Rice Cultivars with Higher Sucrose Synthase Activity Develop Longer Coleoptiles under Submerged Conditions

Akari Fukuda, Satoshi Yoshinaga, Kenji Nagata and Hiroyuki Shiratsuchi

(National Agricultural Research Center for Tohoku Region, Yatsuya, Daisen, Akita 014-0102, Japan)

Abstract : Sucrose synthase, which catalyzes uridine diphosphate (UDP)-dependent cleavage of sucrose into fructose and UDP-glucose, is induced by oxygen deficiency in rice seedlings and is considered to play an important role in energy production under hypoxic conditions. In this study, we analyzed the relationship between coleoptile elongation and sucrose synthase activity in rice (Oryza sativa L.) cultivars under submerged conditions. We also analyzed the activity of α-amylase, which digests starch reserves in the endosperm and is considered to be important for energy production in young seedlings. The results indicated that different rice cultivars had different sucrose synthase and α-amylase activities under submerged conditions. Moreover, sucrose synthase activity in whole seedlings was significantly correlated with coleoptile length under submerged conditions, whereas the correlation between α-amylase activity and coleoptile length was low. Sugar content of shoots differed with the cultivar. Correlation analysis demonstrated that sucrose content was highly correlated with coleoptile length and sucrose synthase activity, but not with α-amylase activity.

Key words : α-Amylase, Coleoptile length, Rice (Oryza sativa L.), Submergence, Sucrose synthase.

It is important to improve seedling establishment in rice sown directly in submerged conditions. Ogiwara and Terashima (2001) grew rice seeds on agarose and reported that the time required for coleoptile emergence after germination is highly correlated with the percentage of seedlings that reach the expanded second leaf stage in experimental nursery boxes. They suggested that rapid elongation of the coleoptile is one of the important factors in seedling establishment. Although rice is a hypoxia-tolerant plant that can germinate and extend its coleoptiles under low-oxygen conditions (Alpi and Beevers, 1983), differences in coleoptile elongation rates have been reported among rice cultivars (Setter et al., 1994). Research to determine why rice cultivars have different coleoptile elongation rates is necessary in order to breed submergence-tolerant cultivars and requires an understanding of plant metabolism under oxygen deficiency. Setter et al. (1994) suggested that coleoptile elongation is related to the rate of ethanol synthesis in rice cultivars grown under oxygen deficiency. However, little is known about other aspects of coleoptile growth, such as sugar metabolism under oxygen deficiency.

Under oxygen deficiency, plants restrict their respiration and alter their metabolism to be more efficient. Specific enzymes related to sugar metabolism are synthesized under oxygen deficiency (Sachs et al., 1980; Geigenberger, 2003). One such major enzyme is sucrose synthase, which is induced transcriptionally and translationally in rice seedlings under oxygen deficiency (Ricard et al., 1991; Kato-Noguchi, 2000).

Sucrose synthase catalyzes the reversible conversion of sucrose and UDP to UDP-glucose and fructose, but sucrose degradation dominates in vivo. Invertase can also break down sucrose, but activity of this enzyme decreases under oxygen deficiency, while sucrose synthase activity increases, suggesting that sucrose synthase is more energy-efficient than invertase (Guglielminetti et al., 1995). Here, we analyzed differences in sucrose synthase activity among different rice cultivars and examined the relationship between coleoptile length and sucrose synthase activity under submerged conditions.

Starch is a major seed reserve for the growth of young plants and is metabolized to sugars by α-amylase (Beck and Ziegler, 1989). Williams and Peterson (1973) found that the activity of α-amylase does not limit shoot dry weight of rice under submerged conditions. However, the relationship between α-amylase activity and coleoptile length under submerged conditions has not been analyzed in detail. In this study, we examined differences in α-amylase activity among rice cultivars under submerged conditions and analyzed the relationship between α-amylase activity and coleoptile length.

