

Source of Respiratory Carbon Evolved from the Shoots of Rice Seedlings in Darkness*

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Abstract : The time course change of the shoot respiration rate, the percentage of currently assimilated carbon in respiration and the amounts of current and reserved compounds in the shoots of rice plants were examined using the steady-state $^{13}\text{CO}_2$ assimilation technique, in an attempt to evaluate the change of respiratory substrates with time during darkness. The shoot respiration exhibited midnight rise of respiration rate (MRR), and then a sudden decrease around 13 hours after the onset of darkness. The percentage of CO_2 derived from ^{13}C -labeled current assimilates in respiration was high during the first 6 hours, and followed by a continuous decrease. Therefore, current assimilates took priority over reserved compounds to support midnight respiration with MRR in the dark period, and as being consumed current photosynthates the respiration using reserved materials increased to compensate the respiration. Since the ^{13}C -labeling and the content of sucrose were high during the early dark period, it was suggested that sucrose was the principal source of respiratory substrates, and thereafter, starch and other compounds gradually became the major source of CO_2 . The integrated value of the percentage of ^{13}C -labeled CO_2 in total respired CO_2 during the first 12 hours was 51%, suggesting respiration dependency on current assimilates was almost equivalent to that on reserved materials.

Key words : ^{13}C , Current assimilate, Diurnal change, Respiration, Respiratory substrate, Rice, *Oryza sativa* L..

暗期にイネ幼植物地上部の呼吸により放出される炭素の由来 : 山岸順子・河内 宏*・米山忠克** (東京大学農学部附属農場・*農業生物資源研究所・**農業研究センター)

要 旨 : 暗期におけるイネ幼植物地上部の呼吸基質の経時変化について、定量的・定性的な解析を試みた。そのために、定濃度・定比活性の $^{13}\text{CO}_2$ を植物体に同化させることにより、新規固定炭素 (Current assimilates) と貯蔵炭素とを区別する方法を用い、呼吸中の新規固定炭素の割合および植物体中の新規固定炭素化合物と貯蔵炭素化合物の含量を経時的に測定した。呼吸速度は暗期中に一時的に上昇 (MRR) し、暗期開始 13 時間後に急激に低下するという経時変化を示した。新規固定炭素由来 (^{13}C 標識) の CO_2 の呼吸中に占める割合は、暗期開始後 6 時間まで比較的高く、その後低下した。このことは、新規固定炭素は貯蔵炭素よりも呼吸に優先的に使われ、MRR を含む呼吸を支えるが、時間と共に消費され、それに伴い、貯蔵炭素化合物が呼吸の主要な基質となることを示している。暗期開始時の植物体内のショ糖含量は高く、また、その新規固定炭素の占める割合が高いことから、初期においてはショ糖が主要な呼吸基質となっているが、時間と共に、テンブソンの他の化合物が次第に主要な呼吸基質になると結論された。暗期開始後 12 時間の総呼吸中に占める ^{13}C 標識 CO_2 の割合は 51% であった。このことは、呼吸に使われる新規固定炭素と貯蔵炭素の割合がほぼ等しいことを示している。

キーワード : イネ, *Oryza sativa* L., 呼吸, 呼吸基質, ^{13}C , 新規固定炭素, 日変化。

Recently it was reported that respiration rate in several plant species during darkness showed change with time, including the midnight rise of respiration (MRR)^{2,9,11,13}. It is probable that the substrate for respiration also changes with time, though the total amount of substrates might continue to decrease from the onset of the dark period. The substrate for respiration during darkness is derived from both the current assimilates, which are

photosynthetically formed in the light period just before darkness, and the reserved compounds. The studies with steady-state $^{14}\text{CO}_2$ labeling of photoassimilates showed that large amounts of current assimilates were consumed as the substrate for respiration during ensuing darkness and that sucrose was consumed before starch, suggesting a change in respiratory substrate with time^{3,4}. However, the quantitative aspects of the contribution of current photosynthates in total respiratory substrate were not studied extensively, except for the roots and nodules of soybean plants^{7,8},

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showing that current assimilates took priority over reserved materials as the substrate for respiration and that the source changed from sucrose to starch with time. In addition, in soybean nodules, more than 80% of respired carbon was derived from current assimilates, while, in roots, 56% was derived from current assimilates⁸⁾. These results indicated that nodule respiration is highly dependent on current assimilates, whereas root respiration is more dependent on carbon compounds reserved in roots.

