

## Effects of Source-to-Sink Ratio on Carbohydrate Production and Senescence of Rice Flag Leaves during the Ripening Period\*

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**Abstract** : Partial removal of spikelets and partial defoliation were conducted in rice at anthesis to evaluate the effects of the source-to-sink ratio (ratio of leaf area to grain number). The ratio was 0.75 in the control plants and ranged from 0.24 to 1.53 for the various treatments. The apparent photosynthetic rate was not influenced significantly by the treatments 12 days after anthesis. Contents of chlorophyll and Rubisco decreased substantially 3 weeks after anthesis, but remained relatively high when the sink size was reduced. Three weeks after anthesis, both the chlorophyll and Rubisco contents were positively correlated with the source-to-sink ratio ( $P < 0.01$ ). Therefore, it appears that a large sink size (i.e., smaller source-to-sink ratio) promotes flag leaf senescence. However, no significant differences in the enzyme activities related to sucrose synthesis were observed among the treatments. In spite of the large differences in sucrose and starch contents in leaf sheath and culm among the treatments, the trends of change in sucrose and starch contents in the flag leaf blades during the ripening period were similar. Thus, the ability of the flag leaves to synthesize sucrose was not greatly reduced during senescence. The nitrogen content of flag leaf blade was negatively correlated with sink size. These results suggest that the difference in leaf senescence under various source-to-sink ratios may be associated with the different rates of nitrogen withdrawal from leaf blades to grains.

**Key words** : Chlorophyll, Leaf senescence, Nitrogen, Photosynthesis, Rice, Rubisco, Sink size, Sucrose synthesis.

葉身および枝梗の部分切除によるソース/シンク比の変化が、登熟期の水稻止葉の物質生産、老化に及ぼす影響：和田義春・三浦邦夫・渡辺和之（宇都宮大学農学部）

**要 旨**：水稻コシヒカリを供試し、開花期に葉身および枝梗の部分切除を行って実験的にソース/シンク比を変化させ、登熟期の止葉の物質生産能力および老化に及ぼす影響を調査した。1 粒当りの開花期葉面積で評価したソース/シンク比は、無処理区で 0.75 であり、処理により 0.24 から 1.53 まで変化した。開花後 12 日目の止葉の光合成速度には処理間で有意な差はみられなかった。止葉のクロロフィル含量とリブコース 1, 5-2 リン酸カルボキシラーゼ/オキシゲナーゼ（ルビスコ）含量は、シンクを小とした区で高く保たれた。開花後 3 週目の各処理区の止葉のクロロフィル含量およびルビスコ含量とソース/シンク比との間には正の相関関係（ $P < 0.01$ ）がみられた。したがって、ソース/シンク比が小、すなわち相対的にシンクが大きいほど止葉の老化が促進されるものと判断された。一方、止葉中のショ糖合成に関与する酵素活性は、ソース/シンク比の違いにより有意な差を生じなかった。登熟期のショ糖およびデンプン含量の消長は、葉鞘＋稈では処理の影響が大であったが、止葉への影響は比較的小さかった。一方、止葉の窒素含量は、シンクが小の区ほど高く保たれた。以上のことから、ソース/シンク比の違いによる止葉の老化の相違は、炭水化物含量の変化に基づくものではなく、窒素の動態の変化によるものと判断された。すなわち、相対的にシンクを大とすると、ソースである葉身からシンクである穂への窒素の再移動が大となりクロロフィル含量、ルビスコ含量の低下が促進されると考えられた。

**キーワード**：イネ、クロロフィル含量、光合成、ショ糖合成、シンクサイズ、窒素、葉の老化、ルビスコ含量。

Sources are plant organs that export photosynthates while sinks are plant parts that import them. During the ripening period of rice plants, grains are the major sink organ. For improving grain yield of rice, it has been frequently debated whether yield is more lim-

ited by the source capacity or by the sink capacity. Yoshida<sup>21)</sup> argued that the photosynthetic capacity can limit rice yield since CO<sub>2</sub> enrichment before or after anthesis increases rice yield. On the other hand, appreciable carbohydrates remain in leaves and culms at the harvest suggesting that sink capacity can limit yield under field conditions<sup>9)</sup>. So far, the main determinant of rice grain yield, whether be sink or source, remains unclear.