Materials and Methods

1. Seed materials

Seeds of all rice cultivars used in this study (Table 1) were harvested from experimental paddy fields of the National Agricultural Research Center for the Tohoku Region (Daisen, Akita, Japan, 39°29'N, 140°30'E). Fully mature seeds (specific gravity >1.15) were
Table 1. Coleoptile length, sucrose synthase activity and α-amylase activity under submerged conditions in the seeds harvested in 2002 (A), 2003 (B) and 2004 (C).

A  
| Cultivar            | Coleoptile length (mm) | Sucrose synthase activity | α-Amylase activity |
|---------------------|------------------------|---------------------------|--------------------|
|                     | shoot                  | endosperm                | shoot              | endosperm              |
| Italica Livorno     | 24.6 ± 4.6             | 7.61 ± 1.09              | 0.87 ± 0.21        | 48.03 ± 10.43          | 40.80 ± 3.33          |
| Arroz da Terra      | 20.6 ± 2.1             | 6.37 ± 0.47              | 0.61 ± 0.08        | 24.08 ± 3.49           | 64.65 ± 8.40          |
| S-201               | 18.5 ± 3.6             | 5.03 ± 0.26              | 0.87 ± 0.41        | 19.49 ± 1.32           | 14.85 ± 2.83          |
| Sensho              | 20.2 ± 3.5             | 4.35 ± 0.29              | 1.36 ± 0.51        | 31.05 ± 6.18           | 20.82 ± 0.70          |
| M-202               | 18.6 ± 3.3             | 4.76 ± 1.35              | 1.01 ± 0.15        | 18.82 ± 4.95           | 17.35 ± 5.71          |
| M-201               | 14.5 ± 4.4             | 4.77 ± 0.25              | 0.85 ± 0.08        | 16.17 ± 0.77           | 12.38 ± 2.14          |
| Calrose             | 15.6 ± 3.5             | 4.09 ± 0.31              | 0.93 ± 0.13        | 15.84 ± 2.18           | 9.41 ± 2.67           |
| Haenuki             | 22.9 ± 3.2             | 5.14 ± 0.46              | 0.60 ± 0.12        | 26.82 ± 6.44           | 21.36 ± 5.27          |
| Fukuhibiki          | 17.8 ± 3.0             | 4.48 ± 0.32              | 0.73 ± 0.19        | 45.10 ± 9.46           | 37.62 ± 4.98          |
| Akitakomachi        | 17.0 ± 2.4             | 4.37 ± 0.87              | 0.62 ± 0.38        | 17.41 ± 2.70           | 23.63 ± 2.60          |
| L-202               | 8.7 ± 2.1              | 2.58 ± 0.27              | 0.80 ± 0.04        | 12.77 ± 2.15           | 5.30 ± 0.41           |
| Bluebelle           | 6.4 ± 1.3              | 1.82 ± 0.10              | 0.55 ± 0.07        | 29.94 ± 5.78           | 19.95 ± 0.87          |
| Blue Bonnet         | 7.7 ± 2.2              | 1.37 ± 0.23              | 0.76 ± 0.23        | 20.49 ± 3.22           | 9.19 ± 1.75           |

B  
| Cultivar            | Coleoptile length (mm) | Sucrose synthase activity | α-Amylase activity |
|---------------------|------------------------|---------------------------|--------------------|
|                     | shoot                  | endosperm                | shoot              | endosperm              |
| Arroz da Terra      | 24.7 ± 3.3             | 5.72 ± 0.27              | 0.26 ± 0.03        | 12.29 ± 1.93           | 61.98 ± 4.91          |
| S-201               | 24.7 ± 5.9             | 5.26 ± 0.31              | 0.48 ± 0.10        | 13.64 ± 3.28           | 15.86 ± 2.36          |
| M-202               | 22.1 ± 3.4             | 5.11 ± 0.77              | 0.40 ± 0.10        | 13.30 ± 3.34           | 16.43 ± 2.44          |
| M-201               | 16.8 ± 3.5             | 3.87 ± 0.24              | 0.53 ± 0.17        | 13.28 ± 3.17           | 14.39 ± 3.22          |
| Calrose             | 18.4 ± 3.5             | 4.81 ± 0.80              | 0.58 ± 0.19        | 15.40 ± 2.74           | 7.60 ± 0.42           |
| Haenuki             | 22.7 ± 2.4             | 4.18 ± 0.34              | 0.31 ± 0.06        | 17.14 ± 3.45           | 28.62 ± 1.76          |
| Fukuhibiki          | 23.3 ± 2.8             | 3.89 ± 0.58              | 0.51 ± 0.14        | 34.79 ± 4.06           | 38.45 ± 6.03          |
| Akitakomachi        | 20.6 ± 2.9             | 4.16 ± 0.31              | 0.36 ± 0.06        | 14.63 ± 3.08           | 20.52 ± 0.85          |
| L-202               | 11.0 ± 2.9             | 3.04 ± 0.44              | 0.39 ± 0.09        | 9.81 ± 0.90            | 7.65 ± 1.34           |

