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Relationship between Export Rate of Photoassimilates and Activation State of Sucrose Phosphate Synthase in Submerged Floating Rice*

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Abstract : Floating rice (*Oryza sativa* L.) responds to submergence by rapid internodal elongation. This stimulated growth needs an increase in the supply of photoassimilates. We investigated the effects of submergence on the export rate of photoassimilates determined by a ^{13}C-tracer experiment and the activity of sucrose phosphate synthase (SPS), a key enzyme in the sucrose biosynthesis pathway, in floating rice. Plants at the 9.5 leaf stage were submerged up to the tip of the 7th leaf blade for 5 days. Export rates of the ^{13}C-photoassimilates at the 9th (9L) and 10th (10L) leaf blades were much higher in the submerged plants than in the control. In order to analyze the activation state of SPS, the activities of SPS were assayed under saturated substrates (Vmax) and limiting substrates plus Pi (Vlimiting). The Vlimiting of SPS of the 9L and 10L were higher in the submerged plants than in the control, whereas the Vmax did not differ between both plots. These results indicate that the SPS of the leaves in the upper position was more highly activated in the submerged plants. Moreover, the Vlimiting of SPS was correlated positively with the export rate. These results suggest that an increase in the Vlimiting of SPS induced by a high activation state might accelerate the export rates of photoassimilates, and that this is probably one of the important factors that support the supply of photoassimilates required for the rapid growth of the sink organs in submerged floating rice.
Key words : ^{13}C, Export rate of photoassimilates, Floating rice, Sucrose phosphate synthase.

深水下における浮イネの光合成産物の転流速度とスクロースリン酸合成酵素の活性化状態との関係 : 平野達也・内田直次**・東　哲司・安田武司** (神戸大学大学院自然科学研究科, ** 神戸大学農学部)
要　旨 : 浮イネは水位の上昇に対して急速に節間を伸長させる．深水下での生長を支えるには，生長部位へより多くの光合成産物を供給することが必要であろう．本研究では，深水下における浮イネ葉身の光合成産物の供給能力を明らかにする目的で，^{13}Cを用いたトレーサー実験による光合成産物の転流速度とスクロース合成経路の鍵酵素であるスクロースリン酸合成酵素（SPS）の活性を調査した．9.5葉期の植物体を第7葉身がほぼ完全に水没する水深で5日間処理を行う深水区と通常の湛水条件で生育させる対照区を設けた．深水区の上位2葉（第9および第10葉身）からの^{13}Cラベル炭素の転流速度は，対照区より著しく高かった．SPS活性に関しては，飽和基質条件下での最大活性（Vmax）と，葉の生理的条件に近い濃度の基質と無機リン酸（活性抑制剤）を加えた条件下の活性（Vlimiting）の2種類の測定を行い，両活性の比較から活性化率を求めた．上位2葉におけるSPSのVmaxは両区でほぼ同じであるのに対し，Vlimitingは深水区が対照区よりも有意に高かった．すなわち，これらの葉身のSPSは深水下でより活性化されていた．また，SPSのVlimitingと^{13}Cの転流速度との間には，処理および葉位の違いを含めて高い正の相関があった．これらの結果から，深水下の浮イネは葉のSPSをより活性化させることにより葉からの光合成産物の転流速度を増加させ，シンク器官の急速な生長を支えているものと考えられる．
キーワード : 浮イネ，光合成産物の転流，^{13}C，スクロースリン酸合成酵素．

Floating or deepwater rice plants respond to submergence by rapid internodal elongation[26] and an acceleration of young leaf expansion[28]. These responses enable the plants to keep part of their shoots above water surface and continue to grow. The enhanced growth of submerged floating rice would need an increase in supply of photoassimilates from the leaves. As the adaptable potentials for supply of photoassimilates, leaves above the water surface in submerged plants have higher photosynthetic activity than those in the control plants[20,29]. In general, source activities in leaves consist of photosynthetic activity and translocating activity of photoassimilates. However, little information on export rate of photoassimilates has been obtained in source leaves of the submerged floating rice plants.

Photosynthetically fixed carbon is par-

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titioned into sucrose and starch in leaves of higher plants : Sucrose is exported to sink organs, whereas starch is stored transiently in chloroplasts. Partitioning of the photosynthetically fixed carbon between sucrose and starch can be altered by source-sink manipulation[7]. When portions of the source leaves are excised or shaded, sucrose content and export of photoassimilates in remaining source leaves increase in bean[1,4] and in soybean[21]. In contrast, when the demand for sucrose is decreased by girdling or fruit removal, photosynthetic rate in the source leaves is observed to decrease, and starch accumulation increases in soybean[2,21] and in *Capsicum annuum* L.[5]. Conversely, provision of leaf sucrose is considered to control the rate of translocation from source leaves[3,15,23]. Sucrose phosphate synthase (SPS) is a key enzyme in the sucrose biosynthesis pathway[11,12,16]. The activation state of SPS is known to be regulated by protein phosphorylation[14].

