Japan. Jour. Crop Sci. 58 (4) : 720—725 (1989)

Respiration of Soybean Plants in Relation to Their Physiological Conditions

III. The characteristics of respiration in leaves and roots under attached and detached conditions*

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Received April 30, 1989

Abstract : Time course changes of respiration rate in a leaf and roots were examined in attached and detached conditions to obtain information of the internal factors determining the time course change of respiration in different organs.

In the attached condition, an expanding leaf showed a rapid increase of respiration around midnight (Midnight rise of respiration, MRR), while, an expanded leaf showed a steady state of respiration around midnight, followed by a rapid decrease, resulting in a vague MRR. The attached roots showed a clear MRR. In the detached condition, the changes in leaf respiration rate were almost the same as in attached condition ; whereas in roots, MRR completely disappeared.

The results suggested that the respiration of a leaf is not affected by other organs, whereas that of roots was strongly dependent on the existence of a shoot. The results also suggested that the internal factors causing MRR existed in a leaf, especially in an expanding leaf.
Key words : Aging, Diurnal change of respiration, *Glycine max* (L.) Merr., Leaf growth, Respiration, Soybean.

ダイズ植物体の生理的条件と呼吸速度との関係　III. 着生および切断した条件下における葉と根の呼吸の特徴：山岸順子・石井龍一・玖村敦彦（東京大学農学部）

要　旨：葉身および根の呼吸速度の経時変化を着生条件と切断条件において比較し，異なる器官における呼吸に経時変化をもたらす体内要因について検討した．

着生条件においては，展開中の葉身の呼吸は深夜において顕著に上昇（Midnight rise of respiration, MRR）したが，展開終了した葉身においては顕著な MRR は見られず，呼吸速度は深夜にはほぼ一定となり，その後急激に低下した．根については，明らかな MRR が認められた．切断条件においては，葉身の呼吸速度は着生条件において見られたものと同様のパターンを維持していたが，根では MRR が完全に消失した．

したがって，根の呼吸は地上部の有無によって著しく影響されるのに比べ，葉身の呼吸は他の器官から独立した傾向を持つことがわかった．さらに，MRR をひきおこす体内要因は，葉，特に展開中の葉に顕著に存在することが示唆された．
キーワード：エイジング，*Glycine max* (L.) Merr.，呼吸，呼吸の経時変化，ダイズ，葉面生長．

In the previous papers[11,12] we reported on the whole plant level, that the time course change of respiration rate of a soybean plant showed a steep and temporary increase around midnight, which was termed as midnight rise of respiration (MRR). MRR was supposed as a reflection of time course changes of nitrogen metabolism occurring in a plant. However, a plant consists of different organs of various ages with different physiological functions, and these organs interact with one another through their whole lives. It is therefore expected that the time course change of respiration differs in each organ. Many researchers[1,2,3,4,6,7,9] have studied on time course changes of respiration rate on the level of single organs like a leaf or roots. Challa[1] showed that there was a difference of respiration not only in its rate, but also in its time course changes between leaves and roots, and among the leaves of different positions in cucumber plants. Ludwig et al.[6] reported that

* A part of this papar was presented at the 178th meeting of the Crop Science Society of Japan, October, 1984.

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a single attached leaf of tomato showed a temporary increase around midnight in a time course change of respiration, which was apparently similar to our MRR in soybean plants[11,12]. Also as for roots, Neales et al.[9] observed a temporary increase of respiration around midnight in wheat. Furthermore, there were many papers[3,4,5,7,9] reporting that reduced light intensity, shortened light period or defoliation treatment reduced the respiration in root to a large extent. These results showed that the time couse changes of respiration rate are different among organs and that there is an interdependence among organs in respiration.

In this paper, we examined the time course changes of respiration in leaves and roots under attached and detached conditions, aiming to obtain information of the internal factors determining the time course change of respiration.