In this paper, we examined the change of the contribution of current assimilates in the total respiratory substrate and the change of the amounts of current and reserved compounds in rice plants using the steady-state $^{13}\text{CO}_2$ assimilation technique previously described⁵⁾, attempting to evaluate the time course change of respiratory substrates.

Materials and Methods

Plant materials ; Rice (*Oryza sativa* L. cv. Hatsuboshi) plants were grown hydroponically on 2.5 liter plastic pots with 45 plants per pot in a growth cabinet controlled at 25°C and 70% RH and illuminated by 12 metal halide lamps at $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ (400–700 nm) during a day (12 hours). The plants were supported with air-tight rubber through small holes on the rids. The nutrient solution was half strength modified Kimura B solution (Table 1) and renewed every other day.

Assimilation of $^{13}\text{CO}_2$ and measurement of respired $^{13}\text{CO}_2$; The method of steady-state $^{13}\text{CO}_2$ assimilation was essentially the same as previously described⁵⁾. The plants (6.5 leaf stage) were transferred into the assimilation cabinet (1×1×1.2 m) and fed $^{13}\text{CO}_2$ (25.9 atom% excess) at $320 \mu\text{l l}^{-1}$ for 10 hours (from 8:00 to 18:00). The temperature, relative humidity and light intensity were the same as described above.

After $^{13}\text{CO}_2$ feeding, the shoots of plants in each pot were enclosed with air-tight acrylic chambers (5 liter) with two (inlet and outlet) ports, and CO_2 -free air⁸⁾ was introduced into the chamber at a flow rate of 500 ml min^{-1} . The outlet port was connected to the infrared $^{13}\text{CO}_2$ analyzer (EX-130S, JASCO, Tokyo, Japan) through multi-channel electromagnetic valves and a sampling pump, to measure $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ concentrations as previously

Table 1. The composition of modified Kimura B solution.

Components	Contents (mg L ⁻¹)
(NH ₄) ₂ SO ₄	48.2
K ₂ SO ₄	15.9
MgSO ₄	65.9
KNO ₃	18.5
Ca(NO ₃) ₂	59.9
KH ₂ PO ₄	24.8
FeCl ₃	5

described⁸⁾. The air in the chamber was stirred with a small fan. Measurement of respiration was conducted for 24 hours under continuous darkness using four chambers. The temperature in the chambers was 25°C.

Analysis of ^{13}C in the tissues ; After $^{13}\text{CO}_2$ feeding, triplicate samples (each 15 plants) were harvested at appropriate intervals and separated into shoots, bases of shoots (shoot sections just below the joint of the second leaf sheath) and roots. All samples were freeze-dried, weighed, ground to a fine powder by a vibrating mill (Model TI-1, Heiko Co. Ltd., Iwaki, Japan) and stored at -50°C until analysis.

Total carbon contents and ^{13}C abundance in tissues was determined as previously described⁵⁾.

A freeze-dried powder was extracted by 80% (v v⁻¹) hot ethanol, and carbon contents and ^{13}C abundance in ethanol-insoluble materials also determined. The concentration and ^{13}C abundance of sucrose (in 80% ethanol extracts) and starch in the residue were also determined as described⁶⁾. The ^{13}C content in the structural materials was estimated by subtracting the ^{13}C content in starch from that in 80% ethanol-insoluble materials. The ^{13}C content in 80% ethanol-soluble materials other than sucrose was also calculated by subtracting the ^{13}C content in sucrose and 80% ethanol-insoluble materials from that in total tissue.

