Changes in Photosynthetic Activity and Export of Carbon by Overexpressing a Maize Sucrose-Phosphate Synthase Gene under Elevated CO₂ in Transgenic Rice

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Abbreviations: [CO₂], CO₂ partial pressure; SPS, sucrose-phosphate synthase.

High carbohydrate accumulation and suppression of photosynthesis have been observed in the leaves of several plants grown under elevated CO₂ partial pressure ([CO₂]) (Sasek et al., 1985; Peet et al., 1986; Yelle et al., 1989; Wong, 1990; Xu et al., 1994). These acclimation responses of plants to elevated [CO₂] may be determined by the rate of sucrose synthesis in the cytoplasm and the subsequent export of sucrose from leaves to sink organs.

Sucrose-phosphate synthase (SPS) is a key enzyme in the sucrose synthesis pathway (Huber and Huber, 1996). The sucrose/starch ratio, which provides an indicator of carbon partitioning, correlated positively with the maximum SPS activity in transgenic rice (Oryza sativa L.) plants containing a maize gene (Zea mays L.) SPS (Ono et al., 1999). A positive correlation was also observed in previous studies in which the maize SPS gene was overexpressed in tomato (Lycopersicon esculentum L.) plants (Caltier et al., 1995). Murchie et al. (1999) mentioned that the difference in the carbohydrate export was only marginal and that the export capacity was not strictly related to the rate of sucrose synthesis. Photosynthetic activity was no significantly different between transgenic and wild-type Arabidopsis plants under ambient [CO₂]. After 10 weeks of CO₂ enrichment, photosynthetic activity decreased in the wild-type plants but not in the transgenic plants (Signora et al., 1998). Tomato and Arabidopsis accumulate starch in their leaves and are thus called starch-formers. In comparison, rice accumulates less starch in its leaf blades and is called a sucrose-former. Overexpression of SPS could have different effects on carbon utilization between these two groups. Under elevated [CO₂], starch accumulates not only in leaf sheaths but also in leaf blades in rice, especially when the N supply to plants is limited.

Abstract: To investigate whether increased sucrose-phosphate synthase (SPS) activity alters photosynthetic activity and/or the export of carbon from leaves under elevated CO₂ partial pressure ([CO₂]), we raised two lines of transgenic rice (H54-9 and H69-7), each overexpressing a maize SPS gene, and wild-type rice under ambient [CO₂] (35 Pa) and elevated [CO₂] (100 Pa). Under ambient [CO₂], no significant difference was observed between the transgenic and wild-type plants in the levels of sucrose or starch in leaves or the photosynthetic activity; but the carbon export rate was higher in H69-7 than in the wild-type. Under elevated [CO₂], SPS activity increased in all plants, but the accumulation of starch was significantly repressed in H54-9, whose SPS activity was about 12.5 times higher than that of the wild-type. The carbon export rate was higher in both transgenic lines than the wild-type. We considered that increased SPS activity in rice plants would promote the export of carbon from leaves and, as a result, starch accumulation in the leaves would be suppressed and/or photosynthetic activity would be promoted under elevated [CO₂].

Key words: Elevated CO₂, Export of carbon, Rice, Sucrose-phosphate synthase, Transgenic plant.

High carbohydrate accumulation and suppression of photosynthesis have been observed in the leaves of several plants grown under elevated CO₂ partial pressure ([CO₂]) (Sasek et al., 1985; Peet et al., 1986; Yelle et al., 1989; Wong, 1990; Xu et al., 1994). These acclimation responses of plants to elevated [CO₂] may be determined by the rate of sucrose synthesis in the cytoplasm and the subsequent export of sucrose from leaves to sink organs.

Sucrose-phosphate synthase (SPS) is a key enzyme in the sucrose synthesis pathway (Huber and Huber, 1996). The sucrose/starch ratio, which provides an indicator of carbon partitioning, correlated positively with the maximum SPS activity in transgenic rice (Oryza sativa L.) plants containing a maize gene (Zea mays L.) SPS gene (Ono et al., 1999). Such a positive correlation was also observed in previous studies in which the maize SPS gene was overexpressed in tomato (Lycopersicon esculentum L.) plants (Caltier et al., 1995; Murchie et al., 1999) and in Arabidopsis thaliana (L.) Heynh. plants (Signora et al., 1998). However, the difference in the sucrose/starch ratio between the transgenic and wild-type in rice plants was smaller than that in tomato or Arabidopsis (Ono et al., 1999).