Materials and Methods

1. Plant materials; Soybean plants (*Glycine max* (L.), Merr. cv. Koganedaizu) were cultured in solution, according to the method previously reported[11], in the controlled greenhouse of the Center of Environment Regulation System for Biology (CERES) of the University of Tokyo. Air temperature and relative humidity were controlled at 25/20°C (day/night) and 70%, respectively. When the fourth trifoliates were fully expanded on the main stem, the plants were transferred to a growth cabinet, where air temperature, relative humidity, light intensity and light period were kept constant at 25°C, 70%, 470μmol m^{-2} s^{-1} (PAR), and 12 hours, respectively. No nodules were observed on the roots of all plants examined.

Measurement of respiration rate; When the fifth trifoliates on the main stem were fully expanded, respiration rates of the fifth trifoliates, the seventh trifoliates, which was still expanding, and roots were measured in attached and detached conditions. For the measurements in detached condition, both expanded and expanding trifoliates were excised at the base of petiole at 10 : 00 and they were kept in the same environmental conditions as in the growing period, with the cut end submerged in distilled water. Another expanded and expanding trifoliates were excised at 18 : 00, and instantly the respiration were measured. The onset of respiration rate measurement at 18 : 00 is corresponding to the onset of dark period during the growing cycle. For the measurement in detached roots, the shoots were cut off at their base at 18 : 00, and the measurement started instantly. The continuous measurement of CO_2 evolution rate was conducted in an environment controlled cabinet. The central leaflet of a trifoliate was mounted in an acrylic chamber ($10\times12\times0.5$cm), and the roots, after sterilized according to the method of Okada[10] were set in a 2 liter acrylic bottle with culture solution. The temperature, dew point and CO_2 concentration in the air introduced to the chambers were maintained at 25°C, 20°C and about 300μl/l respectively. The CO_2 concentration in the sample air from the exit of each chamber was measured by a differential typed infrared CO_2 gas analyzer (ZAP, Fuji electric, Tokyo, Japan), and the measurement was carried out continuously for about 20 hours in dark. The measurement were replicated more than five times in the attached organs and more than ten times in the detached organs.

2. Measurement of leaf area growth; Twenty-eight attached expanding leaves, the seventh trifoliates, were set in the same condition as for the respiration measurement, and four leaves were sampled for leaf area and dry weight measurements every three hours through dark period. The leaf area growth was expressed as the percentage increase of leaf area against initial values in each leaf at 18 : 00.

Results

Time course changes of respiration rate in the expanding leaf is shown in Fig 1. As almost the same results were obtained in the replicated measurements, a typical one from each condition was presented. The expanding leaf, whether attached or detached, showed a clear increase of respiration around midnight (MRR). It should be noticed that MRR was observed even in the detached condition, although the extent was not so large as in attached condition.

On the other hand, the expanded leaf showed a different behavior from that of the expanding leaf (Fig. 2). In the attached condition, respiration rate showed a steep decrease immediately after the onset of the dark period, and remained at a constant level until around