In previous reports, we showed that submergence increased the amount of photoassimilate partitioning to the uppermost internodes the non-emerged part of the growing leaves in floating rice plants[8,9], while it decreased the amount of partitioning to the lower internodes[9]. These alteration in the sink capacities to submergence may affect the translocating activity of the source leaves. In the presnt study, we examined concomitantly the export rate of photoassimilates with ^{13}C-tracer experiment and the activity of SPS in submerged floating rice plants and discussed the relationships between the export rate and SPS activity, especially the activation state. Our results indicate that increase in SPS activation state occurs in the leaf blade above the water surface in submerged floating rice, and that increase in the activity of SPS induced by a higher activation state relates closely to the enhancement of the export rete of labelled carbon.

Materials and Methods

1. Plant materials and submergence treatment

A floating rice variety from Bangladesh, Habiganj Aman II, was used. The plants were the same materials used in the previous report[9] and grown hydroponically outdoors until the 9.5 leaf stage. All the tillers were removed from the plants before submergence treatement. At the 9.5 leaf stage, plants were partially submerged outdoors up to the tip of the 7th leaf blade for five days. After five days of submergence, they were at the 10.3 leaf stage. The elongating uppermost internode (>10 mm) was the 9th internode throughout the experiment. Control plants were grown until the 10.3 leaf stage under ordinary conditions where all the leaves were aerially exposed. Some of the plants were used in a ^{13}C-tracer experiment, and the other plants were used to assay SPS activity and to determine sucrose and starch contents.

2. ^{13}C-tracer experiment

^{13}C-tracer experiment was carried out according to the previously reported method[9]. For two hours from 8 a.m., the ^{13}C-labelled carbon was fed to the submerged and control plants at the 10.3 leaf stage (CO_2 level at 430 ± 20 ppm with about 50% ^{13}C atom). Then, the submerged and control plants were harvested at 0, 6 and 21 hours after the end of $^{13}CO_2$ feeding. Harvested samples were separated immediately into the 7th leaf blade (7L), the 8th leaf blade (8L), the 9th leaf blade (9L), 10th leaf blade (10L) and remaining parts. The amount of labelled carbon in the samples was determined according to the previous report[8].

3. Estimation of the amount of exported labelled carbon

A photosynthesizing leaf gains labelled carbon by fixation and loses it by export as well as respiration[4]. In the present study, though the amount of labelled carbon lost by respiration in each leaf blade was not measured directly, the rate of labelled carbon lost by respiration in the whole plant can be estimated from the amount of labelled carbon that decreases in the whole plant for a certain period. In addition, the total amount of respiration in all leaf blades occupies 25% of that in the whole plant during the vegetative period[27], and little difference in activities of respiration between each fully expanded leaf blade is appreciable[23]. Therefore, the amount of labelled carbon lost by respiration in each leaf blade was estimated using the following equation :

$$E=\Delta\text{leaf}^{13}C-\Delta\text{total}^{13}C\times 0.25\times \text{leaf FW ratio},$$

where E is the amount of exported labelled

carbon in each leaf blade, $\Delta leaf^{13}C$ is the amount of labelled carbon lost in each leaf blade for 6 hours after the end of ^{13}C feeding, $\Delta total^{13}C$ is the amount of labelled carbon lost in the whole plant for 6 hours after the end of ^{13}C feeding and leaf FW ratio is the ratio of fresh weight of each leaf blade to total fresh weight of all leaf blades.

4. SPS assay

Each fully expanded leaf blades above the 6th leaf blade (7L～10L) in the submerged and control plants at the 10.3 leaf stage were harvested at 10 a.m. and frozen immediately in liquid nitrogen. The frozen sample (100 ～200 mg FW) was ground with a mortar and pestle in 1.0 mL of ice-cold 50 mM Mops-NaOH (pH7.0) buffer containing 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.5mg /mL BSA and 0.05% (v/v) Triton X-100. The homogenate was centrifuged at 12,000 ×g for 10 minutes (<4°C). The supernatant was immediately desalted by centrifugal filtration on a Sephadex G-25 (Pharamacia) column equilibrated with the grinding buffer minus EDTA and Triton X-100[6]. SPS activity was assayed under limiting (Vlimiting) and saturated (Vmax) substrate conditions according to method of Huber and Huber[13].

