Mechanism of Varietal Differences in Photosynthetic Rate of Soybean Leaves*

II. Varietal differences in the balance between photochemical activities and dark reaction activities

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INTRODUCTION

In the previous report⁹⁾ I suggested the existence of varietal differences of chloroplasts in soybean leaves both in morphological characters and in photochemical activities. Hill activity of chloroplasts in unit leaf area was closely and positively correlated with light-saturated photosynthetic rate. On the other hand, light-saturated photosynthetic rate is said to be limited by dark reaction activities^{1,5)}. Hence we can naturally suppose that varietal differences in photosynthetic rate may be partly due to the varietal differences in dark reaction activities. Dark reaction activities can be estimated by measuring enzymatic activities of Calvin cycle, but unfortunately rate limiting reactions in the cycle are not identified so clearly as to be beyond doubt. Besides, actually measured enzymatic activities are far lower than expected values from photosynthetic rate of intact leaves⁷⁾, which makes it hard to compare enzymatic activities between varieties.

In the experiments reported herein, intact soybean leaves were used to confirm the relationship between photochemical activities and light-saturated photosynthetic rate and to examine varietal differences in the balance between photochemical activities and dark reaction activities. The relationship between dark reaction activities and light-saturated photosynthetic rate was also discussed.

MATERIALS AND METHODS

Plant materials

Six soybean varieties [*Glycine max* (L.) Merrill] were

used; Harosoy, Wasekogane, Shinanomejiro, Shiromeyutaka, Norin NO. 1 and YL-2-1. YL-2-1 is one of the virescent mutant lines derived from the crossing Madison-Nishiki $\times$ Shiromeyutaka and was offered by Dr. Mikoshiba at Nagano Prefectural Agricultural Experiment Station. Madison-Nishiki is a virescent mutant from Madison. Seeds were sown on July 3 in soil-packed Wagner pots (1/5000 a) with 20 g compound fertilizer (N 3—P₂O₅ 10—K₂O 10) and 8 g magnesium carbonate per pot. Middle leaves of the 3rd to the 8th trifoliate leaves were used. Ages of each leaf were as follows at the experimental days: 3rd trifoliate leaves, 18–19 days from the start of leaf expansion, 4th 16–17 days, 5th 14–15 days, 6th 12–13 days, 7th 11–12 days (just after the completion of expansion), 8th 5–6 days (under expansion).

Apparatus

Photosynthetic rate was measured from 7 to 13 Aug. using a Tōshiba-Beckman differential type infrared CO₂ analyzer, Platass DIA. Intermittent light technique was used principally in this experiment. Photosynthetic rate under intermittent light was measured with the device schematically described in fig. 1. A is a speed-variable drive motor giving 30–300 rpm. One end of the motor axis was connected to a tachometer generator B and rpm was indicated on a tachometer indicator C. The other end was inserted into a black dark box E (about 1 m³) and a transparent acryl disc D ($r \approx 50$ cm) was fixed at the end. Shading plates F ($\theta = 30^\circ$) were fixed on the disc by pairs of small screws. Light period, dark period and the ratio of the two were varied as needed arranging the number and position of the shading plates and motor speed. Light source

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Fig. 1 Diagram of apparatus for measuring photosynthesis under intermittent light

A: Speed-variable drive motor. B: Tachometer generator. C: Tachometer indicator. D: Acryl disc. E: Black dark box. F: Shading plate. G: Slide projector. H: Cylinder. I: Leaf chamber. J: Water chamber. K: Compressor. L: Air dividing bottle. M: Cock. N: Flow meter. O: Infrared CO₂ analyzer. P: Recorder.

G is a 200 W slide-projector, light from which passed a cylinder H and was 42 klx at the leaf position. Soybean leaf is light-saturated at about 25–30 klx⁶⁾. I is a transparent acryl chamber for a single leaf (10 cm × 15 cm × 1.5 cm) and was deposited as near as possible to the rotating disc D in order to make the shade of shading plates as sharp as possible. The leaf chamber was cooled by water running through a water chamber J. Air temperature in the leaf chamber was about 27–29°C. The outdoor air was conducted into the leaf chamber through compressor K, air dividing bottle L, cock M and flow meter N. A portion of the air running out of the leaf chamber was sampled into a Tōshiba-Beckman differential

type infrared CO₂ analyzer O, Plantass DIA. The difference of the CO₂ concentration between the outdoor air and the sampled air from the leaf chamber was recorded on the chart of a recorder P. The full scale of the recorder was 100 ppm CO₂. Reading of small differences in CO₂ concentration was easier than in the case of an absolute type infrared CO₂ analyzer.