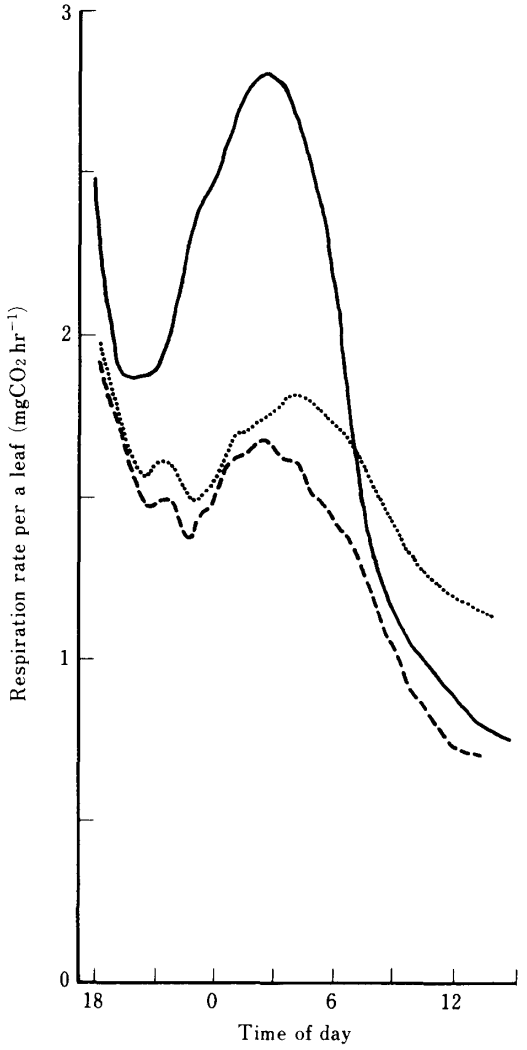

Fig. 1. Time course of respiration rate in an expanding leaf in the continuous dark. ——, attached leaf; — — —, detached leaf cut at the onset of dark period (18 : 00); ·····, detached leaf cut at 8 hours before the onset of dark period (10 : 00). Leaf area at the onset and the end of measurement, and leaf dry weight at the end of measurement were 52.2cm², 77.8cm² and 192mg in the attached leaf, 52.7cm², 60.7cm² and 103mg in the detached leaf cut at 18 : 00, and 40.2cm², 47.4cm², and 102mg in the detached leaf cut at 10 : 00.

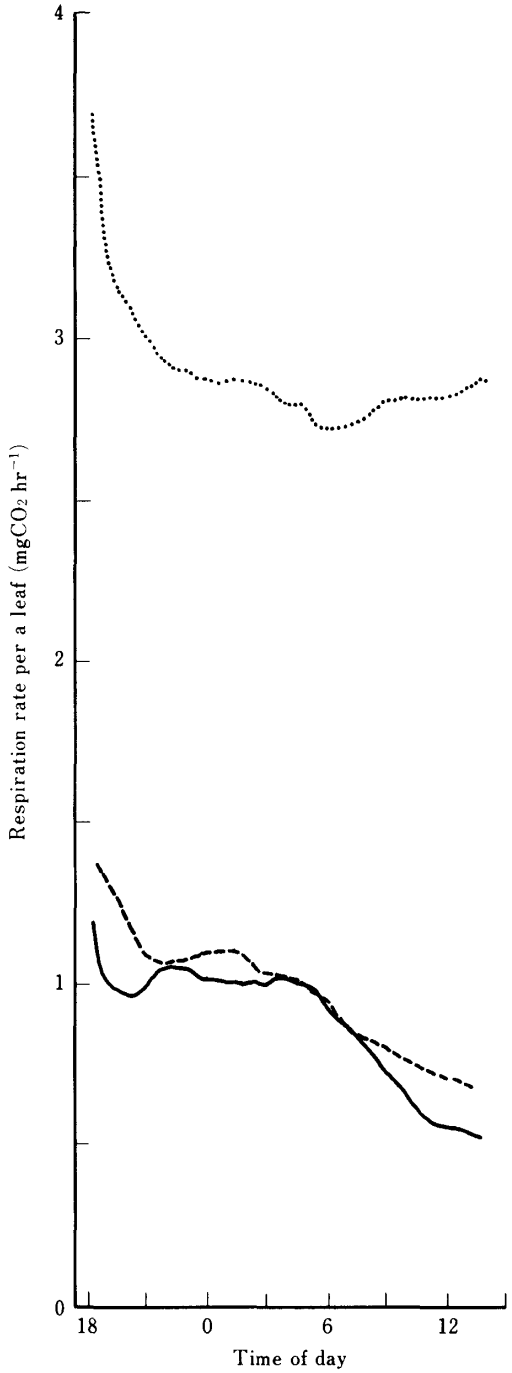

Fig. 2. Time course of respiration rate in an expanded leaf in the continuous dark. ——, attached leaf; — — —, detached leaf cut at the onset of dark period; ·····, detached leaf cut at 8 hours before the onset of dark period. Leaf area and dry weight at the end of measurement were 86.9cm² and 235mg, 83.7cm² and 279mg, and 81.9cm² and 230mg, in the above order, respectively.