C  
| Cultivar            | Coleoptile length (mm) | Sucrose synthase activity | α-Amylase activity |
|---------------------|------------------------|---------------------------|--------------------|
|                     | shoot                  | endosperm                | shoot              | endosperm              |
| Italica Livorno     | 23.2 ± 3.5             | 6.33 ± 0.35              | 1.02 ± 0.31        | 26.55 ± 4.44           | 64.50 ± 12.64         |
| Arroz da Terra      | 16.3 ± 3.9             | 4.11 ± 0.45              | 0.60 ± 0.27        | 9.97 ± 1.39            | 57.55 ± 4.11          |
| S-201               | 23.5 ± 5.3             | 4.20 ± 0.82              | 1.08 ± 0.20        | 14.00 ± 3.27           | 28.11 ± 13.39         |
| Sensho              | 20.1 ± 3.4             | 4.57 ± 0.24              | 0.95 ± 0.02        | 14.56 ± 1.38           | 14.89 ± 2.40          |
| M-202               | 22.5 ± 4.1             | 3.61 ± 0.17              | 0.93 ± 0.20        | 11.31 ± 2.22           | 14.25 ± 0.90          |
| M-201               | 19.3 ± 4.3             | 3.95 ± 0.08              | 1.07 ± 0.20        | 11.35 ± 3.97           | 23.33 ± 7.63          |
| Calrose             | 19.3 ± 3.3             | 3.19 ± 0.22              | 1.02 ± 0.17        | 12.60 ± 1.77           | 15.18 ± 1.10          |
| Haenuki             | 17.2 ± 3.9             | 3.23 ± 0.17              | 1.04 ± 0.18        | 16.27 ± 2.61           | 22.66 ± 3.97          |
| Fukuhibiki          | 20.3 ± 5.2             | 3.63 ± 0.27              | 1.03 ± 0.23        | 27.40 ± 7.95           | 33.15 ± 5.14          |
| Akitakomachi        | 21.1 ± 4.2             | 3.90 ± 0.12              | 0.83 ± 0.19        | 13.97 ± 2.82           | 18.30 ± 3.59          |
| L-202               | 12.3 ± 4.9             | 2.93 ± 0.32              | 1.47 ± 0.12        | 10.83 ± 3.82           | 13.37 ± 1.08          |
| Bluebelle           | 8.5 ± 1.4              | 1.71 ± 0.23              | 0.44 ± 0.10        | 14.60 ± 2.66           | 20.80 ± 3.23          |
| Blue Bonnet         | 8.4 ± 3.6              | 1.50 ± 0.17              | 0.70 ± 0.20        | 12.94 ± 2.45           | 14.49 ± 3.11          |

Correlation with coleoptile length 0.918** 0.241 0.432 0.546

Coleoptile lengths were measured 7 days after germination. Values are the averages of 45 samples with standard deviations. Enzyme activities per shoot and endosperm were measured 4 days after germination. Values are the average of three replicates with standard deviations. ** and * indicate significant correlations at the 1% and 5% levels, respectively.
selected and used for the experiments. The harvested seeds were stored in plastic bottles at 6°C until they were used for the experimental treatments.

