ハツカダイコン胚軸の成長,乾物の分配,糖含量,およびスクロース代謝酵素活性と肥大成長の関係

<table>
<thead>
<tr>
<th>項目</th>
<th>内容</th>
</tr>
</thead>
<tbody>
<tr>
<td>誌名</td>
<td>名城大学農学部学術報告</td>
</tr>
<tr>
<td>ISSN</td>
<td>09103376</td>
</tr>
<tr>
<td>著者</td>
<td>鈴木, 茂敏&lt;br&gt;田口, 頌子&lt;br&gt;森, 裕恵&lt;br&gt;河村, 千賀子&lt;br&gt;野澤, 真理恵</td>
</tr>
<tr>
<td>巻/号</td>
<td>44号</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 13-19</td>
</tr>
<tr>
<td>発行年月</td>
<td>2008年3月</td>
</tr>
</tbody>
</table>
Growth, Dry Matter Partitioning, Sugar Content, and Activities of Sucrose Metabolizing Enzymes in Hypocotyls of Radish (*Raphanus sativus* L.) Plants in Relation to the Thickening Growth.

Shigetoshi Suzuki*, Shoko Taguchi, Hiroe Mori, Chikako Kawamura, and Marie Nozawa

**Summary**

The growth, sugar content, and activities of some sucrose metabolizing enzymes were measured to study the biochemical mechanism of thickening growth of radish plants. Activities of both acid and neutral invertase were high in the early stage of growth and thereafter decreased. Activity of sucrose synthase was low until the 20th day after germination (DAG) and increased on 30 DAG. Increase in activity of sucrose synthase corresponded well with increase in dry weight partitioning to hypocotyls and the specific hypocotyl thickening rate (SHTR), although no correlation was observed between activity of invertase and the thickening growth. Thus, sucrose synthase appeared to be a key enzyme involved in the active thickening growth. Glucose and fructose were the main sugars accumulated in hypocotyls and only a small amount of sucrose was detected. No significant differences in sugar contents and in activities of sucrose synthase were observed between inside and outside tissue of the cambium ring suggesting that there appears to be no difference in sucrose metabolizing activity in both tissues. These results were discussed in relation to the site of unloading of photosynthates and thickening growth in radish hypocotyls.

**Key Words:** radish, thickening growth, unloading, sucrose synthase, acid and neutral invertase.

**Introduction**

The radish is one of the most important vegetable crops in Japan and cultivated extensively at all seasons by selecting varieties and/or under conditions of partially controlled environment. There are varieties of radish cultivars with thickened axis of various shape and color*. However, mechanism of thickening growth of radish plants has not been fully understood. The radish is classified as a root vegetable crop of the xylem-thickening type and morphological and histological studies revealed that activities of the cambium ring, cell division of xylem parenchyma cells, and activity of the secondary cambium play important roles in the thickening growth*. Furthermore, the limited development of lignified vascular elements and the differentiation of abundant thin-walled parenchyma from the cambium derivatives are responsible for the active thickening growth and succulence of the edible root-hypocotyledonary axis*. These histological processes of thickening growth have been explained to be regulated by plant hormones. Among them, auxins and cytokinins are considered to play important roles in the thickening growth of radish plants. Although the bulk of the fleshy axis is composed of water and structural carbohydrates such as cellulose and pectic substances, some reserve materials are also accumulated in the thickened tissue in the form of glucose and fructose, indicating that a large increase in dry weight growth of fleshy axis is dependent on the accumulation of photosynthates translocated from source leaves*. These results suggest that the thickening growth of the radish is regulated by plant hormones in the histological mechanism of the thickening growth and is dependent on dry matter production and
partitioning in the quantitative mechanism of thickening growth.

Usuda et al.\(^8,9\) reported that sucrose is a major sugar translocated from leaves to the fleshy axis of radish plants and that sucrose synthase plays a critical role in the process of breakdown of sucrose and accumulation of reducing sugars in the thickened tissue. They also demonstrated the close relationship between the sink activity expressed by the specific growth rate of hypocotyls and the activity of sucrose synthase. In the fleshy axis of radish plants, the specific site of localization of this enzyme is proposed to be companion cells\(^10\), suggesting the strong possibility that this enzyme is deeply involved in unloading of the photosynthetically produced sucrose, accumulation of glucose and fructose, and then thickening growth of radish plants.