6 : 00. This was followed by a decrease, resulting in disappearance of a clear MRR. In the detached condition, the expanded leaf excised just at the onset of dark period showed a similar time course change of respiration as the attached leaf. Another detached leaf, which was excised at 10 : 00 and exposed to light for 8 hours before the onset of dark period, maintained much higher level of respi-

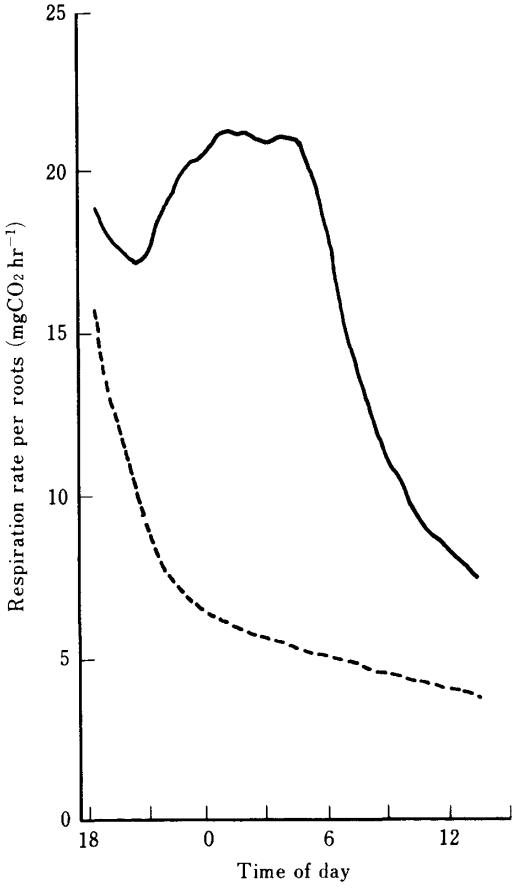

Fig. 3. Time course of respiration rate in roots.
——, attached roots; — — —, detached roots. Dry weights at the end of measurement were 2342mg and 2240mg, for attached and detached condition, respectively.

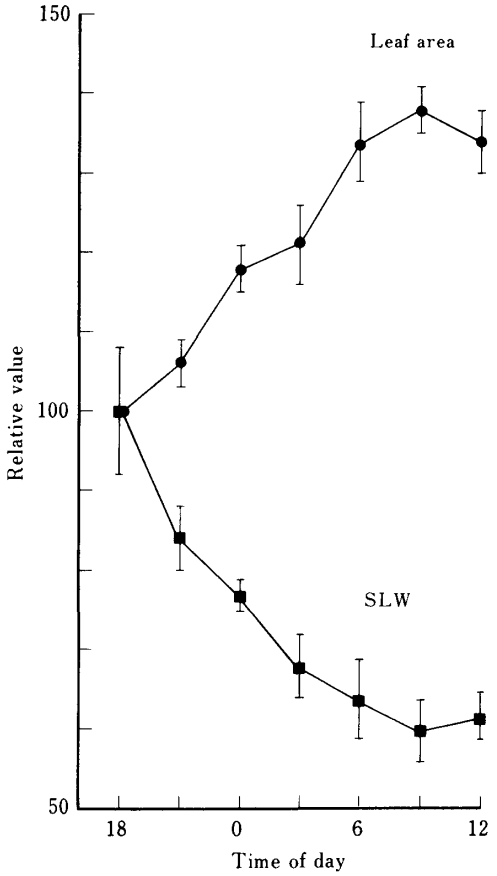

Fig. 4. Changes of leaf area (●) and specific leaf dry weight (SLW, ■) in an expanding leaf. Leaf area was shown as the percentage against initial value in each leaf at 18 : 00 with standard error, and SLW was expressed as the percentage to the mean value of that sampled at 18 : 00. Leaf areas at 18 : 00 were between 30 and 40 cm^2 in all leaves measured. The mean specific leaf weight at 18 : 00 was 508 mg dm^{-2}.

ration throughout the measurement, and showed no second decrease of respiration.