5. Determination of sucrose and starch contents

The frozen sample was ground with a mortar and pestle in 5 mL of 80% (v/v) ethanol. The homogenate was centrifuged at 3,000×g for 15 minutes. The pellet was further extracted with 5 mL of 80% (v/v) ethanol for 15 minutes at 80°C, and the mixture was centrifuged at 3,000×g for 15 minutes. The first and second supernatants were combined, and used to determine sucrose content, while the pellet was used to determine starch content[17].

Results

Figure 1 indicates the changes in the labelled carbon contents of each leaf blade. The immersed 7L of the submerged plants had much lower labelled carbon content than the 7L of the control. No significant differences between both plots were detected in the 8L. The contents of labelled carbon of the 9L and 10L in the submerged plants were higher than those in the control at the end of the ^{13}C

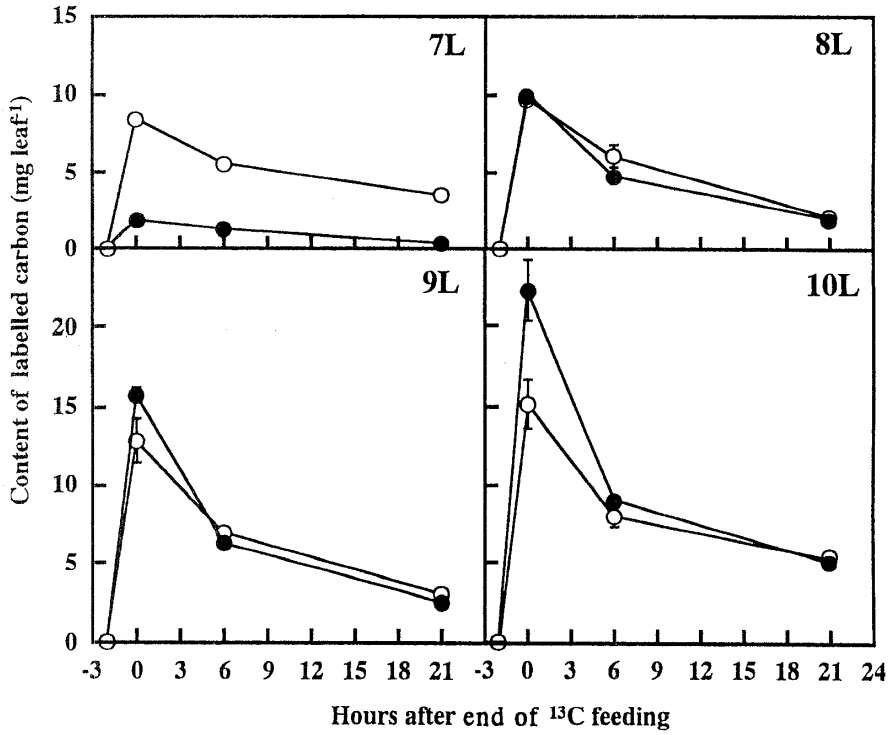

Fig. 1. Changes in content of labelled carbon in each leaf blade in floating rice. Submerged treatment (●) was carried out for 5 days with the 7th leaf blade immersed completely. Plants with all leaves aerially exposed were used as control (○). $^{13}CO_2$ was fed to both plants from 8 a.m. to 10 a.m. Both plants were harvested at 0, 6 and 21 hours after the end of ^{13}C feeding. Vertical bars express ±standard errors (n=5).

feeding (0 h) and then decreased rapidly to the level of the control for 6 hours. Export rate of labelled carbon in each leaf blade was calculated according to the equation described in Materials and Methods. The export rate at the 7L was lower in the submerged plants than in the control (Table 1). On the other hand, the export rates at the 9L and 10L increased significantly under submergence as compared with the control.

Starch and sucrose contents at the 7L were much lower in the submerged plants than in the control (Table 2). Differences between both plots were not appreciable in starch contents at the 8L, 9L and 10L. Sucrose contents at the 9L and 10L were higher in the submerged plants than in the control. Sucrose/starch rataio also increased significantly in the 9L and 10L under submergence as compared with the control.