Experimental technique

Photosynthetic rates under various kinds of intermittent light were measured. Light and dark periods were varied as listed in table 1. Numbers and positions of shading plates on the disc rotating 120 rpm gave the light periods shown in the table. As

the number of shading plates increases, photosynthetic rate decreases. How photosynthetic rate *begins* to decrease depends on the balance between photochemical activities and dark reaction activities, which was illustrated schematically in fig. 2. The abscissa indicates the percentage of incident light quantity compared with the continuous light. The ordinate indicates the percentage of photosynthetic rate compared with the rate under continuous light. With variety A, photosynthetic rate decreases in proportion

Table 1 Light periods and dark periods used in the experiment

No. of shading plates	Position of shading plates	Incident light percentage	Light period (ms)	Dark period (ms)	Light period / Dark period
0		100		0	
2		83	210	42	5/1
4		67	84	42	2/1
6		50	42	42	1/1
10		17	42	210	1/5
11		8	42	462	1/11
12		0	0		

to the decrease in incident light quantity. In this case the relationship between the two activities can be estimated to be photochemical activities $\leq$ dark reaction activities. With variety B, a little decrease in incident light quantity (insertion of short dark periods) brings about decrease in photosynthetic rate, but to a smaller extent than in the case of variety A. In this case the relationship can be estimated to be photochemical activities $>$ dark reaction activities. With variety C, a little decrease in incident light quantity little affects photosynthetic rate. In this case the relationship can be estimated to be photochemical activities $\gg$ dark reaction activities. To avoid varietal effect on the relationship between photosynthetic rate and respiratory rate, upper part of the curves should be compared. In this way varietal differences in the balance between the two reaction activities were examined.

To confirm the relationship between photochemical activities and light-saturated photosynthetic rate, maximum flash yield (max. f.y.) was measured. Because max. f.y. is one of the indicative characters of photochemical activities. F.y. was defined here as the gross photosynthesis brought about by one unit flash (42 klx, 42 ms). It was calculated deviding gross photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) by the number of the unit flashes given in an hour. When the

flashes of longer durations were given, f.y. was calculated regarding the longer flashes as continuous unit ones.

About 30 to 60 min were necessary for a leaf to be stable and fully active in photosynthesis after the insertion of a leaf into a leaf chamber. Photosynthetic rate under continuous saturating light was measured first, then photosynthetic rates under increasing number of shading plates were measured in order.

Sometimes in the course of experiment, photosynthetic rate under continuous light was checked to be sure that it remained constant, otherwise the experiment was interrupted waiting for its recuperation under continuous light.

Light-photosynthesis curve was examined for the reference, using the same leaves employed in the intermittent light experiments. Light intensity was varied piling semi-transparent thin papers on the leaf chamber. Photosynthesis was measured at the following light intensities; 42.0 (100%), 20.0 (48%), 12.2 (29%), 8.9 (21%), 6.0 (14%), and 2.7 klx (6%). Respiration was measured in the dark.

Chlorophyll (a+b) concentration of the leaves used for photosynthesis measurements was checked in the same way as reported before⁹⁾. Some days, six days in the worst case, had elapsed before the analysis after photosynthesis measurements. So that the analytical results are only for the reference.

RESULTS

1. Varietal order of photosynthetic rate under saturating continuous light

Mean photosynthetic rates under saturating continuous light were in the order of Harosoy 31.4 > Wasekogane 30.1 > Shiromeyutaka 28.5 > Shinanomejiro 27.1 > Norin NO. 1 26.5 > YL-2-1 20.2 $\text{mg CO}_2 \text{ dm}^{-2} \text{ h}^{-1}$. The varietal order was the same as in the previous report⁹⁾, where primary leaves were used exclusively, except that the order between Shiromeyutaka and Shinanomejiro interchanged. The identity suggests that primary leaves can almost be representative as to varietal photosynthetic rates. YL-2-1 was about two-third as active as the other normal (not virescent) varieties.

Fig. 2 Schematic illustration of photosynthesis of varieties having different ratios of photochemical activities/dark reaction activities (see text)

2. Relationship between max.f.y. and light saturated photosynthetic rate

F.y. calculated from the definition previously mentioned was dependent on the ratio of light period to dark period as is shown in fig. 3. As the ratio decreased, f.y. increased and in most cases max.f.y. was attained when the ratio was one-fifth. This means that the light energy absorbed in 42 ms under 42 klx light intensity was consumed out in about 252 ms ($42 \text{ ms} \times 6$). Max.f.y. signifies the utilization efficiency of incident light energy under the condition that photochemical reactions limit photosynthetic rate. Max.f.y., therefore, is one of the indicative characters of photochemical activities. Varietal order of max.f.y. was almost the same as that of light-saturated photosynthetic rates and positive and significant correlation was observed between the two characters as is shown in fig. 4. The result coincided well with the previous observation⁹⁾ that Hill activity of chloroplasts in unit leaf area was positively and significantly correlated with light-saturated photosynthetic rate.