In the roots, MRR was clearly observed only in the attached condition (Fig. 3). Detached roots showed continuous decrease of respiration rate with time. It could be, therefore, said that respiration in roots is strongly dependent on the existence of shoot. On the other hand, the leaf, especially the expanding leaf, is independent from other organs with respect of respiration.

In our results, MRR was found evidently in the expanding leaves and in the roots. This suggests that MRR is a characteristic of the respiration in the growing parts of a plant. However, MRR was not necessarily related to the accelerated growth in the plant part, because the expanding rate of the leaf area did not correspond to the change of respiration (Fig. 4).

Discussion

Previous studies[11,12] using a whole plant suggested that there were some internal regulating factors for the time course change of respiration, which could be produced in the precedent light period. This paper, in which the time course change of respiration was examined for separate organs in a plant, gave us more detailed information on the internal factors. In the present experiments, the expanding leaf showed a clear MRR, while the fully expanded leaf showed a vague MRR. The respiration rate in the expanded leaf leveled

off around midnight and was followed by a steep decrease, suggesting that the internal factor could be produced in the young expanding leaf to more extent than in the mature expanded leaf. It could be, therefore, said that the internal factors regulating the time course change of respiration exist commonly in any leaf, although the extent of change varies according to maturity. This idea can be supported by the reports that a temporary increase of respiration was observed in the midnight in the matured leaves of tomato[6], cucumber[1] and soybean[8]. On the other hand, the roots showed a clear MRR in the attached condition, which disappeared in the detached condition. It could be speculated, therefore, that the factors regulating the time course change of respiration, which was produced in the leaves, could be transmitted to the root.

The present paper showed also that MRR is not the reflection of the time course change of respiratory requirement for the leaf area growth. Our previous work[11,12] showed that MRR was related to the change of nitrogen metabolism. Therefore, the internal regulating factor of time course change of respiration should be searched for around nitrogen metabolic change in a leaf, rather than energy requiremnt change for leaf area growth. Challa[1] also studied the relationship between temporary increase in respiration and metabolic change. We will investigate the mechanism of time course change of respiration from the view point mentioned above.

Apart from the behavior of the diurnal change of respiration, the level of respiration rate should be discussed here. There are many papers[1,4,5,7,9] reporting that respiration rate could be regulated by the amount of photosynthetic products. This would be true only for the expanding leaf in this paper. The respiration rate of the expanding leaf was reduced by excision which blocked the influx of photosynthetic products and other substances to the sink leaf from other source organs like fully expanded leaves. However, the fully expanded leaf showed almost the same respiration rate in detached (at the onset of dark period) and attached conditions, in spite of the expectation that respiration rate would be higher in detached than in attached condition, because the level of photosynthetic products should be higher in detached condition where photosynthates did not move out of the leaf. The differences in respiration behavior between the expanding and the expanded leaf suggest that the respiration of a leaf is regulated by some other factors than the level of photosynthetic procducts at least in matured leaves which has a surplus level of photoassimilates as the respiratory substrate ; whereas the respiration in a young leaf, which remains still as a sink organ, would be regulated by the level of photoassimilates and other snubstances. From the above discussion, together with our results obtained previously, it will be suggested that the factors regulating the behavior and respiration rate during the night are produced from the nitrogen metabolism in leaves. We will investigate this point in the next paper.

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