Photosynthates are transported mainly in the form of sucrose; therefore, the rate of sucrose synthesis may be an important factor determining the translocation of photosynthates. Under ambient [CO₂], the estimated export of carbohydrate from source leaves did not differ between the wild-type and transgenic plants overexpressing a maize SPS gene in tomato (Galtier et al., 1993). Even under elevated [CO₂], the high SPS transgenic tomato plants showed no significant increase in photosynthetic activity and only a slight (5%) increase in the rates of carbohydrate export (Murchie et al., 1999). Murchie et al. (1999) mentioned that the difference in the carbohydrate export was only marginal and that export capacity was not strictly related to the rate of sucrose synthesis. Photosynthetic activity was no significantly different between transgenic and wild-type Arabidopsis plants under ambient [CO₂]. After 10 weeks of CO₂ enrichment, photosynthetic activity decreased in the wild-type plants but not in the transgenic plants (Signora et al., 1998). Tomato and Arabidopsis accumulate starch in their leaves and are thus called starch-formers. In comparison, rice accumulates less starch in its leaf blades and is called a sucrose-former. Overexpression of SPS could have different effects on carbon utilization between these two groups. Under elevated [CO₂], starch accumulates not only in leaf sheaths but also in leaf blades in rice, especially when the N supply to plants is limited.
When rice plants are grown under elevated $[CO_2]$, the consequent higher SPS activity is likely to have a larger effect on carbon partitioning than under ambient $[CO_2]$. Increased SPS activities (maximal or activation state) were observed in rice plants grown under elevated $[CO_2]$ (Seneweera et al., 1995; Hussain et al., 1999). Selective SPS activity and maximal SPS activity in plants grown under elevated $[CO_2]$ were 20% and 12% higher, respectively, than those grown under ambient $[CO_2]$ (Hussain et al., 1999). Hussain et al. (1999) concluded that their data were consistent with the hypothesis that up-regulation of leaf SPS may be an acclimation response of rice to optimize the utilization and export of photosynthates with increased rates of $CO_2$ fixation.

To investigate whether the suppression of photosynthesis under elevated $[CO_2]$ would be eliminated in transgenic rice overexpressing a maize SPS gene, we grew transgenic and wild-type rice plants under ambient $[CO_2]$ (35 Pa) and under elevated $[CO_2]$ (100 Pa), then we investigated the levels and export rate of carbon. To our knowledge, this is the first attempt to analyze the effect of enhanced SPS activity on carbon metabolism in rice, a sucrose-former, under elevated $[CO_2]$.

Materials and Methods

1. Plant materials

We used transgenic rice plants (Oryza sativa L. cv. Nipponbare) overexpressing a maize sucrose-phosphate synthase (EC 2.4.1.14; SPS) gene under the control of the promoter of the gene (cab) encoding chlorophyll a/b-binding protein in rice (Ono et al., 1999), and wild-type plants (control). From among the T3 plants, we selected two transgenic plants (H54-9, H69-7) that had the maize SPS protein and higher SPS activity than that of the wild-type rice. Seedlings (T3 plants and wild-type plants) were grown in artificially illuminated growth chambers that were controlled at an irradiance of 400 μmol quanta m$^{-2}$ s$^{-1}$, temperature 25°C, and 60% relative humidity. Seedlings were grown under 35 Pa $CO_2$ (ambient $[CO_2]$) and 100 Pa $CO_2$ (elevated $[CO_2]$) from the time immediately after germination. For the elevated $[CO_2]$ treatment, $CO_2$ was injected into the uppermost fully expanded leaves on each of four tillers per plant. Each pot was covered with a transparent polyvinyl chloride bag in which 3 mL of 7.3 M phosphoric acid was added to 100 mg $Ba^{13}CO_3$ to generate $^{13}CO_2$. The leaves absorbed $^{13}CO_2$ photosynthetically for 90 min. One leaf was sampled from each plant immediately, 1d, 3d and 7d after feeding (7.5-8.5 hr light). Leaves were frozen in liquid $N_2$ and weighed. Small portions in the center of leaves were ground completely. Then 200–500 μg of the dried samples were used for $^{13}C$ analysis. The carbon and $^{13}C$ contents were determined with an elemental analyzer (NC2500, CE Instruments, Milano, Italy) and a mass spectrometer (Delta Plus system, Finnigan MAT, Bremen, Germany). The labeled $^{13}C$ content (g) was calculated according to the following equation:

Labeled $^{13}C$ content = (total carbon atom content) $\times$ ($^{13}C$ atom % excess) $\times 13 \times 10^{-2}$, where total carbon atom content (mol) is the sum of numbers of moles for $^{12}C$ and $^{13}C$, and $^{13}C$ atom % excess (10$^2$ mol mol$^{-1}$) is the difference between the values of ($^{12}C$/$^{13}C$+$^{12}C$) in plants fed $^{13}CO_2$ and plants not fed $^{13}CO_2$.

2. Carbohydrate analysis

The uppermost fully expanded leaves were sampled at 6:00 p.m. for the measurements of photosynthetic rate and to estimate the maximum accumulation of photosynthates. Starch and sucrose were extracted as described previously (Ono et al., 1999) and measured with commercial kits (Starch No. 207748, Sucrose/D-glucose No. 139041, Boehringer Mannheim GmbH, Mannheim, Germany).

3. Gas-exchange measurement

The $CO_2$ assimilation rate was measured with a portable gas exchange system (LI-6400; LICOR Inc., Lincoln, NE, USA). Measurements were made on intact, uppermost fully expanded leaves from 9:00 a.m. to noon. When photosynthetic activity would be maximal. Light was provided by an LED source (red/blue, 6400-02 LED source; Li-Cor Inc., Lincoln NE, USA). Photosynthetic activity was measured under both growth conditions (photo flux density 400 μmol quanta m$^{-2}$ s$^{-1}$, leaf temperature 25°C, and reference $[CO_2]$ of 35 or 100 Pa).

4. Feeding of $^{13}C$ and sampling

To investigate the rates of carbon export, we covered plants with aluminum foil except for the uppermost fully expanded leaves on each of four tillers per plant. Each pot was covered with a transparent polyvinyl chloride bag in which 3 mL of 7.3 M phosphoric acid was added to 100 mg $Ba^{13}CO_3$ to generate $^{13}CO_2$. The leaves absorbed $^{13}CO_2$ photosynthetically for 90 min. One leaf was sampled from each plant immediately, 1d, 3d and 7d after feeding (7.5-8.5 hr light). Leaves were frozen in liquid $N_2$ and weighed. Small portions in the center of leaves were ground completely. Then 200–500 μg of the dried samples were used for $^{13}C$ analysis. The carbon and $^{13}C$ contents were determined with an elemental analyzer (NC2500, CE Instruments, Milano, Italy) and a mass spectrometer (Delta Plus system, Finnigan MAT, Bremen, Germany). The labeled $^{13}C$ content (g) was calculated according to the following equation:

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5. Measurements of SPS activity

SPS activities were measured in the leaves sampled at 1 d after $^{13}C$ feeding, according to the method of Lunn and Hatch (1997) with a slight modification (Ono et al., 1999). Maximal SPS activity was calculated as described...
by Ono et al. (1999).

6. Statistical analyses

Differences in the photosynthetic rates and the amounts of carbohydrates between the transgenic and wild-type plants under the two CO₂ partial pressures were tested with a two-way ANOVA by using the Statistix 7 statistical software package (Analytical Software, Tallahassee, FL, USA). Two main factors: CO₂ treatment and SPS overexpression, and their interaction were tested against the random variation between the plants, and the combination means were compared with each other by using Tukey's method based on the Studentized range statistic at the rejection level of 0.05.

Results

1. SPS activity

Under ambient [CO₂] condition, maximum SPS activity in the transgenic rice was about 6.3 times (H54-9) and 8.6 times (H69-7) higher than that in the wild-type rice (Table 1). Each line had a higher SPS activity under elevated [CO₂] than under ambient [CO₂]. The SPS activity of the wild-type rice under elevated [CO₂] was about twice as high as that under ambient [CO₂]. The SPS activities in the transgenic lines H54-9 and H69-7 were about 12.5 times and 8.2 times that of wild-type rice under elevated [CO₂].

