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Respiration of Soybean Plants in Relation to Their Physiological Conditions

IV. Time course changes in nitrogen metabolism in a leaf*

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Abstract : Time course changes in nitrogen transformation among nitrogenous compounds in a detached leaf of soybean was examined in relation to respiration rate.

Nitrogen content in protein continued to increase for 12 hours from the onset of dark period. This apparent protein synthesis showed a high peak between 0 : 00 and 3 : 00 in an expanding leaf, and a low peak in an expanded leaf, followed by a steep decrease in both leaves. This trend of protein synthesis fitted to the time course change of respiration rate. The time of maximum protein synthesis rate corresponded to that of midnight rise of respiration appearance. It was, therefore, supposed that the time course changes in respiration which has been examined in the previous papers, is the reflection of diurnal changes in protein synthesis rate in a leaf.
Key words : Amino acid, Diurnal change of respiration, *Glycine max* (L.) Merr., ^{15}N, Nitrogen metabolism, Protein, Respiration, Soybean.

ダイズ植物体の生理的条件と呼吸速度との関係　IV. 葉における窒素代謝の経時変化：山岸順子・石井龍一・玖村敦彦（東京大学農学部）

要　旨：葉における各種窒素化合物中の窒素含量の変化を経時的に調べ，それと呼吸速度の変化とを対比させることによって，窒素代謝と呼吸との相互関係を検討した．

タンパク中の窒素含量は，暗期開始後12時間増加し続けたが，そのみかけの合成速度には経時変化がみられ，0時から3時の間に最大となり，その後急激に低下した．この変化は，特に展開中の葉において顕著であった．このタンパク合成速度にみられた経時変化は，既報の呼吸の経時変化と非常によく一致することから，呼吸の経時変化はタンパク合成速度の経時変化を反映しているものと考えられた
キーワード：アミノ酸，*Glycine max* (L.) Merr.，呼吸，呼吸の経時変化，^{15}N，ダイズ，タンパク，窒素代謝．

In the previous papers[6,7] we reported that a soybean plant showed a sharp increase of respiration around midnight (Midnight rise of respiration, MRR), when the plant was supplied with sufficient nitrogen. This suggested that MRR was closely related to nitrogen metabolic change in a plant. Penning de Vries[2,3] reported that more than half of the growth respiration was directed to the biosynthesis of nitrogenous compounds, and more than half of maintenance respiration was directed to the turnover of protein in a corn plant. Because the content of nitrogen in a soybean plant is higher than in a corn plant, the cost of biosynthesis of nitrogenous compounds is probably higher in a soybean plant. Steer[4,5] reported in *Capsicum annuum* L. that the protein synthesis showed a diurnal change. These results imply that time course changes of respiration are closely linked to those of protein biosynthesis.

In this roport, we examined the nitrogen metabolism in a detached soybean leaf to elucidate the interrelationship between respiration and the nitrogen metabolism by chasing the nitrogen partitioning in different nitrogen compounds in a leaf using ^{15}N.

Materials and Methods

1. Plant materials : Soyben plants (*Glycine max* (L.), Merr. cv. Koganedaizu) were cultured according to the method as previously reported[8], in the growth cabinet where air temperature, relative humidity, light intensity

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Table 1. Relative nitrogen partitioning between NaOH and EtOH fractions in the extracts of an expanding leaf.

Time	Non-labeled N (%)		Labeled N (%)		Total N (%)	
	NaOH	EtOH	NaOH	EtOH	NaOH	EtOH
18 : 00	81.1	18.9	34.0	66.0	79.9	20.1
21 : 00	85.0	15.0	50.2	49.8	84.2	15.8
0 : 00	87.6	12.4	56.4	43.6	87.1	12.9
3 : 00	95.1	4.9	75.1	24.9	94.6	5.4
6 : 00	96.0	4.0	85.2	14.8	95.8	4.2
10 : 00	89.1	10.9	80.2	19.8	88.9	11.1

Table 2. Relative nitrogen partitioning between NaOH and EtOH fractions in the extracts of an expanded leaf.

Time	Non-labeled N (%)		Labeled N (%)		Total N (%)	
	NaOH	EtOH	NaOH	EtOH	NaOH	EtOH
18 : 00	87.4	12.6	38.7	61.3	85.7	14.3
21 : 00	88.5	11.5	48.0	52.0	87.7	12.3
0 : 00	89.4	10.6	52.9	47.1	87.8	12.2
3 : 00	92.0	8.0	66.7	33.3	91.4	8.6
6 : 00	93.3	6.7	65.1	34.9	92.6	7.4
10 : 00	80.1	19.9	49.8	50.2	79.4	20.6

and light period were kept constant at 25°C, 70%, 470μmol m^{-2} s^{-1} (PAR) and 12 hours, respectively. When sixth trifoliate on the main stem was fully expanded, ^{15}N labeled nitrate was fed in the form of $Na^{15}NO_3$ (10.8 atom%) to the plants through roots for six hours during the latter half of the light period. As no nodules were observed on the roots, the only source of nitrogen was nitrate in the culture solution. When dark period began, thirty five of both fully expanded sixth trifoliates and expanding eighth trifoliates were cut at the base of petiole and they were placed in a bottle, with the cut end immersed in distilled water under dark condition.