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It has been shown in several crop species that the rate of photosynthesis changes in response to sink demand<sup>4,11)</sup>. King et al.<sup>7)</sup> showed in wheat that ear removal causes a reduction in flag leaf photosynthetic rate and subsequent darkening of other leaves leads to recovery of the rate. One of the possible mechanisms for these source-sink relationships is the feedback inhibition of photosynthesis<sup>4,11)</sup>. When the sink demand is small, excess carbohydrates are accumulated in leaves, which in turn causes feedback inhibition of photosynthesis. Sawada et al.<sup>13)</sup> found significant negative correlations between photosynthetic activity and sucrose and starch contents in the leaves, using single rooted leaf of soybean as a source-sink model. In rice, Weng and Chen<sup>20)</sup> reported a significant negative correlation between photosynthetic rate and nonstructural carbohydrate content in the leaves by leaf sheath cooling treatment. Furthermore, it has been suggested that leaf senescence is induced in some ways by a depletion of assimilates<sup>2,15)</sup>. Contrary to this, it has also been suggested that accumulation of soluble carbohydrates and starch could lead to the induction of leaf senescence<sup>2,8)</sup>. For field grown rice plants, these relations are not too clear. When sink size is reduced by removing panicle or spikelets, the nitrogen content in the leaves remains high while photosynthetic activity often shows a different trend<sup>3,16)</sup>. Therefore, it would be useful to study photosynthesis activity, nitrogen content, and carbohydrate accumulation in leaf blades simultaneously under different source-to-sink ratios in the field conditions.

In most plants, photosynthetically fixed carbon must be converted to sucrose before translocation, and the process of sucrose synthesis within the leaves can also limit source activity. Furthermore, leaf sheath and culm, which are located along the translocation pathway, can accumulate large amount of carbohydrates. Subsequently, the carbohydrates accumulated in these tissues may affect the source-sink relation in rice plant too.

The purposes of this study were as follows: (1) to determine photosynthetic rates and carbohydrate contents of rice flag leaf blades under different source-to-sink ratios during the ripening period and investigate their relationships, (2) to determine the influence on

flag leaf senescence of different source-to-sink ratios with special references to photosynthetic capacity and sucrose synthesis and investigate their relationships with the contents of carbohydrates and nitrogen in the leaf blades.

## Materials and Methods

### Plant material

Twenty-five-day old rice seedlings (*Oryza sativa* L., cv. Koshihikari) were transplanted in the experimental field of Utsunomiya University on May 23, 1988. Line spacing was 30 cm  $\times$  15 cm with four plants in a hill. The compound fertilizer (4 g N  $\cdot$  m<sup>-2</sup>, 5 g P<sub>2</sub>O<sub>5</sub>  $\cdot$  m<sup>-2</sup> and 6 g K<sub>2</sub>O  $\cdot$  m<sup>-2</sup>) was mixed with the soil four days before transplanting. Ammonium sulfate (2 g N  $\cdot$  m<sup>-2</sup>) was applied as top-dressing on July 13 and on August 20. Anthesis was on August 27 and flag leaf emerged 15 days before anthesis. One hundred and twenty hills, which had an averaged tiller number of  $18 \pm 1$  at anthesis, were used in this study.

### Treatment

The experiment was laid-out in completely randomized design. At anthesis, the 120 hills selected were randomly grouped into six subgroups (20 hills/group). In each hill, treatment was applied to 4 tillers which had similar length of flag leaf blade and panicle. The treatments were as follows: (1) Control, (2) all leaves other than flag leaf were clipped (Source 1), (3) all leaves other than flag and second leaves were clipped (Source 2), (4) 4th to 7th primary rachis branches from the top were removed (Sink 1), (5) 4th and 5th primary rachis branches from the top were removed (Sink 2), (6) all leaves other than flag leaf were clipped and 4th to 7th primary rachis branches from the top were removed (Sink 1 + Source 1). Grain numbers and leaf area per tiller for these treatments are shown in Table 1.

