed relatively constant in K (Usuda, unpublished data). For WC the development of ray parenchyma cells, where SuSy was not detected, was considerable after 7 d of growth (Table 2). Therefore, images based on immuno-histological reactions did not coincide perfectly with the results of SuSy quantification from the total soluble proteins extracted. SuSy was lacking in the hypocotyl of both cultivars at 3 DAS with both detection methods (Fig. 1, Usuda et al. 1999a, Usuda et al. 1999b). These results suggest that SuSy plays an important role in sink development.

The different isoforms of SuSy, found in a number of plant species, are differentially regulated with respect to time, space and inducibility (Sturm et al. 1999). In the case of monocotyledonous plants such as maize (Koch et al. 1996), wheat (Maraña et al. 1990) and barley (Guerin and Carbonero 1997), two isoymes of SuSy have been characterized. Recently, three isoforms of the protein have been identified for rice (Wang et al. 1999), mung bean (Arai et al. 1992), sugar beet (Hesse and Willmitzer 1996) and Chenopodium (Godt et al. 1996) suggest only one isoform of SuSy. Alignment of the amino acid sequences of SuSy from the different species showed high homology among the products of the different genes. To our knowledge.

Table 2  Relative proportion, cell size and cell number of different parenchyma tissues in White Cherrish and Kosena hypocotyl at 13 d after sowing

<table>
<thead>
<tr>
<th></th>
<th>White Cherrish</th>
<th>Kosena</th>
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<tbody>
<tr>
<td>Total parenchyma (mm²)</td>
<td>13.95±3.43</td>
<td>1.58±0.47 *</td>
</tr>
<tr>
<td>Pericycle, cambium, phloem parenchyma % of parenchyma</td>
<td>46.0±3.1</td>
<td>56.8±1.9 *</td>
</tr>
<tr>
<td>Cell size (µm²)</td>
<td>735±268</td>
<td>282±32</td>
</tr>
<tr>
<td>Cell number</td>
<td>8,803±1,568</td>
<td>3,147±642 *</td>
</tr>
<tr>
<td>Ray                  % of parenchyma</td>
<td>41.8±1.5</td>
<td>21.3±3.1 *</td>
</tr>
<tr>
<td>Cell size (µm²)</td>
<td>1,378±266</td>
<td>554±265 *</td>
</tr>
<tr>
<td>Cell number</td>
<td>4,231±198</td>
<td>645±116 **</td>
</tr>
<tr>
<td>Xylem parenchyma     % of parenchyma</td>
<td>12.2±2.3</td>
<td>21.9±4.9</td>
</tr>
<tr>
<td>Cell size (µm²)</td>
<td>303±46</td>
<td>197±20 *</td>
</tr>
<tr>
<td>Cell number</td>
<td>5,446±1,325</td>
<td>1,783±786 *</td>
</tr>
</tbody>
</table>

Transverse surface areas of the different tissues are expressed in % of the total parenchyma. Mean cell sizes and cell numbers were determined from sections stained with toluidine blue. Sections are originating from a region located at 5 mm from the base of the shoots. Values (n = 3) are mean ± standard deviation. Significance are the result of a t-test comparing White Cherrish and Kosena (*p < 0.05, **p < 0.01).

Fig. 6 Simplified model of sucrose partitioning in radish plants. Sucrose (Suc) is transported in the sieve elements (SE) of the phloem, transformed by sucrose synthase (SuSy) into fructose (Fru) and UDP-glucose (UDPG) in the companion cells (CC). Sugars are utilized in the parenchyma cells of the storage root for sink development and storage or exported towards a different sink.
knowledge, there is no report concerning isoforms of SuSy in radish. For histo-immunological localization in radish hypocotyl an antiserum produced from mung bean SuSy was used, it reacted with a polypeptide having a molecular mass of about 92 kDa (Usuda et al. 1999a, Usuda et al. 1999b). This antiserum was specific enough to reveal the localization of SuSy but did not allow to draw any conclusion concerning isoforms.

The present study showed that, during the radish hypocotyl thickening, SuSy was mainly localized in the companion cells. This observation is essentially in agreement with the previous findings (see Introduction) confirming the idea brought by Nolte and Koch (1993) that the breakdown of sucrose by SuSy enzyme occurs in the companion cells and that hydrolyzed products of fructose and UDP-glucose are utilized for sink development. Our observations, however, revealed the absence of complete cell specificity; SuSy was also observed in the xylem parenchyma and some cortical cells (see Fig. 2). Koch et al. (1992) showed that SuSy was most prevalent in the stele and apex of intact maize root and also showed that change in SuSy localization does not stem from strict cell-specific distribution. SuSy distribution was shifted to the epidermal cells when the roots were in contact with an external medium enriched in glucose. With transgenic potato plants, Zrenner et al. (1995) suggested that SuSy was crucial for tuber development. Expression of one of the isoforms, in leaves and roots of potato plants, provided evidence for a role of SuSy in vascular function while the expression of the other isoform in all tissues except for the periderm of tuber indicated a crucial role in sink development (Fu and Park 1995). These results indicate that SuSy is not only active in the vascular tissues (mainly in the companion cells of the phloem) for transport of sucrose between source and sinks but also in other parenchyma cells. Namely, part of the sucrose conveyed by the companion cells is also transported to other cell, including xylem parenchyma cells, providing substrates for cell wall biosynthesis, energy and storage to sustain sink development (see Fig. 6). SuSy was also localized in some cortical cells at certain stage (especially at 7 DAS, see Fig. 1A, 2A) but the functions of SuSy in those cells and its heterogeneity within the cortical parenchyma are unknown.

Due to the lower rate of cell division and cell enlargement in K compared to WC (Fig. 1, Table 1 and 2), the utilization of photosynthetate in K hypocotyl appeared to be lower than in WC. This probably induced the relatively high concentration of sucrose measured in the hypocotyl of K (Usuda et al. 1999b). The gradient of sucrose concentration between the source leaves and sink hypocotyl is decreased, implying transport of the photosynthetate to other sinks of developing leaves (Fig. 6) particularly for K which has larger shoots compared to WC (Fig. 2 in Usuda et al. 1999b).

In summary, our results confirm that both enzymatic activity of SuSy in companion cells, creating a gradient of sugar, and the activities of cell division and cell enlargement are the important factors that control sink development and regulate the partitioning of photosynthetate between sinks. SuSy in xylem parenchyma may also provide the material for vessel development, which is essential for plant growth and development.

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References


Sucrose synthase distribution