2. Growth conditions
   (1) Coleoptile growth, enzyme activities and sugar content under submerged conditions (Experiment 1)

   Coleoptile length, sucrose synthase activity, α-amylase activity and sugar content were analyzed using seeds harvested in 2002, 2003 and 2004 (Tables 1 and 2). Thirteen rice cultivars that had different rate of seedling growth (Ogiwara and Terashima, 2001), cultivars from Japan, Akitakomachi, Fukuhibiki, Haenuki and Sensho, cultivars from USA, Bluebelle, Blue Bonnet, Calrose, L-202, M-201, M-202 and S-201, a cultivar from Italy,Italic Livorno, and a cultivar from Portugal, Arroz da Terra, were used as materials. Because of the loss of the harvested seeds, only nine cultivars, Akitakomachi, Arroz da Terra, Calrose, Fukuhibiki, Haenuki, L-202, M-201, M-202 and S-201, were harvested in 2003. The seed-storage periods from harvest to experiment were 18 months, 12 months and 6 months for the seeds harvested in 2002, 2003 and 2004, respectively.

   The coleoptile length was measured on an agarose bed by the method described by Ogiwara et al. (1998). Four mL of 4 g L⁻¹ agarose were prepared in test tubes (10 mm diameter, 100 mm long), and 15 tubes were packed in a polycarbonate pot (70 mm in diameter, 51 mm in length, cover 70 mm in length with a vent hole; “Agripot”, Iwaki Glass Co. Tokyo, Japan) and sterilized by autoclaving. Seeds were dehulled and sterilized for 1 hr in 50-fold diluted antiformin containing 100 μL L⁻¹ Triton X-100. After initial sterilization, seeds were washed with sterilized water three times and soaked in sterilized water for 48 hr at 16°C. One germinated seed with a 0.5 mm long coleoptile was placed in each tube 5 mm below the surface of the agarose gel, then sterilized water (3 mL) was added to the tube. The distance from the embryo to the water surface was 35 mm. Tubes were returned to the Agripot and incubated at 16°C in the dark. After 7 days, coleoptiles were photographed and their lengths measured.

   For enzyme analysis, seeds were dehulled, sterilized as described above, and soaked in sterilized water at 16°C for 48 hr. Ten germinated seeds with 0.5 mm coleoptiles were selected and incubated under submerged conditions (imbibed in 3.5 cm deep water) in an Agripot. After incubation at 16°C for 4 days in darkness, seedlings were stored at −80°C until analysis of sucrose synthase activity, α-amylase activity and sugar content.

   (2) Seedling growth under aerobic and submerged conditions (Experiment 2)

   Six cultivars with different coleoptile-elongation rates were used (see Figs. 3 and 4). The seeds harvested in 2002 were used 12 months after harvest. Seeds were dehulled and sterilized as described above. After sterilization, seeds were soaked in sterilized water at 25°C for 48 hr. Ten germinated seeds with 0.5 mm long coleoptiles were selected and incubated under aerobic conditions (sown on wet filter paper) or submerged conditions as described above in an Agripot. After incubation at 16°C for 24 hr in the dark, seedlings were stored at −80°C until the analysis of sucrose synthase activity, α-amylase activity and sugar content.

   (3) Comparison of sucrose synthase activity in long- and short-coleoptile cultivars under aerobic and submerged conditions (Experiment 3)

   We used two long-coleoptile cultivars (Italica Livorno and Arroz da Terra) and two short-coleoptile cultivars (Bluebell and Blue Bonnet) for the experiments. Seeds were harvested in 2005 and stored for 4 months until they were used in the experiments. Seeds were dehulled, sterilized, and incubated under aerobic and submerged conditions in Agri,ots, as described above. After germination, seeds were incubated for 1, 3, 5 and 7 days, and seedlings were then stored at −80°C until analysis of sucrose synthase activity.