In the thickened fleshy axis of the radish, phloem elements differentiate in the meristematic tissues of xylem parenchyma (tertiary phloem) and outward from cambium ring (secondary phloem)\(^1,2\). In the experiment of Usuda et al.\(^8,9,10\), however, they used young radish plants in which the tertiary phloem tissue did not fully develop. A large transversal area of the xylem parenchyma tissue inside the phloem ring in the fleshy axis of fully thickened radish plants indicate the important role of the tertiary phloem in the unloading of sucrose. Hence, it is of interest to make clear the difference of sucrose metabolizing activity between phloem tissues arising from different origins.

In this paper, we confirmed the close relationship between sucrose synthase activity and thickening growth of radish plants, and compared sugar content and sucrose synthase activity in the inside and outside tissue of the cambium ring.

Materials and Methods

Plant materials

Radish (Raphanus sativus L. 'Comet' or 'French Breakfast') seeds were germinated on wet filter paper in the dark at 25°C for 24 h. Three germinated seeds were selected for uniformity and planted in a black plastic pot (9 cm diam. and 300 cm\(^2\) capacity) filled with premixed soil (vermiculite: peat moss: carbonated rice hull = 5 : 4 : 1). Plants were grown in a phytotron (S-206W, Koito Industries, Ltd., Japan) at 23/18°C (day/night) under a natural light condition. After the first true leaf fully expanded, one seedling per pot was remained for measurement of growth, sugar content, and enzyme activity. Plants were irrigated with tap water before seeding emergence and thereafter supplied daily with a nutrient solution containing 1.125 mM of MgSO\(_4\), 7H\(_2\)O, 0.5 mM of KH\(_2\)PO\(_4\), 0.375 mM of Ca(H\(_2\)PO\(_4\))\(_2\), 2H\(_2\)O, 1.5 mM of Ca(NO\(_3\))\(_2\), 4H\(_2\)O, 7.5 mM of KNO\(_3\), and microelements (Fe; 3, Mn; 0.5, B; 0.5, Zn; 0.05, Cu; 0.02, Mo; 0.01 ppm). The 'Comet' plants were sampled on 5, 10, 20, and 30 days after germination (DAG) for measurement of growth and enzyme activity. The 'French Breakfast' plants were harvested on 30 DAG and transverse sections of about 0.5 cm in thickness were prepared from thickened hypocotyls, from which the inner portion encircled by the cambial ring (inside tissue) and the outer portion between the cambial ring and periderm (outside tissue) were taken for the measurement of sugar content and sucrose synthase activity. The 'French Breakfast' is a variety with cylindrical thickened tissue from which both tissues can be separated easily.

Quantitative growth analysis

Ten plants were sampled for measurement of growth. Fresh weight of each shoot and hypocotyl, its length and diameter were recorded (data not shown), before being dried in a ventilated oven at 80°C for 3 days. Calculation and statistical analysis of specific growth rate (SGR) were carried out using the pairing method on the basis of sequential order of plant size\(^11,12\). SGR is the rate of increase in total dry weight per plant with the unit of g·g\(^{-1}\)·day\(^{-1}\) or day\(^{-1}\) and calculated as given by

\[
SGR = \frac{1}{W} \frac{dW}{dT} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}
\]

where W is plant total dry weight and dW is the increment of dry weight during the period dT (5 or 10 days). W\(_1\) and W\(_2\) are dry weight of plant on day t\(_1\) and t\(_2\), respectively. Specific hypocotyl thickening rate (SHTR) was also calculated using the same equation, in which W was replaced with hypocotyl diameter.

Measurement of enzyme activity

Enzymatic activities of sucrose synthase and invertase were determined as described by Usuda et al.\(^9\). A 500–mg of fresh tissue was taken from hypocotyls and homogenized in a extraction buffer containing 50 mM HEPES-KOH (pH 7.8), 1 mM EDTA, 5 mM DTT, 0.3 mM (p-amidinophenyl) methanesulfonyl fluoride, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg ml\(^{-1}\) chymostatin, 10 μg ml\(^{-1}\) leupeptin, 2% (w/v) glycerol and 0.5% (w/v) BSA with a chilled pestle and mortar. The crude homogenate was used for assays of invertase activity, which is the sum of activities of soluble and insoluble form of the enzyme as described by Usuda et al.\(^9\). The crude homogenate was dialyzed twice for 3 hr each against the buffer containing 50 mM HEPES-KOH (pH 7.8), 1 mM EDTA and 1 mM DTT. The volume of the
homogenate was brought to 4 ml and the homogenate was used for the assay of invertase activity. After centrifugation at 12,500 × g for 9 min, the supernatant was assayed for sucrose synthase activity. Neutral and acid invertase and sucrose synthase activities were measured as described by Usuda et al.⁹. The amount of glucose produced in the measurement of invertase activity was determined enzymatically using the glucose assay kit from Boehrnger Mannheim Biochemicals. Activity of sucrose synthase was assayed by measuring the rate of sucrose breakdown reaction.