### Photosynthesis measurement

Leaf gas exchange was studied with a portable infrared CO<sub>2</sub> analyzer (model LCA2, Shimadzu-ADC). Measurements were taken between 10:00 and 12:00 on the flag leaves of treatments 1, 2, 4 and 6 in the field 12 days after anthesis. Photon flux density was between 1300 and 1500  $\mu$ mol  $\cdot$  m<sup>-2</sup>  $\cdot$  s<sup>-1</sup>, and leaf temperature was  $28 \pm 1^\circ\text{C}$ .

### Determination of chlorophyll (Chl) and ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) contents and activities of sucrose synthesis enzymes

The middle portion of four flag leaf blades from each treatment were harvested between 12:00 and 12:30 and placed into an ice box. The sampling was done weekly in the field. Each leaf blade was separated to 2 parts: one part was used for chlorophyll extraction in 90% methanol and chlorophyll content was determined according to Schmid<sup>14)</sup>, and the other part was used for enzyme extraction by grinding in cold motor and pestle with a medium containing 50 mM HEPES-NaOH (pH 7.7), 20 mM KCl, 5 mM dithiothreitol and 5% insoluble polyvinyl pyrrolidone with the aid of acid-washed quartz sand. The leaf extract was centrifuged at 20,000 g for 15 minutes at 4°C and the supernatant was used for the assays of Rubisco and enzymes of sucrose synthesis. Rubisco content was determined immunologically as described in a previous report<sup>18)</sup>. Cytoplasmic fructose bisphosphatase (FBPase) activity was assayed at pH 7.5 according to Kelly et al.<sup>6)</sup>. Sucrose phosphate synthase (SPS) and sucrose synthase (SS) activities were assayed as described by Rufty et al.<sup>12)</sup>.

### Determination of sucrose, starch and nitrogen contents

These measurements were conducted for the plants from treatments 1, 2 and 4 only. Four tillers from each treatment were sampled between 12:00 and 12:30. After leaf area was quickly determined with an automatic

area meter, each tiller was separated into four parts, namely, flag leaf blade, other leaf blades, leaf sheath + culm, and panicle. These samples were killed at 105°C for 30 minutes then dried at 80°C for a week. After dry weight was determined, four duplicate samples were combined and milled. Sugars were extracted in hot water at 60°C as stated by Aoki<sup>1)</sup>. Starch was solubilized by dimethylsulfoxide. Contents of glucose, sucrose and starch were determined enzymatically (F-kit, Boehringer Mannheim). Nitrogen content was determined by an automatic N/C analyzer (NC-80, Sumitomo Chemicals Co. Ltd., Tokyo).

## Results

### 1. Source-to-sink ratio

Source-to-sink ratios in the various treatments were evaluated by the ratio of total leaf area at anthesis divided by grain numbers (Table 1). The ratio of control plants was 0.75, and the ratios of treated tillers ranged from 0.24 in treatment 2 to 1.53 in treatment 4. This range of ratios is much larger than that ordinarily occurring in Japonica rice.

### 2. Photosynthetic rate

Although the differences in photosynthetic rate among the treatments were not statistically significant, the photosynthetic rates of all treated tillers were lower than that of control tillers (Table 2). Stomatal conductances of all treated tillers were also lower compared to that of control tillers.

### 3. Contents of Chl and Rubisco

Chl content in the flag leaf blades increased

Table 1. Grain number and leaf area per tiller for control plants and plants altered in source-to-sink ratio.

The source-to-sink ratios were altered by partial defoliation (Source), by partial removal of spikelets (Sink), or by combination both.