3. Analysis of sucrose synthase activity

   Ten shoots (including coleoptiles, embryos and scutella) and endosperms were collected from the seeds using a scalpel and ground in an ice-cooled glass mortar with 2 mL of extraction buffer (100 mM Heps-KOH, pH 7.5, 1 mM EDTA, 5 mM MgCl₂, 5 mM dithiothreitol, 10 mM NaHSO₃). After centrifugation at 10,000 x g for 10 min, the supernatant was collected. The solution was desalted by passage through a PD-10 desalting column (GE Healthcare UK Ltd., Buckinghamshire, England) equilibrated with extraction buffer. The amount of protein in the solution was measured using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories Inc., Hercules, CA, USA). Sucrose synthase activity was assayed in the direction of sucrose cleavage. Four hundred μL of reaction mixture (50 mM Heps-KOH, pH 7.0, 2 mM MgCl₂, 1 mM EDTA, 15 mM KCl, 25 mM sucrose, 1 mM UDP) containing 10 μg of protein were incubated at 30°C for 30 min. All controls lacked UDP. Reactions were terminated by heating for 2 min in a boiling water bath. The amount of fructose released was measured with an F-kit (Roche Diagnostics Co., Basel, Switzerland). One unit (U) of activity was defined as the amount of enzyme required to release 1 μM of fructose in 1 min under the assay conditions.

4. Analysis of α-amylase activity

   The Ceralpha kit (Megazyme Co. Ltd., Sydney, Australia) was used to assay α-amylase activity of the extracted proteins. One U of activity was defined as the amount of enzyme, in the presence of excess
Table 2. Sucrose, glucose and fructose contents per shoot under submerged conditions in the seeds harvested in 2002 (A), 2003 (B) and 2004 (C).

### A

| Cultivar         | Sucrose | Glucose | Fructose |
|------------------|---------|---------|----------|
| Italica Livorno  | 43.8 ± 3.7 | 13.8 ± 4.0 | 11.9 ± 4.5 |
| Arroz da Terra   | 44.9 ± 3.3 | 25.8 ± 2.5 | 26.2 ± 3.6 |
| S-201            | 50.3 ± 8.1 | 24.4 ± 2.5 | 23.8 ± 2.2 |
| Sensho           | 27.4 ± 2.8 | 15.4 ± 2.5 | 10.4 ± 4.1 |
| M-202            | 48.1 ± 8.0 | 23.3 ± 3.2 | 22.8 ± 3.5 |
| M-201            | 24.7 ± 5.9 | 12.3 ± 2.4 | 12.3 ± 3.1 |
| Calrose          | 29.1 ± 3.8 | 8.6 ± 0.7  | 7.5 ± 2.0  |
| Haenuki          | 34.4 ± 5.4 | 12.6 ± 2.7 | 10.2 ± 1.5 |
| Fukuhibiki       | 25.8 ± 3.0 | 10.1 ± 2.4 | 8.0 ± 2.6  |
| Akitakomachi     | 37.7 ± 2.3 | 17.3 ± 2.1 | 15.3 ± 4.2 |
| L-202            | 13.4 ± 2.1 | 4.8 ± 1.4  | 5.2 ± 1.9  |
| Bluebelle        | 11.0 ± 4.5 | 5.5 ± 2.4  | 5.9 ± 2.7  |
| Blue Bonnet      | 7.6 ± 1.2  | 3.5 ± 0.6  | 3.7 ± 1.3  |

Correlation with coleoptile length: 0.820** 0.661* 0.537
Correlation with sucrose synthase activity: 0.833** 0.679* 0.601*
Correlation with α-amylase activity: 0.355 0.313 0.258

### B

| Cultivar         | Sucrose | Glucose | Fructose |
|------------------|---------|---------|----------|
| Italica Livorno  | 52.0 ± 15.5 | 27.7 ± 5.7 | 29.9 ± 5.1 |
| Arroz da Terra   | 54.3 ± 2.1 | 15.8 ± 1.6 | 18.1 ± 2.4 |
| S-201            | 44.4 ± 7.7 | 16.6 ± 1.6 | 15.3 ± 2.9 |
| Sensho           | 37.0 ± 1.5 | 11.4 ± 1.5 | 7.7 ± 0.9  |
| M-202            | 50.5 ± 7.6 | 19.4 ± 2.5 | 21.5 ± 3.2 |
| M-201            | 34.7 ± 51  | 18.2 ± 2.5 | 19.7 ± 3.3 |
| Calrose          | 42.5 ± 6.5 | 20.2 ± 1.4 | 20.8 ± 2.8 |
| Haenuki          | 52.1 ± 2.9 | 22.8 ± 1.1 | 21.1 ± 2.6 |
| Fukuhibiki       | 38.5 ± 5.3 | 13.1 ± 2.4 | 10.2 ± 2.2 |
| Akitakomachi     | 50.3 ± 7.6 | 17.8 ± 2.9 | 15.2 ± 3.5 |
| L-202            | 14.2 ± 2.7 | 7.2 ± 2.0  | 8.8 ± 2.7  |
| Bluebelle        | 16.9 ± 1.9 | 6.8 ± 0.8  | 7.6 ± 1.2  |
| Blue Bonnet      | 10.4 ± 1.6 | 2.8 ± 1.2  | 3.9 ± 1.1  |

