Photosynthetic Activity in the Developing Cotyledon of Soybean Seeds

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We examined whether developing cotyledons of soybean seed had photosynthetic activities. The cotyledons evolved oxygen under illumination and the activity was inhibited by 3-(3',4'-dichlorophenyl)-1,1-dimethylurea. The rate of oxygen evolution decreased during the development of seeds; about $30 \mu\text{mol O}_2 \cdot \text{mg chlorophyll}^{-1} \cdot \text{hr}^{-1}$ at the early developing stage and about $10 \mu\text{mol O}_2 \cdot \text{mg chlorophyll}^{-1} \cdot \text{hr}^{-1}$ at the late developing stage. The rate of oxygen uptake remained at an almost constant level of $40 \mu\text{mol O}_2 \cdot \text{mg chlorophyll}^{-1} \cdot \text{hr}^{-1}$ throughout the development. Photosynthetic $^{14}\text{CO}_2$ -fixation by the cotyledon was observed. Ribulose biphosphate carboxylase was immunochemically detected in the developing cotyledons. These results show that functional photosynthetic apparatus is present in the developing cotyledons of soybean seeds.

Photosynthesis in the organs related to reproduction is important in the yield of crops. In cereals, photosynthates from these organs, such as ears and flag leaves, contribute to the production of storage substances.^{1~3)} Pod walls of the crops photosynthesize.^{4~10)} The development of rape seeds proceeds after defoliation⁷⁾; this is sustained mainly by the photosynthate of the pod.^{7,8)} Photosynthetic reassimilation of respiratory CO_2 has been reported in the pod wall.^{9,10)}

We have reported^{11,12)} that amyloplasts in developing cotyledons of soybean seed have a lamella structure similar to that of chloroplasts in matured leaves. However, little is known about photosynthetic activities of the developing cotyledon of dicotyledonous seeds. We report here the occurrence of photosynthetic activity in the developing soybean cotyledon, and its possible contribution to the crop production is discussed.

MATERIALS AND METHODS

Plant material. Soybean plants (*Glycine max.* cv. Enrei) were grown in pots at 25/20°C day/night and 70% humidity in an environment-controlled glass house under natural light. The pots were filled with 300 ml of a 4:2:4:1 (v/v) mixture of vermiculite, perlite, peat moss, and fine gravel which were supplied with a nutrient solution (1 g of Magamp K (N:P₂O₅:K₂O=6:40:5, W. R. Grace Co., Tennessee, USA)) and 3 g of magnesia lime twice a week. The fresh weights of seeds increased during development as previously observed.¹¹⁾ A seed which weighed 0.2 g was at the age of about 25 DAF and one which weighed 0.6 g was about 50 DAF.

Uptake and evolution of oxygen by developing cotyledon. The cotyledon was cut latitudinally into slices of about 1 mm thickness after removing the pod wall and seed coat. Four slices were put into the well of an oxygen electrode (Rank Brothers, U.K.) with 2.5 ml of 0.1 M HEPES-NaOH, pH 7.6. The rate of oxygen uptake was measured in the dark at 25°C and then the reaction mixture was illuminated with a tungsten projector lamp about 5 min after the addition of the slices. The light intensity was $800 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ PAR. The rate of O_2 evolution was

Abbreviations: DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; PAR, photosynthetically active radiation; RUBPCase, ribulose biphosphate carboxylase; PEPCase, phosphoenol pyruvate carboxylase; Chl, chlorophyll; DAF, days after flowering.

calculated by subtracting the rate of oxygen uptake in the dark from the rate at about 5 min after illumination. The Chl content was measured spectrophotometrically.¹³⁾

Light-dependent $^{14}\text{CO}_2$ -fixation of soybean cotyledon. Developing cotyledon (about 40 DAF) was cut into slices of about 1 mm thick, and 40 mg of the slices (equivalent to 18 μg of Chl) was incubated in 0.5 ml of 50 mM HEPES-NaOH, pH 7.6, containing 1 mM $\text{NaH}^{14}\text{CO}_3$ (1.2 $\mu\text{Ci}/\mu\text{mol}$) in the presence or absence of 0.2 mM DCMU for 20 min at 25°C under illumination. Two ml of methanol was added to the reaction mixture to end the reaction, and the slices were homogenized in the mixture. Then 0.1 ml of 5% acetic acid was added to 0.2 ml of the homogenate to remove the unincorporated carbon. The residual ^{14}C was measured by a Packard 3255 liquid scintillation counter.

Immunoassay of RUBPCase in the developing cotyledon. The immunoassay was done by the method of Ouchterlony.¹⁴⁾ The antibody against the large subunit of RUBPCase from maize leaves was a generous gift from Dr. Sugiyama of Nagoya University. Developing soybean seeds (1.34 g, 40 DAF), soybean leaves (0.5 g), or maize leaves (0.5 g) were homogenized in 2 ml of 10 mM potassium phosphate, pH 7.2, containing 0.15 M NaCl, 0.1% NaN_3 , and 10 mM dithiothreitol. After centrifugation at $10,000 \times g$ for 20 min, 10 μl of the clear supernatant was put into each hole of the agarose gel, surrounding the center hole of anti-RUBPCase. The gel was stained with 0.5% amido black 10B in 90% methanol and 10% acetic acid.

RESULTS

Oxygen evolution in developing soybean seed under illumination

Slices of the developing soybean cotyledon took up respiratory oxygen in the dark (Fig.

FIG. 1. Effects of Light and DCMU on Oxygen Exchange in Developing Soybean Cotyledons. (Age of the slices was about 40 DAF.)