Treatment	Grain number* (tiller <sup>-1</sup> )	Leaf area* (cm <sup>2</sup> • tiller <sup>-1</sup> )	Source-to-sink ratio (cm <sup>2</sup> • grain <sup>-1</sup> )
1. Control	123 ± 6	92.4 ± 4.9	0.75
2. Source 1	110 ± 4	26.1 ± 1.6	0.24
3. Source 2	122 ± 4	54.3 ± 1.3	0.44
4. Sink 1	62 ± 1	94.5 ± 4.7	1.53
5. Sink 2	103 ± 3	98.3 ± 3.1	0.95
6. Source 1 + Sink 1	61 ± 4	24.8 ± 1.3	0.41

\* Values represent the mean ± SE of 4 samples at the anthesis (Aug. 27).

slightly (10–15%) up to the second week after anthesis and then started to decrease except in treatment 4 (Table 3). Rubisco content showed a decreasing trend from anthesis in all treatments. At the third week from anthesis, however, significant differences in the contents of Chl and Rubisco among the treatments were observed. Treatment 4, which has a reduced sink or a higher source-to-sink ratio, tended to have relatively higher Rubisco and Chl contents than other treatments. Both the

contents of Chl and Rubisco at this time were significantly correlated with the source-to-sink ratio ( $P < 0.01$ , Fig. 1). In other words, the rate of flag leaf senescence, as reflected in Chl and Rubisco content, is slower in the treatment with a reduced sink or higher source-to-sink ratio.

#### 4. Activities of enzymes involved in sucrose synthesis

In all treatments, cytoplasmic FBPase activity began to decrease from anthesis. On the contrary, SPS and SS activities increased slightly during the two to three weeks following anthesis (Table 3), and remained high thereafter. No significant difference was observed in the activities of these enzymes among treatments at each stage. On a leaf area basis, the extractable enzyme activity was lower in FBPase than in SPS after anthesis.

#### 5. Sucrose and starch content

The patterns of change in sucrose and starch content in the flag leaf blade and leaf sheath + culm for treatments 1, 2 and 4 are shown in Fig. 2. The sucrose content of flag leaf blade exhibited maximum values ( $7.2 \text{ mg} \cdot \text{leaf blade}^{-1}$ ,  $3 \text{ g} \cdot \text{m}^{-2}$ ) at anthesis and three weeks after anthesis. It decreased during the first week and then began to increase until third week after anthesis and then decreased again. This pattern was similar in all 3 treatments. The starch content of the flag leaf

Table 2. Apparent photosynthetic rate (AP) and stomatal conductance ( $g_s$ ) of flag leaves from control plants and plants altered in source-to-sink ratio. Measurements were conducted on 12th day after anthesis.

Treatment	AP* ( $\text{mg} \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ )	$g_s$ * ( $\text{cm} \cdot \text{sec}^{-1}$ )
1. Control	28.6	1.27
2. Source 1	26.3	1.12
4. Sink 1	27.6	1.02
6. Source 1 + Sink 1	24.8	1.06
	NS**	NS**

\* Values represent the mean of 6 replications.

\*\* NS indicates not significant at  $P = 0.05$ .

Table 3. Changes in chlorophyll and Rubisco content activities of enzymes of sucrose synthesis during rice flag leaf development under different source-to-sink ratios.

Weeks after treatment	Treatment	Content		Enzyme activity		
		Chlorophyll ( $\text{mg} \cdot \text{dm}^{-2}$ )	Rubisco	FBPase ( $\mu\text{mol} \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ )	SPS	SS
0	1. Control	3.2* bcd**	26.7 a	109.9 a	87.2 cd	30.3 b
2	1. Control	3.6 abc	24.3 ab	62.1 b	109.9 abc	32.2 ab
	2. Source 1	3.7 ab	22.8 b	62.4 b	109.7 abc	30.5 b
	4. Sink 1	3.9 a	24.5 ab	64.5 b	125.4 a	35.5 ab
	6. Source 1 + Sink 1	3.6 ab	22.3 b	60.9 b	118.3 ab	34.5 ab
3	1. Control	3.0 cd	14.7 cd	44.0 b	90.6 bcd	28.3 b
	2. Source 1	2.6 d	12.7 d	43.3 b	89.3 cd	32.8 ab
	4. Sink 1	3.6 abc	18.2 c	55.5 b	104.2 abcd	43.9 a
	6. Source 1 + Sink 1	2.7 d	13.4 d	50.7 b	77.9 d	37.3 ab