2. Photosynthetic rates

The elevation of [CO₂] significantly increased photosynthetic rates in all lines (P < 0.001). The photosynthetic rate under ambient [CO₂] was not significantly different between transgenic (high SPS rice) and wild-type rice (Fig. 1), whereas the photosynthetic rate under elevated [CO₂] was significantly higher in the high SPS rice H54-9 than in the wild-type rice (Fig. 1).

Chlorophyll content on a leaf area basis did not differ significantly among wild-type rice and the two high SPS rice lines (data not shown).

3. Levels of photosynthates

Levels of photosynthates were expressed on a fresh weight basis. Under ambient [CO₂] the sucrose level was higher than the starch level in all lines, but no significant difference between high SPS rice and wild-type rice was observed in the sucrose level (Fig. 2b). Under elevated [CO₂], starch level in the leaves of wild-type rice; the starch level was significantly higher than under ambient [CO₂] (Fig. 2a). On the other hand, in high SPS rice, the starch level did not increase under elevated [CO₂], and the starch level in H54-9 was about half that in the wild-type rice (Fig. 2a). The sucrose level did not differ significantly between high SPS rice and
and wild-type rice under either elevated or ambient [CO$_2$] (Fig. 2b). The only significant difference was observed only between the wild-type plants under ambient [CO$_2$] and H54-9 plants under elevated [CO$_2$].

4. Rates of carbon export

The $^{13}$C content in the fed leaf decreased rapidly after feeding (Fig. 3), showing that most of the $^{13}$C exported to other leaves or was lost by respiration. On 1 d after the $^{13}$C feeding under ambient [CO$_2$], about 40% of $^{13}$C was retained in the fed leaves in the wild-type rice and the high SPS rice H54-9 but only 27% in the high SPS rice H69-7 (Fig. 3a). About 10% of the $^{13}$C remained in the fed leaves in all lines on day 3, and the relative amount of $^{13}$C retained in the fed leaves had not changed significantly in any line until day 7. Under elevated [CO$_2$], although 35% of the $^{13}$C was retained in the fed leaf of wild-type rice on day 1, only 24% and 26% of $^{13}$C was retained in the fed leaves of high SPS rices H54-9 and H69-7, respectively, on day 1 (Fig. 3b). By day 3 after the feeding, the retained $^{13}$C had decreased to 15% in wild-type rice and high SPS rice H69-7 and to 9% in high SPS rice H54-9. On day 7, about 10% of the $^{13}$C remained in the fed leaves of wild-type rice and high SPS rice H69-4 and 7% in those of high SPS rice H54-9.

Discussion

In the high SPS rice H54-9, the rate of carbon export was higher (Fig. 3b), starch accumulation was lower (Fig. 2a) and photosynthetic activity (Fig. 1) was higher under elevated [CO$_2$] than under ambient [CO$_2$]. Under elevated [CO$_2$], the production of photosynthates increases because of the increase in CO$_2$ available for assimilation. Rice plants adapt to the increased production of photosynthates by increasing SPS activity and this is expected to be closely related to the export of photosynthates. Seneweera et al. (1995) and Hussain et al. (1999) observed increased SPS activity under elevated [CO$_2$]. We too observed an increase in SPS activity under elevated [CO$_2$], not only in wild-type rice but also in high SPS rice (Table 1). Since the accumulation of photosynthates could reduce photosynthetic activity (e.g. Neals and Incoll, 1968), we consider that the increased export of photosynthates, in turn, should increase photosynthetic activity in high SPS rice.

We do not know why the maximal SPS activity in our transgenic rice was higher under elevated [CO$_2$] than under ambient [CO$_2$]. We used the cab promoter of rice to overexpress a maize SPS gene. Sheen (1990) reported that the photosynthetic products, sucrose and glucose, repressed the transcriptional activity of the maize cab promoter. In this study, sucrose levels in leaves tended to decrease rather than increase under elevated [CO$_2$] (Figs. 2b). These results suggested that the expression of the maize SPS might not be repressed at the transcriptional level and cause higher SPS activity under elevated [CO$_2$].