Correlation with coleoptile length: 0.810** 0.778** 0.654*
Correlation with sucrose synthase activity: 0.656* 0.729** 0.664*
Correlation with α-amylase activity: 0.458 0.481 0.500

### C

| Cultivar         | Sucrose | Glucose | Fructose |
|------------------|---------|---------|----------|
| Italica Livorno  | 55.3 ± 2.4 | 25.4 ± 1.2 | 25.4 ± 1.8 |
| Arroz da Terra   | 40.7 ± 4.7 | 14.5 ± 0.9 | 11.5 ± 1.9 |
| S-201            | 44.4 ± 9.3 | 13.5 ± 2.8 | 13.3 ± 3.1 |
| M-202            | 29.1 ± 1.4 | 13.7 ± 1.4 | 14.8 ± 1.5 |
| Calrose          | 27.0 ± 3.5 | 4.9 ± 1.8  | 4.1 ± 0.8  |
| Haenuki          | 34.0 ± 1.7 | 13.4 ± 0.6 | 11.7 ± 1.2 |
| Fukuhibiki       | 34.5 ± 0.3 | 16.5 ± 3.4 | 12.9 ± 1.8 |
| Akitakomachi     | 35.5 ± 4.4 | 11.4 ± 0.8 | 9.2 ± 1.1  |
| L-202            | 16.5 ± 3.0 | 5.8 ± 0.8  | 7.4 ± 0.7  |

Correlation with coleoptile length: 0.851** 0.710* 0.507
Correlation with sucrose synthase activity: 0.619** 0.498 0.433
Correlation with α-amylase activity: 0.622 0.817** 0.703*

Sugar contents were measured 4 days after germination. Values are the average of three independent replicates with standard deviations. Correlations with coleoptile length, sucrose synthase activity and α-amylase activity in whole seedlings (including shoots and endosperms, plotted in Table 1) were analyzed. ** and * indicate significant correlations at the 1% and 5% levels, respectively.
α-glucosidase and glucoamylase, required to release 1 μM of p-nitrophenol from blocked p-nitrophenyl maltoheptaoside in 1 min.

5. Analysis of sucrose, glucose and fructose content

Ten shoots (including coleoptiles, embryos and scutella) were ground in an ice-cooled glass mortar with 2 mL of extraction buffer, as described above. After centrifugation at 10,000 x g for 10 min, the supernatant was heated in a water bath at 80°C for 15 min to inactivate the enzymes. The sucrose, glucose and fructose contents were analyzed using an F-kit.