2. Determination of ^{15}N : Five central leaflets of the above mentioned trifoliates were sampled every three hour, and submerged in 80% ethanol at 77°C for 5 minutes. Then the leaflets were homogenized with a pestle and mortar on ice. After centrifugation, ethanol soluble fractions were collected. It was evaporated and then added with a sodium citrate buffer (pH 2.2). Etanol and sodium citrate buffer soluble fraction (EtOH fraction) containing amino acids, peptides and inorganic nitrogen compounds, was used for the determination of ^{15}N and for the analysis of amino acids. The analysis of amino acids was made with an amino acid auto analyzer (MLC-703, Atto, Tokyo, Japan). Sodium citrate buffer insoluble fraction was extracted with chloroform, and chloroform soluble fraction ($CHCl_3$ fraction) was used for ^{15}N determination in lipids and pigments. Sodium hydroxide solution (1N) was added to ethanol insoluble residue, and it was left in room temperature for one day with frequent shaking. The supernatant (NaOH fraction) was served for the determination of ^{15}N in protein. The ^{15}N content in each fraction was determined by the optical emission spectrometric method, with ^{15}N analyzer (NIA-1, JASCO, Tokyo, Japan). All the data were shown as the means of two replications. Nitrogen contents were determined with C-N corder (MT-500, Yanagimoto, Kyoto, Japan) in ten lateral leaflets sampled at the onset of dark period. Nitrogen compounds were surveyed only in NaOH and EtOH fractions, because more than 99% of the nitrogen was recovered of both expanded and expanding leaves.

Results

Nitrogen partitioning between NaOH and EtOH fractions was shown for the expanding and the expanded leaf in Table 1 and 2, respectively. Here we assumed that the con-

tent of total nitrogen was constant through the measurement, because the measurement was conducted in the detached condition. Therefore, we determined the total nitrogen contents only at 18 : 00 when the measurement started. The mean values with standard deviations of the total nitrogen obtained were 59.1 ± 1.5mg gDW^{-1} in the expanding leaf, and 50.7 ± 1.3mg gDW^{-1} in the expanded leaf. At that time, the mean values of the percentage of ^{15}N labeled nitrogen in the total nitrogen with standard deviations were $2.15 \pm 0.29\%$ in the expanding leaf and $2.79 \pm 0.81\%$ in the expanded leaf. As shown in Table 1 and 2, nitrogen, irrespective of labeled or non-labeled, decreased in EtOH fraction, with increase in NaOH fraction until 6 : 00. This suggests that nitrogen was transferred from EtOH fraction to NaOH fraction during this period. Especially, a considerable amount of labeled nitrogen was transferred from EtOH to NaOH fraction. However, after 6 : 00, nitrogen in EtOH fraction recovered, suggesting that an adverse transfer of nitrogen from NaOH fraction to EtOH fraction occurred. These trends were observed in both expanding and expanded leaves. It was, therefore, considered that protein synthesis overwhelmed its breakdown until 6 : 00, but after 6 : 00 a reverse relation occurred. Fig. 1 shows the increasing rates in percentage of nitrogen content in NaOH fraction. This figure clearly shows that the apparent rate of protein synthesis reached a maximum during three hour period, 0 : 00 to 3 : 00, in both expanding and expanded leaves. The content of each amino acid is shown for an expanding leaf (Table 3) and for an expanded leaf (Table 4). In an expanding leaf, the total amino acid content decreased gradually after the intiation of the dark period until 6 : 00, but recovered greatly at 10 : 00. On the other hand, the total amino acid content in an expanded leaf showed a steep decrease immediately after the onset of the dark period followed by rapid recovery which lasted until the end of the measurements. These time course changes of total amino acid contents were due to a common phenomenon seen in the change in the contents of all sorts of amino acids.