\* Values represent the mean of 4 replications.

\*\* In each column, common letters indicate insignificant difference by Duncan's new multiple range test at  $P = 0.05$ .

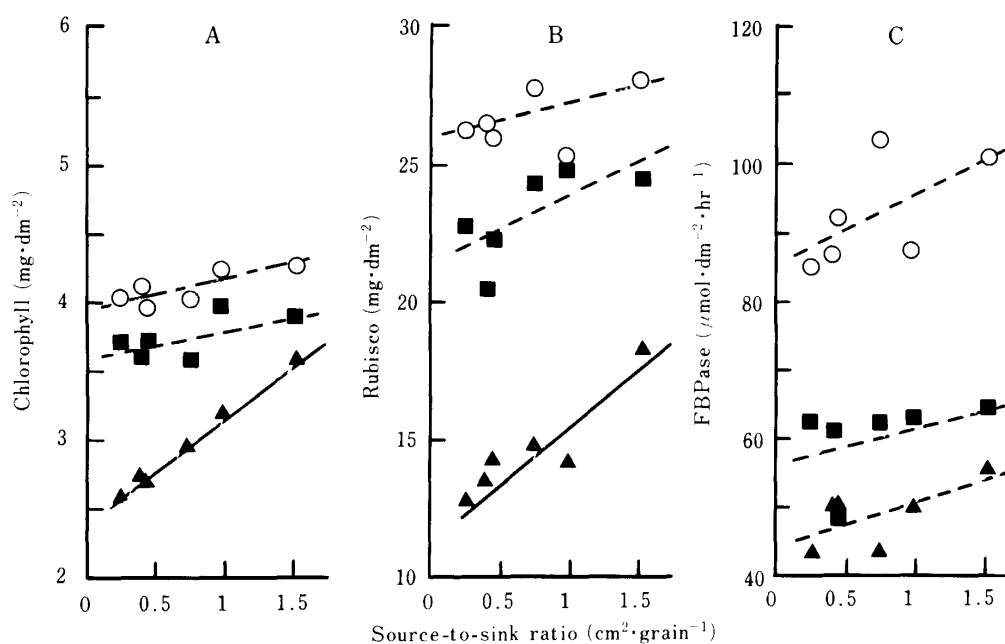


Fig. 1. Relations between source-to-sink ratio and chlorophyll content (A), Rubisco content (B), and cytoplasmic FBPase activity (C).  
○ : 1 week, ■ : 2 weeks, ▲ : 3 weeks after anthesis.  
Solid and broken lines indicate significant correlation (at  $P=0.01$ ) and insignificant correlation, respectively.