Results

1. Relationships between enzyme activities and coleoptile length under submerged conditions

The relationship between the coleoptile length and enzyme activities under submerged conditions were analyzed. Because of the environments around harvests might affect the enzyme activities and growth rate of seeds, the seeds harvested in 2002, 2003 and 2004 were examined (Table 1) to analyze whether or not the correlations between coleoptile length and enzyme activities differed among the seeds harvested in different years. The differences in coleoptile length among cultivars were analyzed in the agarose bed experiment. At the time of coleoptile measurement under submerged conditions on the agarose bed, the first leaf had not emerged, and no coleoptiles had reached their final length in any cultivar, but the lengths of the coleoptiles differed among cultivars (Table 1). When seedlings were incubated under aerobic conditions, coleoptile length was too short to measure (data not shown). Activities of sucrose synthase and α-amylase in shoots (including coleoptiles and embryos) and endosperms were analyzed for each cultivar (Table 1). Sucrose synthase activity was higher in the shoot than in the endosperm (Table 1). There was a significant correlation between coleoptile length and sucrose synthase activity in the shoots from the seeds harvested in all three years (Table 1). No significant correlation was found between α-amylase activity of the shoot or the endosperm and coleoptile length (Table 1). The relationships between the enzyme activities of whole seedlings (including shoots and endosperms) and coleoptile length were also analyzed (Figs. 1 and 2). Sucrose synthase activity in whole seedlings was significantly correlated with coleoptile length for seeds harvested in all three years (Fig. 1). The activity of α-amylase in whole seedlings
was significantly correlated with coleoptile length only for seeds harvested in 2002 (Fig. 2A), but not for seeds harvested in 2003 or 2004 (Fig. 2B and C).

2. Relationship among sugar content, coleoptile length and enzyme activity under submerged conditions

Sucrose, glucose and fructose contents of shoots were analyzed under submerged conditions. To analyze whether the relationships among sugar contents, coleoptile length and enzyme activities differed among the seeds harvested in different years or not, the seeds harvested in 2002, 2003 and 2004 were examined (Table 2). The content of sugars varied with the cultivar (Table 2). The coleoptile length significantly correlated with the sucrose content and glucose content for seeds harvested in all three years (Table 2). Fructose content was significantly correlated with coleoptile length only for seeds harvested in 2004 (Table 2C), but not for seeds harvested in 2002 or 2003 (Table 2A and B). There was also a significant correlation between sucrose content and sucrose synthase activity for seeds harvested in all three years (Table 2). Glucose and fructose contents were significantly correlated with sucrose synthase activity for seeds harvested in 2002 and 2004 (Table 2A and C), but not for seeds harvested in 2003 (Table 2B). Sucrose content did not show a significant correlation with α-amylase activity (Table 2). Glucose and fructose
content had significant correlation with \( \alpha \)-amylase activity in the seeds harvested in 2003 (Table 2B), but not in those harvested in 2002 and 2004 (Table 2A and C).

3. Sucrose synthase activity, \( \alpha \)-amylase activity and sugar contents of shoots under aerobic and submerged conditions

The sucrose synthase and \( \alpha \)-amylase activity in shoots were analyzed under aerobic and submerged conditions (Fig. 3A and B). To analyze the difference among cultivars, the seeds of five long-coleoptile cultivars, Arroz da Terra, Calrose, Fukuhibiki, Haenuki and S-201, and a short-coleoptile cultivar, Blue Bonnet (Table 1), were used. Blue Bonnet had less sucrose synthase activity than the other cultivars (Fig. 3A). Haenuki and Fukuhibiki had higher \( \alpha \)-amylase activities (Fig. 3B). Two-way analysis of variance conducted between cultivars and treatment (aerobic or submerged) showed that sucrose synthase activity under submerged conditions was significantly higher than that under aerobic conditions (Fig. 3A). \( \alpha \)-Amylase activity was significantly lower in shoots grown under submerged than in aerobic conditions (Fig. 3B).

The contents of sucrose, glucose and fructose in the shoots of six cultivars under aerobic and submerged conditions were analyzed (Fig. 4). Blue Bonnet had less sucrose, glucose and fructose than other cultivars (Fig. 4). Under submerged conditions, cultivar differences in glucose and fructose contents were enhanced (Fig. 4B and C). Two-way analysis of variance between cultivars and treatment (aerobic or submerged) showed that sucrose contents were significantly lower under submerged conditions (Fig. 4A), while glucose and fructose contents were significantly higher under submerged conditions (Fig. 4B and C).

4. Sucrose synthase activity with time under aerobic and submerged conditions

Time course of sucrose synthase activities in shoots were analyzed under aerobic and submerged conditions (Fig. 5). To analyze whether or not the long- and short coleoptile cultivars showed different tendency, we used the cultivars that had long coleoptiles, Italica Livorno and Arroz da Terra, and short coleoptiles, Bluebell and Blue Bonnet (Table 1) for the experiments. Sucrose synthase activity per shoot increased both under aerobic and submerged conditions (Fig. 5). Italica Livorno and Arroz da Terra had higher sucrose synthase activity than Bluebell and Blue Bonnet both under aerobic and submerged conditions (Fig. 5).