The changes of nitrogen contents in NaOH fraction, amino acids in EtOH fraction, and residual EtOH fraction, which was supposed

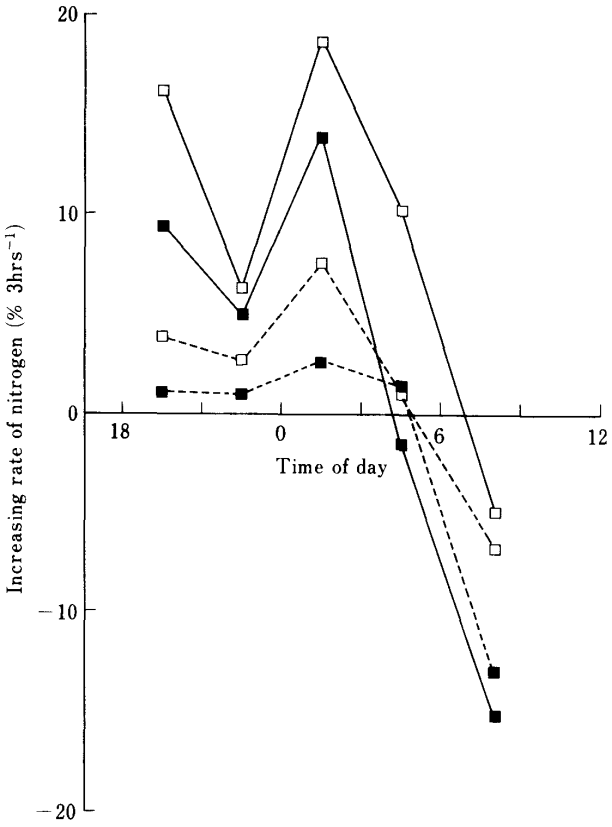

Fig. 1. Time course change of increasing rate of nitrogen in NaOH fraction. ——, labeled nitrogen; ------, non-labeled nitrogen. □, expanding leaf; ■, expanded leaf.

to consist of inorganic and peptide nitrogen, are shown in Fig. 2. In an expanding leaf, the change of nitrogen content in amino acids was very small, and that in NaOH fraction was adversely related to that of residual EtOH fraction. This suggests that amino acids synthesis from inorganic nitrogen, and its consumption for protein synthesis, were in equilibrium. In an expanded leaf, the trend is basically the same as in an expanding leaf, although the magnitude of the change is not so large.

Discussion

The time course change of apparent protein synthesis in a soybean plant was found quite similar to that of respiration rate in an expanding leaf[8]. Especially, the time when apparent protein synthesis reached the peak fitted to that when MRR was observed. These results strongly suggest that the time course change of respiration could be the reflection of metabolic

Table 3. Time course changes in contents of amino acids in expanding leaf.

Amino acid	Amino acid content per dry weight of a leaf (μmol/g)					
	18 : 00	21 : 00	0 : 00	3 : 00	6 : 00	10 : 00
Asp	5.57	10.06	5.52	5.13	6.67	20.02
Thr+Ser+Gln+Asn	35.04	18.68	17.49	13.35	14.27	17.57
Glu	14.92	14.44	12.25	11.66	13.47	16.22
Pro	nd	nd	nd	nd	nd	nd
Gly	0.10	nd	0.13	nd	0.29	0.12
Ala	7.95	3.69	6.95	6.25	5.86	6.82
Cys	nd	nd	nd	nd	nd	nd
Val	0.95	1.02	0.90	0.89	0.80	3.13
Met	nd	nd	nd	nd	nd	nd
Ile	nd	nd	nd	nd	nd	1.48
Leu	nd	nd	nd	nd	nd	1.62
Tyr	0.93	0.99	1.06	1.11	1.28	1.42
Phe	0.36	0.86	1.01	0.65	0.63	1.12
γ-ABA	5.20	3.15	3.24	2.15	3.47	4.38
Lys	0.75	0.47	0.83	0.93	0.73	3.07
His	0.52	0.32	0.23	nd	0.31	0.35
Total	72.30	53.68	49.61	42.12	46.78	77.34

nd : not detected.

Table 4. Time course changes in contents of amino acids in expanded leaf.

Amino acid	Amino acid content per dry weight of a leaf (μmol/g)					
	18 : 00	21 : 00	0 : 00	3 : 00	6 : 00	10 : 00
Asp	8.40	8.81	16.07	11.39	17.16	14.44
Thr+Ser+Gln+Asn	77.12	18.47	40.97	35.84	33.51	40.66
Glu	26.75	13.01	17.20	20.52	25.70	24.80
Pro	nd	nd	nd	nd	nd	nd
Gly	nd	nd	nd	nd	nd	nd
Ala	17.53	5.58	9.61	11.65	11.34	11.27
Cys	nd	nd	nd	nd	nd	nd
Val	1.39	0.59	0.40	0.78	0.79	0.79
Met	nd	nd	nd	nd	nd	0.09
Ile	0.70	nd	nd	nd	nd	0.24
Leu	0.39	nd	nd	nd	0.12	0.48
Tyr	0.64	0.29	0.62	0.61	0.35	0.46
Phe	2.64	0.79	0.62	0.41	0.57	0.46
γ-ABA	10.07	4.83	5.17	8.04	7.26	7.53
Lys	0.21	0.20	0.29	0.56	0.22	0.29
His	0.70	0.32	0.35	0.26	0.25	0.44
Total	146.52	52.89	91.29	90.05	97.24	101.95

nd : not detected.

change of nitrogen in a leaf.