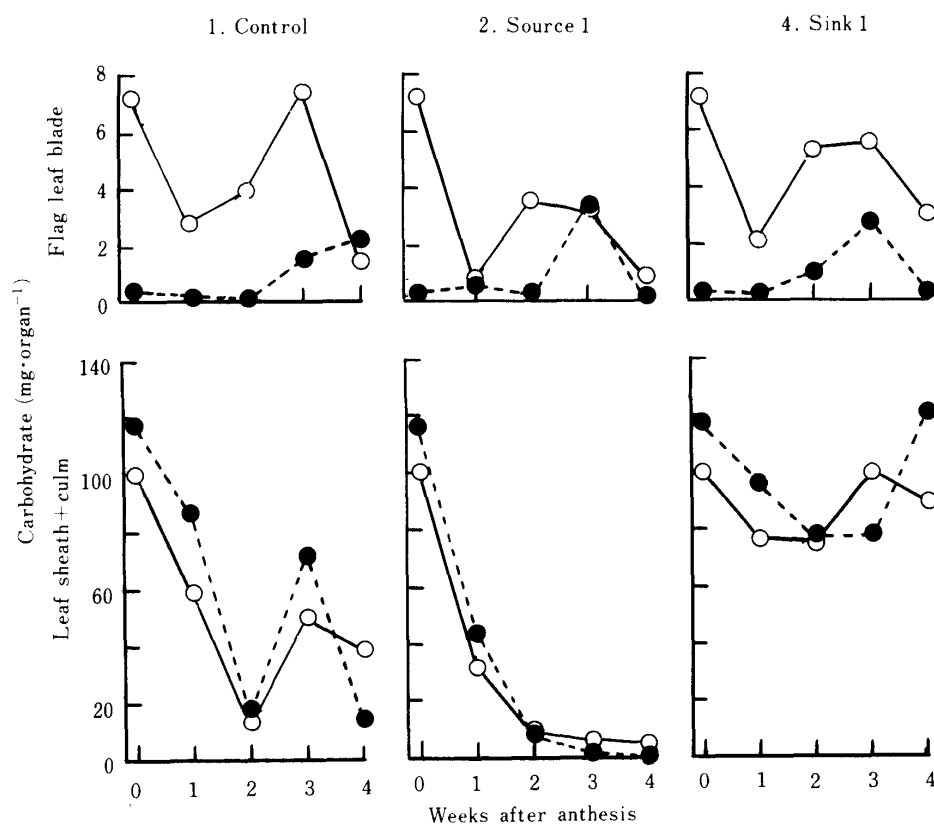


Fig. 2. Changes in sucrose (○) and starch (●) contents in flag leaf blade and leaf sheath + culm.

blade was low until second week after anthesis and then began to increase. The maximum value of starch in the flag leaf blade was about  $3 \text{ mg} \cdot \text{leaf blade}^{-1}$  ( $1.2 \text{ g} \cdot \text{m}^{-2}$ ). The sucrose and starch contents of leaf sheath + culm during the ripening period were almost in parallel. Differences in the pattern among treatments were significant. In the control plants (treatment 1), both parameters decreased in the second week and then began to increase. In the plants with reduced source size (treatment 2), both parameters decreased drastically in the second week to less than 10% of their initial values and stayed low thereafter. In the plants with reduced sink size (treatment 4), both parameters decreased slightly during the 2nd week after anthesis and the values were more than 60% of their initial values at anthesis. The contents of sucrose and starch were much higher in leaf sheath + culm than in flag leaf blade.

## 6. Nitrogen content

The nitrogen contents in all vegetative plant parts decreased over the sampling period (Fig. 3). However, the nitrogen content of flag leaf blade remained at a higher level in treatment 4, which has a smaller sink size.

## Discussion

In this study, we found that treatments with reduced sink (treatment 4) or with reduced source (treatment 2) did not affect apparent photosynthetic rates (Table 2) and related leaf components such as Chl and Rubisco (Table 3) until the second week after anthesis. The decreases in Chl and Rubisco content from the second to third week after anthesis were larger in the treatments with a reduced source size or smaller source-to-sink ratios (Fig. 1). Therefore, it is apparent that larger sink size promotes flag leaf senescence during the ripening period of rice plant. This conclusion is in good agreement with earlier studies<sup>3,5,14,19</sup>. In the present study, the enzymes involved in sucrose synthesis showed differential responses during flag leaf senescence, but none of them was affected by the treatments (Table 3). The nitrogen content of the flag leaf blade was lower in the treatment with reduced sink size (Fig. 3). Thus, the promotion of leaf senescence by a larger sink size may be due to a greater withdrawal of nitrogenous compounds from the source leaf.