Were the different sucrose synthase activities among cultivars caused only by the amount of total proteins in shoots or not? To answer this question, we analyzed the sucrose synthase activities per mg protein under aerobic and submerged conditions (Fig. 6). Sucrose synthase activity per mg protein increased under submerged conditions in all cultivars from 1 to 3 days after germination (Fig. 6B). Under submerged conditions, Italica Livorno and Arroz da Terra had higher sucrose synthase activity per mg protein than Bluebell and Blue Bonnet (Fig. 6B). Under aerobic conditions, sucrose synthase activity per mg protein decreased in Italica Livorno and Arroz da Terra (Fig. 6A). Seven days after germination, all cultivars had similar sucrose synthase activity per mg protein under aerobic conditions (Fig. 6A), but different activities under submerged conditions (Fig. 6B).
Discussion

Analysis of the physiology and metabolism of rice plants in relation to their tolerance to submergence is needed for breeding to improve seedling establishment. In this study, we analyzed the relationship among the activities of the hypoxic enzyme sucrose synthase, and α-amylase, sugar content, and coleoptile length of rice cultivars under submerged conditions. The rice cultivars examined had different sucrose synthase activity under submerged conditions (Table 1), and sucrose synthase activity was strongly correlated with coleoptile length for seeds harvested in all three years (Fig. 1), whereas α-amylase activity was not (Fig. 2).

The question then was whether the difference in sucrose synthase activity among cultivars was the result or the cause of the different rates of coleoptile elongation. Sucrose synthase activity per mg protein in the long-coleoptile cultivars, Italica Livorno and Arroz da Terra, was higher than that in the short-coleoptile cultivars, Bluebell and Blue Bonnet, under submerged conditions (Fig. 6B). This suggests that the differences in sucrose synthase activity were not just caused by the total amount of protein. The long-coleoptile cultivars may have a better system to enhance sucrose synthase activity per mg protein than short-coleoptile cultivars under submerged conditions, and sucrose synthase activity may be important for metabolism and coleoptile growth under submerged conditions.

Sucrose synthase cleaves sucrose more efficiently than invertase requiring less ATP (Guglielminetti et al., 1995; Bologna et al., 2003). Under submerged, low-oxygen conditions, plants face serious obstacles to energy production. Rice cultivars with higher sucrose synthase activity may use energy more efficiently, leading to enhanced coleoptile growth. Pondweed (Potamogeton distinctus), a hypoxia-tolerant plant, increases transcription of sucrose synthase in elongating turions under oxygen deficiency (Harada et al., 2005). Sucrose synthase also produces UDP-glucose needed for cellulose synthesis (Haigler et al., 2001), which could result in enhanced coleoptile elongation.

A possible reason for the difference in sucrose synthase activity among cultivars may be the amount of sucrose in shoots. Sucrose content of shoots was highly correlated with sucrose synthase activity and coleoptile length for seeds harvested in all three years (Table 2). The transcription of two rice sucrose synthase genes, RSus1 and RSus3, is enhanced by feeding with sucrose (Huang et al., 1996). In our study, a larger supply of sucrose might also have caused the increase in sucrose synthase activity leading to enhanced coleoptile growth.

What causes the differences among cultivars in sucrose content of shoots? Sucrose is synthesized in the scutellum by sucrose phosphate synthase (Nomura et al., 1969; Chávez-Bárcenas et al., 2000) and transported to the embryo and coleoptile through the phloem. The activity of sucrose synthesis in the scutellum and the sucrose loading to the phloem may affect sucrose content of the shoot. However, activity of α-amylase, which digests starch in seeds and supplies glucose for synthesis of sucrose in the scutellum, was not correlated with sucrose content of the shoot (Table 2). Under submerged conditions, α-amylase activity is unlikely the limiting factor in sucrose supply and coleoptile elongation.

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