In transgenic tomato overexpressing a maize SPS gene and wild-type tomato under ambient [CO$_2$], no significant difference was observed in estimated carbohydrate export from the source leaves (Galtier et al., 1995), and under elevated [CO$_2$], the export rate was only slightly (5%) higher in transgenic tomato (Murchie et al.,
Table 1. Maximal SPS activity in the most recently fully expanded leaves of transgenic rice overexpressing a maize SPS gene and of wild-type rice. Plants were grown under ambient [CO$_2$] (35 Pa) or elevated [CO$_2$] (100 Pa).

| Plant type | Maximal SPS activity (µmol mg$^{-1}$ chlorophyll h$^{-1}$) |
|------------|----------------------------------------------------------|
|            | Ambient [CO$_2$]                        | Elevated [CO$_2$]                         |
| Wild-type  | 50.3±4.2                                          | 97.5±14.0                                    |
| H54-9      | 317.0±45.5                                        | 1216.8±178.7                                  |
| H69-7      | 431.3±30.2                                        | 804.0±169.8                                   |

Values are means and standard errors of three plants for wild-type rice and six plants for high SPS rice under ambient [CO$_2$], and of three plants (one leaf of each plant) for wild-type rice and high SPS rice under elevated [CO$_2$].

1999). Although the reported methods of estimating the export of photosynthates were different from our method, the effect of overexpressing a maize SPS on export of photosynthates was larger in rice than in tomato. Tomato accumulates starch in source leaves even under ambient [CO$_2$] (Worrell et al., 1991; Galtier et al., 1995; Murchie et al., 1999). In tomato, starch might accumulate to a level that exceeds the demand, and other regulatory mechanisms, such as activity of the sucrose transporter, might counteract the advantage of the high SPS activity under elevated [CO$_2$], as mentioned by Murchie et al. (1999). On the other hand, in rice, which is a sucrose-former, it is possible that the export of sucrose from leaf blades is closely linked to the activity of sucrose synthesis when the development of sink in the plant is not limited. The extents of photosynthetic carbon metabolism and the enzyme activities including sucrose metabolism under elevated [CO$_2$] are different among species (Moore et al., 1998). These results suggested that the extent of increased SPS activity on carbon metabolism might differ depending on species, especially the kind of carbohydrates accumulated in leaves.

We observed a positive correlation between maximal SPS activity and sucrose/starch ratio in our previous study (Ono et al., 1999). However, we did not observe a significant difference in either sucrose or starch content between wild-type and high SPS rice under ambient [CO$_2$] (Figs. 2a, b) in this study. In the previous study, the flag leaves in the reproductive stage of the plants grown in soil culture were used (Ono et al., 1999), but in this study, we used the uppermost fully expanded leaves in the vegetative stage of the plants grown hydroponically. Our group proposed that the effects of elevated [CO$_2$] on carbon metabolism might vary depending on the growth stage in rice plants (Aoki et al., 2003) and the growth condition mainly determines the characters of plants. Probably, the differences in growth condition and/or stage might cause the difference in results between this and previous study.

Preliminary experiments showed that the development of panicles was more rapid in the high SPS rice than in the wild-type (data not shown). We did not compare the final dry mass and yield in high SPS rice with those in wild-type rice. Further study is necessary to investigate whether or not the yield in high SPS rice increases under elevated [CO$_2$].

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References

Aoki, N., Ono, K., Sasaki, H., Seneweera, S.P., Sakai, H., Kobayashi, K. and Ishimaru, K. 2003. Effects of elevated CO$_2$ concentration on photosynthetic carbon metabolism in flag-leaf blades of rice before and after heading. Plant Prod. Sci. 6: 52-58.

Galtier, N., Foyer, C.H., Huber, J., Voelker, T.A. and Huber, S.C. 1993. Effects of elevated sucrose-phosphate synthase activity on photosynthesis, assimilate partitioning, and growth in tomato (Lycopersicon esculentum var UC222B). Plant Physiol. 101: 535-543.