Figure 2 indicates SPS activities assayed under two different reaction mixtures (Vlimiting and Vmax) in each leaf blade. Both activities at the 7L were reduced extremely by submergence. Vmax at the 8L, 9L and 10L in

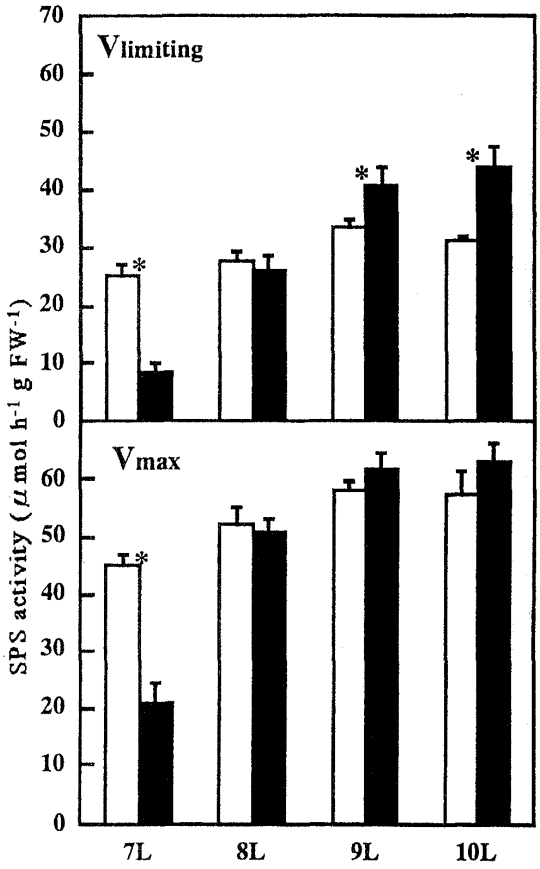

Fig. 2. Effect of submergence on Vmax and Vlimiting of SPS of each leaf blade in floating rice at the end of ^{13}C feeding. Submerged treatment (■) was carried out for 5 days with the 7th leaf blade immersed completely. Plants with all leaves aerially exposed were used as control (□). SPS was assayed using two different reaction mixtures, saturated substrate (Vmax) and limiting substrate plus Pi (Vlimiting) by the method of Huber et al. (1989). Vertical bars express + standard errors (n=3). * indicates significant difference at 1% level between control and submergence.

Table 1. Effects of submergence on export rate of labelled carbon in each leaf blade for 6 hours after ^{13}C feeding.

Treatment	Leaf position			
	7L	8L	9L	10L
	mg C h^{-1} g FW^{-1}			
Control	1.96	3.03	5.31	6.58
Submergence	0.10**	4.29	8.21**	12.11**

Data are expressed as the means of five replicates.
** ; significant at 1% level between control and submergence.

Table 2. Effects of submergence on sucrose and starch content of each leaf blade at the end of ^{13}C feeding.

	Treatment	Leaf position			
		7L	8L	9L	10L
Sucrose (mg g FW^{-1})	Control	28.6	38.0	42.1	43.9
	Submergence	5.3**	37.8	54.4*	54.0*
Starch (mg g FW^{-1})	Control	3.48	3.14	2.87	2.75
	Submergence	0.65**	3.27	2.94	2.55
Sucrose/starch	Control	8.2	12.1	14.6	16.0
	Submergence	8.2	11.6	18.5*	21.2**

Data are expressed as the means of five replicates.
*, ** ; significant at 5% and 1% levels between control and submergence, respectively.

the submerged plants were almost the same with those in the control. On the other hand, the 9L and 10L of the submerged plants had significantly higher Vlimiting than the control. SPS activation state as Vlimiting/Vmax increased int the upper two leaves (9L and 10L) while it decreased in the submerged 7L (Table 3).

Discussion

The upper two leaves had much higher export rate of photoassimilates in the submerged plants than in plants grown under ordinary conditions (Fig. 1, Table 1). These facts indicate the the fully expanded leaves of the upper position in the submerged plants have a high potential to supply the photosynthetically fixed carbon to the other sink organs. One of the important factors controlling the export rate of photoassimilates from source leaves in the rate of sucrose formation[3,15,23]. In fact, sucrose contents as well as sucrose/starch ratios of the 9L and 10L were higher in the submerged plants than in the control (Table 2), indicating that sucrose formation rates of the upper two leaves increase under submergence.

SPS is a key enzyme in the sucrose biosynthesis pathway[11,12,16]. In addition, SPS activity is known to be regulated by protein phosphorylation in many species[10,13,14]. Phosphrylated SPS (inactivated SPS) has a decreased affinity for glucose 6-phosphate (activator) and an increased affinity for Pi (inhibitor), so that the kinetic effect of phosphorylation is only apparent when SPS activity is assayed under more physiological conditions of limiting substrates in the presence of Pi rather than under saturated substrate conditions[24]. Thus, we investigated the effect of submergence on SPS activities under two different reaction mixtures, saturated substrates (Vmax) and limiting substrates plus Pi (Vlimiting).