All data of ^{13}C abundance was translated to the percentage of labeled carbon by the following equation ;

$$\text{Percentage of labeled carbon (\%)} = \frac{{}^{13}\text{C atom\% excess in component}}{{}^{13}\text{C atom\% excess in administered CO}_2} \times 100$$

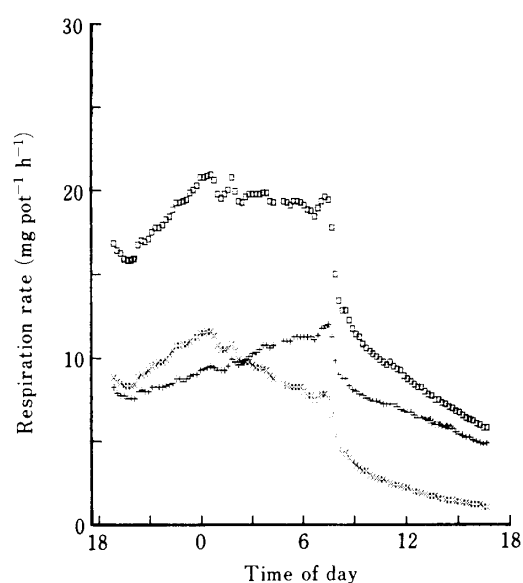


Fig. 1. Time-course of respiration rate in shoots.

□ ; total CO₂, + ; non-labeled CO₂,
× ; ¹³C-labeled CO₂.

Absolute amounts of labeled carbon were calculated by total carbon content in the sample multiplied by percentage of labeled carbon divided by 100.

Results

Respiration of shoots; The respiration rates changed with time as illustrated in Fig. 1. The rates decreased just after the onset of the dark period followed by increase until midnight, and they kept almost constant values for the next seven hours before a sudden decrease which lasted until the end of the measurement. This phenomenon was observed in many plant species^{2, 9, 11, 13}). The time course changes of ¹³C-labeled and non-labeled CO₂ respired from shoots were obviously different. The evolution of ¹³C-labeled CO₂ increased until midnight and then decreased, while the evolution of non-labeled CO₂ continuously increased for 13 hours after the onset of darkness. From 7:00 a.m., when the light period should start in the normal growing cycle, both ¹³C-labeled and non-labeled respired CO₂ decreased rapidly.

The percentage of ¹³C-labeled CO₂ in respired CO₂ was almost constant, between 51% and 56%, during the first six hours, and then continued to decrease until the end of measurement (Fig. 2).

Partitioning of carbon into individual plant

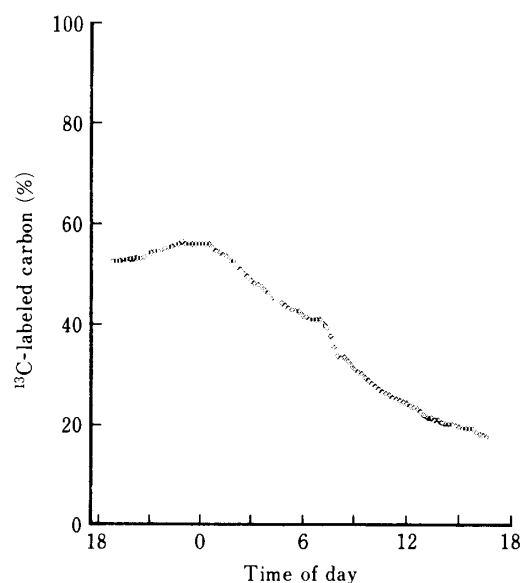


Fig. 2. The percentage of ¹³C-labeled CO₂ in the respired CO₂.

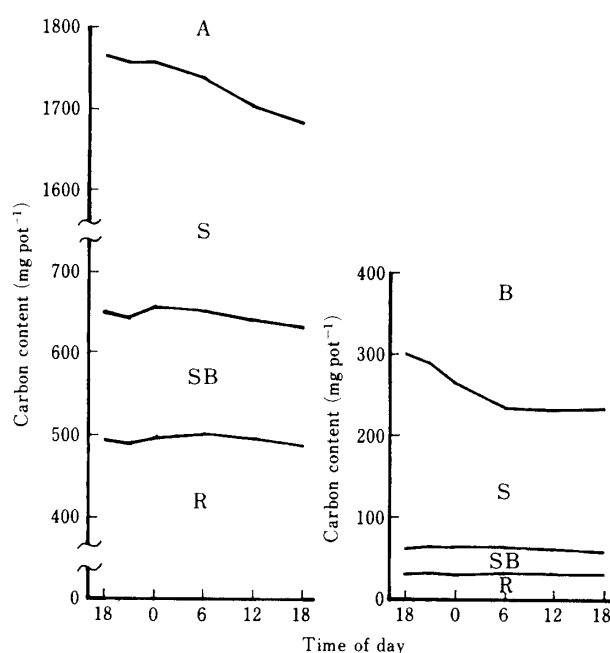


Fig. 3. Carbon contents in individual plant parts.