Rubisco, which accounts for about half of the total leaf soluble protein, was reported to be one of the most degradable proteins under nitrogen deficient conditions<sup>17</sup>. At the third week after anthesis, Rubisco content was positively correlated with the source-to-sink ratio (Fig. 1). Therefore, it is concluded that the difference in leaf senescence among treatments with various source-to-sink ratios was caused by the different rates of nitrogen withdrawal from source leaves to the sink grains.

In rice plant, Weng and Chen<sup>20</sup> reported a significant negative correlation between photosynthetic rate and nonstructural carbohydrate content in the leaves above a threshold of  $7 \text{ g} \cdot \text{m}^{-2}$ , below which no correlation was found. In this study, the maximum values of sucrose and starch in the flag leaf blade were about  $3 \text{ g} \cdot \text{m}^{-2}$  and  $1.2 \text{ g} \cdot \text{m}^{-2}$ , respectively. Furthermore, the fluctuation patterns of sucrose and starch contents in the flag leaf blade during senescence did not vary substantially among treatments with different source-to-sink ratios. Therefore, feedback inhibition of photosynthesis probably did not occur in these treatments with source-to-sink ratio ranging from 0.24 to 1.53 in the field. Larger differences in carbohydrate contents were

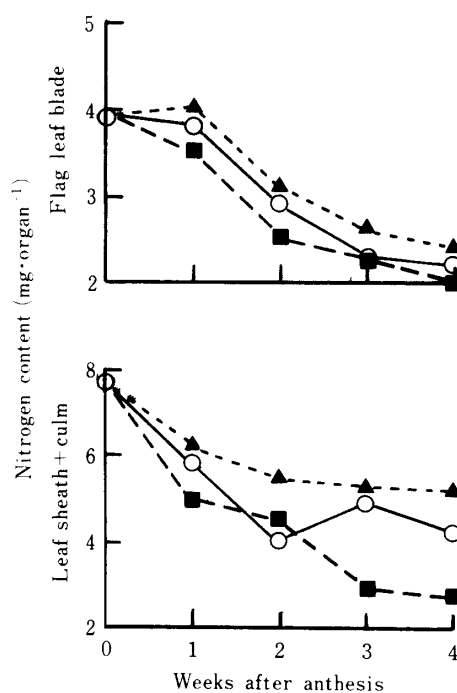


Fig. 3. Changes in nitrogen content in flag leaf blade and leaf sheath + culm.  
○: 1. Control, ■: 2. Source 1, ▲: 4. Sink 1.

observed in leaf sheath and culm. The absolute values of sucrose and starch contents in these tissues were much higher compared to the flag leaf blade. This suggests that leaf sheath and culm acting as vegetative sinks diminish the effects of reproductive sink demand on carbohydrate contents in the source leaf blade in rice plant.

The lower photosynthetic rates exhibited by all treated tillers relative to the control tillers (Table 2) may be attributed to the lower stomatal conductances. Ishihara et al.<sup>5)</sup> and Nakaya et al.<sup>10)</sup> also reported reduced photosynthetic rates about 10 days after panicle removal in rice plants. Therefore, apparent photosynthetic rate after sink or source manipulations may be affected by stomatal behavior through the influence of hormones and this relationship needs to be studied further.