**Carbohydrate analysis**

Plant materials were stored in a deep freezer at -30°C and lyophilized to determine sugar content. Lyophilized tissues were then combined and pulverized to a fine powder by using a mortar and pestle. A 100-mg powdered sample was extracted with 15 mL of boiling 80% ethanol for 15 min and the extract was centrifuged at 3,000 rpm for 10 min, with these procedures repeated four times after each centrifugation. Ethanol in the supernatants was evaporated in boiling water. After precipitating proteins with 3 N Ba(OH)₂ and 5% ZnSO₄, the volume was adjusted to 50 mL with distilled water. Total soluble sugar was assayed by the anthron method. Sucrose, glucose, and fructose were determined enzymatically using the sucrose/glucose/fructose assay kit from Boehrnger Mannheim Biochemicals.

**Results and Discussion**

The growth stage of radish plants after emergence can be divided into two stages.⁶ In the first stage of 15 days after emergence, foliage leaves are the strongest sink and dry matters are partitioned preferentially to foliage leaves. In the second stage of 15-30 days after emergence, thickening and dry weight growth of hypocotyls are stimulated. Our experiments also confirmed these findings (Fig. 1). In this experiment, dry weight partitioning to hypocotyls was increased on 30 DAG (Fig. 1) and the specific hypocotyl thickening rate (SHTR) was enhanced on 20-30 DAG (Fig. 2c). The high SGR on 10-20 and 20-30 DAG was due to the active growth of foliage leaves and hypocotyls, respectively (Fig. 2a, b).

Changes in the activity of sucrose metabolizing enzymes are shown in Fig. 3. Activity of both acid and neutral invertase was high in the early stage of growth and thereafter decreased during the experimental period (Fig. 3a). Activity of sucrose synthase was low until 20 DAG and increased on 30 DAG (Fig. 3b). Several researchers have reported similar results⁸,⁹,¹⁰. They also indicated that sucrose synthase play a critical role in the thickening growth of the radish because a close relationship was observed between sink strength of thickened tissue and sucrose synthase activity. As shown in Fig. 2c and 3b, increase in activity of sucrose synthase corresponded well with increase in SHTR. Since no correlation was observed between activity of invertase and the thickening growth, sucrose synthase would be a key enzyme involved in the second growth stage. However, the activities of both invertases were approximately the same as that of sucrose synthase on 30 DAG. Activity of invertase estimated in this experiment using crude homogenate was considered to be the sum of activities of soluble and insoluble

---

**Fig. 1.** Changes in the relative dry weight of each organ expressed as percentage of total dry weight.
There are several isoforms of invertase with pH optima for sucrose breakdown in acid and neutral or slightly alkaline range. Isoforms of acid invertase are known to localize in vacuole and cell wall as soluble acid invertases, whereas neutral or alkaline invertase is found in cytosol. As reported in carrot and potato plants, apoplastic invertase would play an important role in unloading of photosynthates and then thickening growth in radish plants. Furthermore,

Fig. 2. Specific growth rate (SGR) and specific hypocotyl thickening rate (SHTR). (a) SGR of a whole plant; (b) SGR of a hypocotyl; (c) specific hypocotyl thickening rate (SHTR). Data represent average of 10 measurements and error bars denote 95% confidence limits of means (n = 10) by Fisher’s LSD test.

Fig. 3. Changes in activities of invertases (a) and sucrose synthase (b) of the extracts from whole thickened tissues of hypocotyls. Data represent averages of three measurements and error bars denote SE.
the role of invertases exhibiting high activity during the early stage of radish growth is unclear in this experiment. Further study is needed to make clear the role of invertase in radish thickening.