We calculated, according to Penning de Vries[1], the amount of evolved CO_2 which is required for apparent protein synthesis.

$$CO_2\text{evolution} = \frac{\text{Apparent protein synthesis} \times 6 \times \text{CPF}}{\text{PV}}$$

where CPF is carbon dioxide production factor, which is the weight of carbon dioxide produced divided by the weight of substrate required for carbon skeletons and energy production. PV is production value, which is the weight of the end product divided by the weight of substrate required for carbon skele-

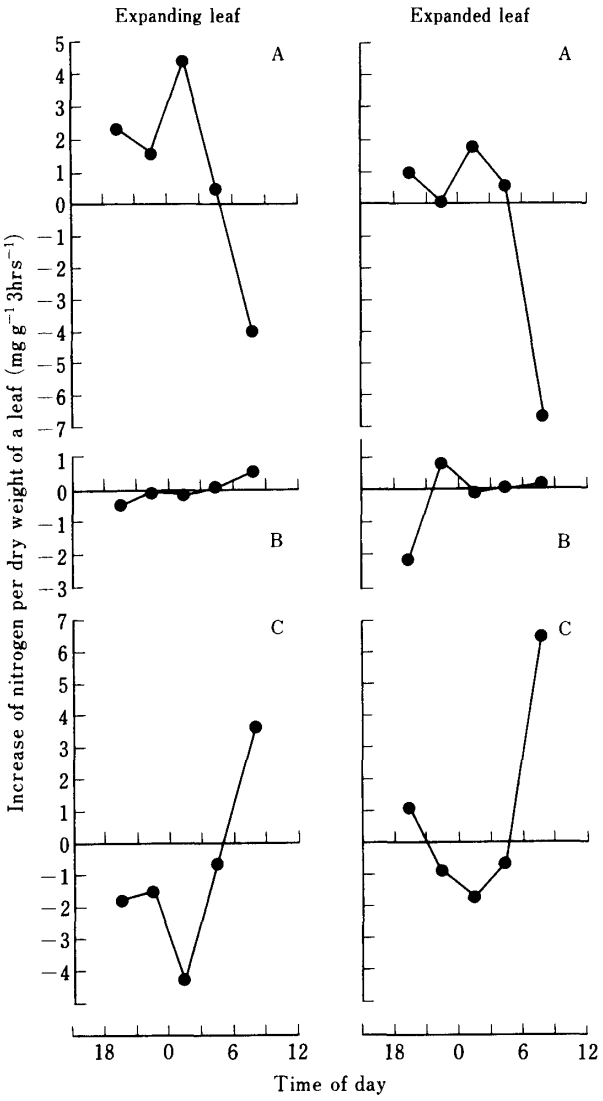

Fig. 2. Time course change of nitrogen increase in NaOH fraction (A), in amino acids of EtOH fraction (B) and in residual substances of EtOH fraction (C).

tons and energy production. By assuming that protein content is six times of nitrogen content, and that protein was synthesized from glucose and nitrate, PV and CPF were obtained as 0.604 (g g^{-1}) and 0.252 (gCO_2 gDW^{-1}), respectively.

The theoretical CO_2 evolution rates obtained by above equation, were 1.43mgCO_2 gDW^{-1} hr^{-1} and 3.70mgCO_2 gDW^{-1} hr^{-1}, for pre-(from 21 : 00 to 0 : 00) and post-(from 0 : 00 to 3 : 00) midnight, respectively, with as much difference as 2.27 mgCO_2 gDW^{-1} hr^{-1} between pre- and post-midnight. We also calculated the difference of actual respiration rates between pre- and post-midnight, from the previously reported data[8], and got the value in the range from 1.50 to 4.40 mgCO_2 gDW^{-1} hr^{-1}, which is fitted to the value obtained theoretically. It suggests that MRR is probably caused by the accelerated rate of protein synthesis around midnight.

The apparent protein synthesis in an expanded leaf showed almost the same trend as that in expanding leaf, although the fluctuation was small. We discussed in the previous papar[8] that internal processes causing MRR in leaf were common in both expanding and expanded leaves. Our results in apparent protein synthesis in expanded leaf well coincide with this idea.

It was concluded from these results that protein synthesis in a leaf apparently changed with time in the dark period with a peak between 0 : 00 and 3 : 00, and that this change might be the cause of the diurnal change of respiration characterized by MRR.

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