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### References

1. Aoki, S. 1985. Determination of carbohydrates and free amino acids in tea plants with immobilized enzymes and H<sub>2</sub>O<sub>2</sub> electrode. *Jpn. J. Crop Sci.* 54 : 235—240\*.
2. Araus, J.L., L. Tapia, R. Calafell and E. Lopez 1987. Carbohydrate accumulation and senescence onset in field-grown flag wheat leaves during grain filling. *Plant Physiol. Biochem.* 25 : 549—556.
3. Cho, D., S. Yokoi and Y. Murata 1981. Studies on the photosynthesis and dry matter production of rice plants. III. Influence of whole or partial removal of panicles at heading on photosynthesis, dry matter production and yield components. *Jpn. J. Crop Sci.* 50 : 67—71\*.
4. Herold, A. 1980. Regulation of photosynthesis by sink activity—the missing link. *New Phytol.* 86 : 131—144.
5. Ishihara, K., H. Nakaya and T. Hirasawa 1989. Effect of panicle removal on production and distribution of dry matter and flag leaf photosynthesis in rice plants. *Jpn. J. Crop Sci.* 58 (Extra issue 2) : 105—106\*\*.
6. Kelly, G.J., G. Zimmermann and E. Latzko 1982. Fructose-bisphosphatase from spinach leaf chloroplast and cytoplasm. In Wood, W.A. ed., *Methods in Enzymology*. 90. Academic Press, London. 371—378.
7. King, R.W., I.F. Wardlaw and L.T. Evans 1967. Effect of assimilate utilization on photosynthetic rate in wheat. *Planta* 77 : 261—276.
8. Lazan, H.B., E.W.R. Barlow and C.J. Brady 1983. The significance of vascular connection in regulating senescence of the detached flag leaf of wheat. *J. Exp. Bot.* 34 : 726—736.
9. Murata, Y. and S. Matsushima 1975. Rice. In Evans L.T. ed., *Crop Physiology. Some Case Histories*. Cambridge Univ. Press, London. 73—99.
10. Nakaya, H., K. Ishihara and T. Hirasawa 1989. Physiological response to flag leaf photosynthesis to panicle removal in rice plants. *Jpn. J. Crop Sci.* 58 (Extra issue 2) : 107—108\*\*.
11. Neales, T.F. and L.D. Incoll 1968. The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf : A review of hypothesis. *Bot. Rev.* 34 : 107—125.
12. Rufty, T.W. Jr., P.S. Kerr and S.C. Huber 1983. Characterization of changes in activities of enzymes involved in sucrose biosynthesis. *Plant Physiol.* 73 : 428—433.
13. Sawada, S., T. Hayakawa, K. Fukushi and M. Kasei 1986. Influence of carbohydrates on photosynthesis in single rooted soybean leaves used as a source-sink model. *Plant Cell Physiol.* 27 : 591—600.
14. Schmid, G.H. 1971. Origin and properties of mutant plant : yellow tobacco. In San. Pietro, A. ed., *Methods in Enzymology*. 23. Academic Press, London. 171—194.
15. Thomas, H. and J.L. Stoddart 1980. Leaf senescence. *Ann. Rev. Plant Physiol.* 31 : 83—111.
16. Tsuno, Y. and T. Shimizu 1962. Studies on Yield forecast in main crops. VI. On the relation between nitrogen content in leaves and photosynthetic ability of rice plant at ripening stage. *Proc. Crop Sci. Soc. Japan* 30 : 325—328\*.
17. Uchida, N., Y. Wada and Y. Murata 1982. Studies on the changes in photosynthetic activity of a crop leaf during its development and senescence. II. Effect of nitrogen deficiency on the changes in the senescing leaf of rice. *Jpn. J. Crop Sci.* 51 : 577—583\*.
18. Wada, Y. 1988. Changes with senescence in photosynthetic characteristics of a rice leaf. *Special Bulletin of the College of Agriculture, Utsunomiya University* 49 : 1—44\*.
19. ——— and G. Wada 1991. Varietal difference in leaf senescence during ripening period of advanced Indica rice. *Jpn. J. Crop Sci.* 60 : 529—536.
20. Weng, J.H. and C.Y. Chen 1991. Effect of accumulated nonstructural carbohydrates on photosynthesis of rice leaves. *Jpn. J. Crop Sci.* 60 : 320—321\*\*.
21. Yoshida, S. 1973. Effects of CO<sub>2</sub> enrichment at different stages of panicle development on yield components and yield of rice. *Soil. Sci. Plant Nutr.* 19 : 311—316.

\* In Japanese with English summary.

\*\* In Japanese. Translated by the present authors.