1, addition of the slices of the cotyledon; 2, light on; 3, addition of 0.1 mM DCMU.

1). When illuminated, the rate of oxygen uptake gradually decreased to reach a constant level after 5 min of light. The addition of 0.1 mM DCMU increased the rate of oxygen uptake to the original level in the dark. Because DCMU is a specific inhibitor of photosynthetic electron transport,¹⁵⁾ the light-dependent suppression of oxygen uptake is ascribed to photosynthetic oxygen evolution. Thus the results indicate that developing soybean cotyledons are photosynthetically competent.

Changes in the rates of oxygen evolution and uptake in cotyledons during development

Rates of oxygen exchange varied during the development of cotyledons as shown in Fig. 2. The rate of oxygen evolution at an early developing stage, where a cotyledon weighed

FIG. 2. Changes in Rates of Oxygen Uptake and Evolution of Developing Soybean Cotyledons during Maturation.

The rates were measured as described in the text. The seed maturation process is shown by weight increases. A seed which weighed 0.2 g was at the age of about 25 DAF and one which weighed 0.6 g was about 50 DAF.

0.2 g, was about $30 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{hr}^{-1}$. The rate decreased as the seed weight increased or maturation proceeded, and a cotyledon of 0.6 g at a late developing stage had an oxygen evolution rate of about $10 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{hr}^{-1}$. The rate of oxygen uptake remained at an almost constant level of $40 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{hr}^{-1}$ throughout the maturation.

Light dependent CO₂-fixation of developing soybean cotyledons

Light-dependent CO₂-fixation in slices of soybean cotyledon was also detected by measuring the incorporation of ¹⁴CO₂ into acid involatile products. The incorporation rate under illumination was $2.31 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{hr}^{-1}$ as compared with a dark fixation rate of $0.46 \mu\text{mol O}_2 \cdot \text{mg Chl}^{-1} \cdot \text{hr}^{-1}$. DCMU specifically reduced the light fixation. Rates of CO₂-fixation were 0.83 and $0.37 \mu\text{mol O}_2 \cdot \text{mg}$

$\text{Chl}^{-1} \cdot \text{hr}^{-1}$ in the light and dark, respectively, in the presence of 0.2 mM DCMU. The rate of CO₂-fixation is considerably lower than that of O₂ evolution. This may be ascribed to reassimilation of endogeneous CO₂. At any event, the results show that soybean cotyledons can fix CO₂ photosynthetically.

Detection of RUBPCase protein in cotyledons of soybean seeds

The third line of evidence for the occurrence of photosynthetic apparatus in developing soybean cotyledons was obtained with an antibody against the large subunit of RUBPCase. The double radial immunodiffusion technique of Ouchterlony was used (Fig. 3). Immunoprecipitin lines were observed not only with extracts from maize and soybean leaves, which contain chloroplasts, but also with extracts from soybean cotyledons. This indicate that RUBPCase protein that is immunochemically the same as the enzyme from maize leaves is present in developing soybean cotyledons.

DISCUSSION

Photosynthesis by soybean seeds has been studied by Quebedeaux and Chollet¹⁰⁾ and Sambo *et al.*¹⁶⁾ Sambo *et al.* measured the CO₂ exchange of intact and deseeded soybean pods, and concluded that photosynthesis in the seeds and hulls refixed some 50~70% of the CO₂ respired by these tissues. Quebedeaux and Chollet reported that gross photosynthesis of the pod was observed but the net photosynthesis was negligible.¹⁰⁾ It is important to measure the photosynthetic capacity of each part of the soybean pod, such as the pod wall, seed coat, and cotyledon to understand the role and function of photosynthesis in whole pod. Our work indicates that developing cotyledons of soybean seeds have a significant photosynthetic activity.

The finding that developing soybean cotyledons fix CO₂ and evolve O₂ under illumination indicates that the cotyledons of soybean seed had chloroplasts competent in photo-

FIG. 3. Double Immunodiffusion Experiment Showing Precipitin Lines of RUBPCase in Developing Soybean Cotyledons.

The center well contained $10 \mu\text{l}$ of antibody of the large subunit of RUBPCase.

Extracts from soybean cotyledons, maize leaves, and soybean leaves were placed in peripheral wells; 1 and 4, soybean cotyledon; 2 and 5, maize leaves; 3 and 6, soybean leaves.

synthesis.

The rate of O₂ evolution per mg Chl was found to decrease during maturation. We suggest the following explanation for the change in the photosynthetic activity of the cotyledon during the maturation. At the early stage of development, sunlight penetrates inside the seeds and illuminates chloroplasts in the thin cotyledon. As the cotyledon gradually thickens and enlarges by the accumulation of storage substances during maturation, the intensity of light reaching chloroplasts is weakened, resulting in a gradual loss in the photosynthetic activity of chloroplasts inside the cotyledon.

Activities of RUBPCase and PEPCase have been observed in developing seeds of several crops.^{5,6,17,18)} Singal and Singh¹⁷⁾ found a high activity of PEPCase in developing chickpea pods, and Shimotsubo *et al.* reported changes in the activities of these enzymes in rape seeds¹⁸⁾ during the maturation. It was reported that pod walls of soybean seeds had the two enzyme activities.¹⁰⁾ In this work, RUBPCase, an enzyme of the CO₂-fixation cycle, was immunochemically detected in developing cotyledons of soybeans. Refixation of respiratory CO₂ by these enzymes using photoenergy in the cotyledon may reduce the loss of carbon in the seed.

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