A ; non-labeled carbon, B ; labeled carbon. S ; shoots, SB ; shoot bases, R ; roots.

parts; The distribution of labeled and non-labeled carbon in each plant part is shown in Fig. 3. The total amount of labeled carbon at the end of feeding period was 303.6 mg, of which 79% was in shoots, about 10% in shoot bases and about 10% in roots. The content of both labeled and non-labeled carbon in roots and shoot bases remained almost constant

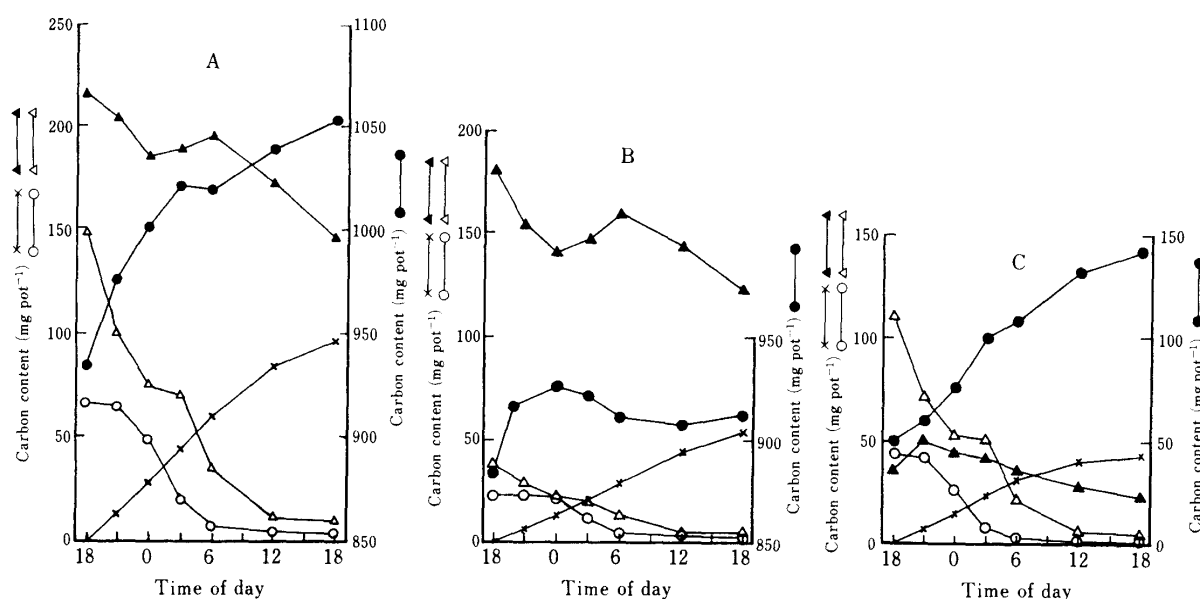


Fig. 4. Carbon contents in chemical fractions of shoots. A ; total carbon, B ; non-labeled carbon, C ; labeled carbon. ○ ; starch, ● ; structural materials, △ ; sucrose, ▲ ; ethanol soluble compounds other than sucrose, × ; integrated respiration.

throughout the experiment period. The content of labeled carbon in shoots, however, decreased for the first 12 hours, while that for non-labeled carbon did so throughout the measurement period. During the 12 hours of darkness, the labeled carbon content in whole plants was reduced to 234.7 mg, 77% of the initial value. This indicated that 23% of the current assimilates were consumed as respiratory substrate during first 12 hours of the dark period.

Distribution of carbon in chemical fractions of shoots ; The labeled carbon content in the structural materials was continuously increased with time, while the non-labeled carbon was almost constant, except during the first three hours (Fig. 4). A large part of the labeled carbon was found in sucrose at the onset of the dark period. Almost 80% of this was respired or transferred to other plant parts during the following 12 hours of the dark period. The labeled carbon in starch decreased with time after a three-hour lag period, while the non-labeled carbon in starch started to decrease after a six-hour lag period. Most of the starch, both labeled and non-labeled, was degraded during the first 12 hours of the dark period. There was a considerable amount of carbon, especially non-labeled carbon, in the ethanol soluble fraction during the experiment period.