The export of labelled carbon was correlated positively with the Vlimiting of SPS (Fig. 3). In addition, a correlation also existed between the Vmax of SPS and the export rate of labelled carbon ($r=0.817$, $p<0.01$). Therefore, it is unclear which, the Vlimiting or Vmax of SPS, relates more closely to the export rate of photoassimilates. However, no appreciable difference in the Vmax of SPS between the treatments was observed in the upper two leaves in which the export rate was accelerated by submergence (Fig. 2), while these leaves had a higher activation state of SPS in the submerged plants than in the control (Table 3). Consequently, the Vlimiting of SPS increased in the upper two leaves under submergence as compared to the control (Fig. 2). Therefore, we conclude that the increase in the Vlimiting of SPS resulting from the high activation state, rather than the

Table 3. Effects of submergence on SPS activation state (Vlimiting/Vmax) of each leaf blade.

Treatment	Leaf position			
	7L	8L	9L	10L
	%			
Control	55.1	53.0	57.8	54.6
Submergence	40.9**	51.0	65.9*	69.4**

Data are expressed as the means of three replicates.

*, ** ; significant at 5% and 1% levels between control and submergence, respectively.

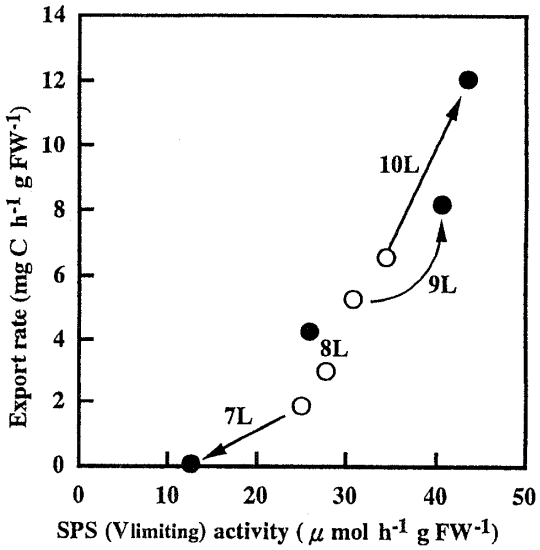

Fig. 3. Relationship between export rate and SPS Vlimiting activities of each leaf blade in floating rice. Submerged treatment (●) was carried out for 5 days with the 7th leaf blade immersed completely. Plants with all leaves aerially exposed were used as control (○). In the leaves except for the 8L, significant differences between both plots (represented by arrows) were detected in both Vlimiting of SPS and export rate. The correlation coefficient ($r=0.949$) is significant at 0.1% level.

Vmax of SPS, is one of the causative factors that promote the export rate of labelled carbon and sucrose/starch ratio of the upper leaves in submergd plants.

The contents of labelled carbon of the 9L and 10L were higher in the submerged plants than in the control at the end of the ^{13}C feeding (Fig. 1). This result indicates that the upper leaves in the submerged plants have higher activity of photosynthesis than those in the control, and correspond to the other reports[20,29]. This increased capacity of photosynthesis also might relate to the enhancement of export rate and sucrose contents of the 9L and 10L in the submerged plants. The rate of CO_2 assimilation in non-floating rice leaves under saturated irradiance and ambinet air conditions is limited by the ribulose-1,5-bisphosphate carboxylase/oxygenase capacity[18], not by sucrose synthesis enzymes such as SPS[19], while the activation state of SPS rises progressively as the rate of photosynthesis increases[24]. Therefore, the relationships between the Vlimiting of SPS and the photosynthetic capacity are unclear in this study. Further investigation of the relationships between the Vlimiting of SPS and the photosynthetic activity in submerged floating rice will be necessary.

Seneweera et al.[22] showed that high CO_2 concentration increased the elongation rate of expanding blades in rice plants, and that the elongation rate was closely correlated with Vlimiting of SPS in the uppermost expanded blade. Similarly, submergence induces the rapid elongation of uppermost internode[9], and thus in terms of the relationship between the increased growth in sink organs and the Vlimiting of SPS in source leaves, our results would support their report. Furthermore, submergence increases the amount of import of photoassimilates into the elongating uppermost internode and alters the partitioning patterns of photoassimilates to each sink organ[8,9,20]. In addition to their alterations in the sink capacities, the present results indicate that the increase in the Vlimiting of SPS probably plays an important role in the supply of photoassimilates to the rapidly growing sink organs in the submerged floating rice plants. This function may be at last one of the important factors that enable floating rice to adapt to submergence.

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