Sugar content in the inside and outside tissue of the cambium ring is shown in Fig. 4. A large amount of total sugar accumulated in the thickened tissue by as much as 50% of dry weight. Glucose and fructose were the main sugars accumulated in hypocotyls and only a small amount of sucrose was detected as reported previously, indicating a rapid breakdown of sucrose transported from source leaves to hypocotyls. No significant difference was observed in the sugar content between the inside and outside tissue of the cambium ring. No significant difference was also observed in activity of sucrose synthase between both tissues (Fig. 5). These results indicate that there are no differences in sucrose

![Fig. 4. Sugar content in the tissue of thickened hypocotyls. Plants were harvested on 30 DAG and transverse sections of about 0.5 cm in thickness were prepared from thickened hypocotyls, from which the inner portion encircled by the cambium ring (inside tissue) and the outer portion between the cambium ring and periderm (outside tissue) were taken for the measurement of sugar content. Data represent averages of three measurements and error bars denote 95% confidence limits of means (n = 3) by Fisher's LSD.]

![Fig. 5. Sucrose synthase activities in the tissue of thickened hypocotyls. Plants were harvested on 30 DAG and transverse sections of about 0.5 cm in thickness were prepared from thickened hypocotyls, from which the inner portion encircled by the cambium ring (inside tissue) and the outer portion between the cambium ring and periderm (outside tissue) were taken for the measurement of sucrose synthase activity. Data represent averages of three measurements and error bars denote SE.]
metabolizing activity between both tissues. Rouhier and Usuda reported the specific localization of sucrose synthase in the companion cell by the histo-immunological study using an antiserum from mung bean. Since sucrose synthase was also observed in xylem parenchyma cells, they proposed that part of sucrose unloaded in the phloem tissue would be transported to xylem parenchyma tissue and broken down for active thickening growth. However, the phloem tissue in which they observed the sucrose synthase localization in the 13 days old plants appears to be the secondary phloem developing outward from the cambium ring. In the actively thickening plant during the second growth stage, the tertiary phloem tissues arise and develop in the xylem parenchyma tissue. As shown in Fig. 5 in the companion cell by the histo-thickening plant during the second growth stage, the tertiary phloem tissues arise and develop in the xylem parenchyma tissue in actively thickening radish plants of the second growth stage. Hence, there is a possibility that the xylem parenchyma cells in which they found localization of sucrose synthase would be developing tertiary phloem tissues. In such case, there appears to be at least two sites of unloading in the fleshy thickened axis; the secondary phloem tissue outside the cambium ring and the tertiary phloem tissue in xylem parenchyma. As shown in Fig. 5, there appears to be no difference in sucrose metabolizing activity in both sites. However, a large transversal area of the xylem tissue inside the phloem ring in the fleshy axis of radish plants, which is a typical root vegetable crop of the xylem-thickening type, would accumulate a large amount of glucose and fructose in xylem parenchyma tissue via sucrose breakdown by sucrose synthase in the tertiary phloem tissue. Further studies are needed to clarify localization of sucrose synthase in the xylem parenchyma tissue in actively thickening radish plants of the second growth stage.

**Literature Cited**


ハツカダイコン胚軸の成長，乾物の分配，糖含量，およびスクロース代謝酵素活性と肥大成長の関係

鈴木茂敏，田口順子，森裕恵，河村千賀子，野澤真理恵

ハツカダイコンの肥大成長の生理学的機能を明らかにする目的で，成長，乾物重の分配，糖含量，およびスクロース代謝酵素活性を調べた。酸性および中性インペルターゼ活性は成長初期で高く，その後減少した。スクロース合成酵素活性は発芽後20日目まで低く，30日目に増加した。スクロース合成酵素活性の増大は，胚軸への乾物の分配と肥大成長速度の増加とよく対応したが，インペルターゼ活性と肥大成長の間には相関が見られなかった。これらから，スクロース合成酵素は活発な肥大成長に重要な役割を果たしていることが考えられた。グルコースとフルクトースは胚軸に蓄積する主要な糖であり，スクロースの蓄積はわずかであった。また，形成層の内部と外部で組織中の糖含量およびスクロース合成酵素活性には違いは認められなかったことから，これらの組織におけるスクロース代謝活性に違いは認められないことが示された。これらの結果について，胚軸における光合成産物の積み下ろし（unloading）部位と肥大成長との関連において考察した。