Galtier, N., Foyer, C.H., Murchie, E., Aldred, R., Quick, P., Voelker, T.A., Thepenier C., Lasceve, G. and Betche, T. 1995. Effects of light and atmospheric carbon dioxide enrichment on photosynthesis and carbon partitioning in the leaves of tomato (Lycopersicon esculentum var.) plants over-expressing sucrose phosphate synthase. J. Exp. Bot. 46: 1333-1344.

Huber, S.C. and Huber, J.L. 1996. Role and regulation of sucrose-phosphate synthase in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 431-444.

Hussain, M.W., Allen, L.H. Jr. and Bowes, G. 1999. Up-regulation of sucrose phosphate synthase in rice grown under elevated CO$_2$ and temperature. Photosyn. Res. 60: 199-208.
Lunn, J.E. and Hatch, M.D. 1997. The role of sucrose–phosphate synthase in the control of photosynthetic partitioning in *Zea mays* leaves. Aust. J. Plant Physiol. 24: 1-8.

Makino, A., Mac, T. and Ohira, K. 1988. Differences between wheat and rice in the enzyme properties of ribulose–1,5-bisphosphate carboxylase/oxygenase and the relationship to photosynthetic gas exchange. Planta 174: 30-38.

Moore, B.D., Cheng, S.-H., Rice, J. and Seemann, J.R. 1998. Sucrose cycling, rubisco expression, and prediction of photosynthetic acclimation to elevated atmospheric CO₂. Plant Cell Environ. 21: 905-915.

Murchie, E.H., Sarrobert, C., Contard, P., Betsche, T., Foyer, C.H. and Galtier, N. 1999. Overexpression of sucrose–phosphate synthase in tomato plants grown with CO₂ enrichment leads to decreased foliar carbohydrate accumulation relative to untransformed controls. Plant Physiol. Biochem. 37: 251-260.

Nakano, H., Makino, A. and Mac, T. 1997. The effects of partial pressures of CO₂ on the relationship between photosynthetic capacity and N content in rice leaves. Plant Physiol. 115: 191-196.

Neals, T.F. and Incoll, L.D. 1968. The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: a review of the hypothesis. Bot. Rev. 34: 107-125.

Ono, K., Ishimaru, K., Aoki, N., Takahashi, S., Ozawa, K., Ohkawa, Y. and Ohtani, R. 1999. Characterization of a maize sucrose–phosphate synthase protein and its effect on carbon partitioning in transgenic rice plants. Plant Prod. Sci. 2: 172-177.

Peet, M.M., Huber, S.C. and Patterson, D.T. 1986. Acclimation to high CO₂ in monococious cucumbers. II. Carbon exchange rates, enzyme activities, and starch and nutrient concentrations. Plant Physiol. 80: 63-67.

Sasek, T.W., Delucia, E.H., Strain, B.R. 1985. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO₂ concentrations. Plant Physiol. 78: 619-622.

Seneweera, S.P., Barra, A.S., Barkow, E.W. and Comroy, J.P. 1995. Diurnal regulation of leaf blade elongation in rice by CO₂. Plant Physiol. 108: 1471-1477.

Shren, J. 1990. Metabolic repression of transcription of higher plants. Plant Cell 2: 1027-1038.

Signora, L., Galtier, N., Skot, L., Lucas, H. and Foyer, C.H. 1998. Overexpression of sucrose phosphate synthase in *Arabidopsis thaliana* results in increased foliar carbohydrate accumulation in plants after prolonged growth with CO₂ enrichment. J. Exp. Bot. 49: 669-680.

Wong, S.C. 1990. Elevated atmospheric partial pressure of CO₂ and plant growth. II. Nonstructural carbohydrate in cotton plants and its effect on growth parameters. Photosyn. Res. 23: 171-180.

Worrel, A.C., Bruneau, J.M., Summerfield, K., Boersig, M. and Voelker, T.A. 1991. Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. Plant Cell 3: 1121-1130.

Xu, D.-Q., Gifford, R.M. and Chow, W.S. 1994. Photosynthetic acclimation in pea and soybean to high atmospheric CO₂ partial pressure. Plant Physiol. 106: 661-671.

Yelle, S., Besson, R.C. Jr. and Trudel, M.J., Gosselin A. 1989. Acclimation of two tomato species to high atmospheric CO₂. I. Sugar and starch concentration. Plant Physiol. 90: 1465-1477.