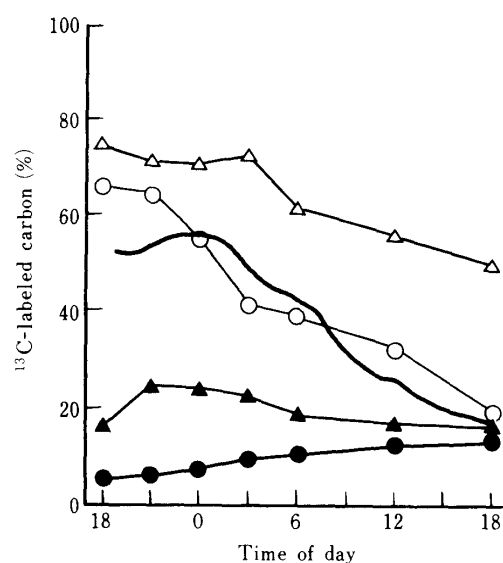


Fig. 5. The percentages of labeled carbon in the total carbon in chemical fractions of shoots.

○—○ ; starch, ●—● ; structural materials, △—△ ; sucrose, ▲—▲ ; ethanol soluble compounds other than sucrose, — ; respired CO₂.

The percentage of labeled carbon in sucrose was high throughout the dark period and showed a decrease from 3:00 a.m., and the percentage of labeled carbon in starch was high at the beginning of the dark period but steeply decreased with degradation (Fig. 5).

Discussion

The percentage of ^{13}C -labeled CO_2 in respired CO_2 was high (more than 50%) for the first 6 hours of dark period and then decreased due to the decrease of labeled CO_2 and the increase of non-labeled CO_2 . This suggests that current photosynthates took priority over reserved carbon to support respiration in the early dark period and as being consumed current photosynthates the respiratory loss of reserved materials was increased to compensate the total respiration. MRR was observed for the first 6 hours during which the amount of labeled CO_2 respired increased and current photosynthates, mainly sucrose, were consumed actively as the substrates of respiration. Since the percentage of labeled carbon in sucrose was much higher than that in respiratory CO_2 , other low labeled compounds, like ethanol soluble compounds could also be consumed as respiratory substrate. When starch degradation started, they would be consumed as respiratory substrates in addition to sucrose. The percentage of ^{13}C -labeled carbon in starch decreased with time, indicating that the newly synthesized starch was preferentially broken down to the reserved one. Starch content in shoots was almost depleted after 12 hours of darkness, and major respiratory substrate should have changed to other compounds which have low percentages of ^{13}C -labeled carbon like ethanol-soluble compounds other than sucrose. These changes of respiratory substrates were, as Amthor¹⁾ mentioned, probably caused by the difference in accessibility of substrates to respiratory pathways, because respiratory substrates were localized spatially within tissues, cells and organelles in plant in addition to chemical distribution.

The sudden decrease of respiration rate from the time when the normal light period started was related to the decrease in both labeled and non-labeled respired CO_2 and corresponded well to the time when starch was almost degraded in shoots. However, this decrease was so abrupt and steep that starch depletion cannot be the only causative factor. In soybean plants¹¹⁾, the same decrease was observed, and it was reported that the time of its onset did not change even if the respiration rate was accelerated by higher temperature

and the respiratory substrates largely consumed and exhausted earlier. McCree¹⁰⁾ found the same phenomenon in white clover and grain sorghum, and it was also observed by us in young rice plants (unpublished data). These results suggested that the sudden decrease was not caused by the depletion of the remaining compounds which could be used for respiratory substrate. Therefore, the mechanism of decrease would be related to other factors like nitrogen metabolism as suggested in soybean leaves¹²⁾.

The integrated value of the percentage of ^{13}C -labeled CO_2 in respired CO_2 during the first 12 hours of the dark period was 51%. This value was similar to the case of soybean root respiration (56%)⁸⁾. Therefore, it is concluded that the dark respiration of plant tissues depends equally on current assimilates and reserved materials.

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