Fig. 3 Relationship between the ratio of light period to dark period and flash yield
 ● Harosoy ○ Wasekogane □ Shinanomejiro △ Shiromeyutaka ■ Norin No. 1
 × YL-2-1
 Varietal symbols are common in all the figures and tables unless indicated otherwise.

Max.f.y.s. were also correlated with Hill activity of chloroplasts which were measured previously⁹⁾ (fig. 5). It is worth mentioning that Shinanomejiro had smaller light-saturated photosynthetic rate than its expected value from its max.f.y. in spite of the fact that max.f.y.s. were, as a whole, significantly correlative with light-saturated photosynthetic rates.

3. The balance between photochemical activities and dark reaction activities

Mean relative photosynthetic rates under various kinds of intermittent light were shown in fig. 6. The abscissa indicates relative incident light (100%:

Fig. 4 Correlation between maximum flash yields and light-saturated gross photosynthetic rates. Varietal symbols are the same as in fig. 3.

Fig. 5 Correlation between Hill activity of chloroplasts in unit leaf area and maximum flash yield. Varietal symbols are the same as in fig. 3.

continuous light) varied by increasing number of shading plates (ref. table 1). The ordinate indicates relative photosynthetic rate or respiratory rate (100%: photosynthetic rate under continuous light). Variance analysis (table 2) revealed the existence of significant varietal differences in relative photosynthetic rates. But Tukey's test^{b)} revealed that in most cases varietal differences existed only between the variety YL-2-1 and others. In other words, among normal five varieties there existed little varietal differences in relative photosynthetic rate. From the interpretation of the curves in fig. 2. I guess here that there was little varietal differences in the balance between photochemical activities and dark reaction activities as a whole. That is to say the variety having higher photochemical activities had the tendency to have also higher dark reaction activities.

On the other hand, the curve of YL-2-1 was quite different from those of the other normal varieties. The curve can be interpreted as to show that the ratio between the two activities, photochemical activities/dark reaction activities, was smaller than the other normal varieties.

Statistical analysis denied in most cases the existence

Fig. 6 Relative photosynthetic rate under intermittent light. Relative photosynthetic rates were averaged in six leaves from 3rd to 8th leaf. Varietal symbols are the same as in fig. 3.

of varietal difference between Shinanomejiro and other normal varieties in the balance between the two reaction activities, but the curve of the variety Shinanomejiro was a little different from those of the other normal varieties. As will be referred later, this variety had the largest photosynthetic rate under weak light, but the varietal order went down as the light intensity increased (ref. fig. 9). The light-saturated photosynthetic rate was smaller than its expected value from its max.f.y. (ref. fig. 4). These observations suggest that in Shinanomejiro the ratio may be higher than the other four normal varieties, which would have been revealed with smaller statistical errors.

Varietal differences in the balance between the two reaction activities can be estimated in another way, from the consumption pattern of captured light energy during a flash. Varietal consumption patterns were estimated as follows. Let us suppose the case of Harosoy explained in fig. 7. The abscissa indicates a light period followed by five dark periods. The area A, B or C indicates f.y.s. obtained during each corresponding period. F.y.s. in Harosoy were as follows (ref. fig. 3). Max.f.y. 5.19×10^{-5} mgCO₂, f.y. of the case 42 ms L+42 ms D 4.15×10^{-5} mgCO₂, f.y. of continuous light 3.50×10^{-5} mgCO₂. Max.f.y. was measured when five dark periods followed a flash, hence can be expressed as A+B+C (A+B+C=5.19 unit omitted). Under continuous light only A

Table 2 Statistical analysis of relative photosynthetic rates in fig. 6. Varietal symbols are the same as in fig. 3.

Incident light percentage	F value of variance analysis		Tukey's test ^{c)}
	Variety	Age	
83	6.07** ^{a)}	0.30	×—(●○□△■)
67	10.13**	0.22	×—(●○□△■) □—●
50	10.30**	0.14	×—(●○□△■)
17	11.12**	0.45	×—(●○□△■)
8	22.09**	8.91* ^{b)}	×—(●○□△■)
0	4.24**	2.65*	×—(● □△■)

a) Significant at 1% level.

b) Significant at 5% level.

c) Significant varietal differences were observed between varieties connected by line.

is effective ($A=3.50$). Under alternative flashings 42 ms L+42 ms D, $A+B$ is effective ($A+B=4.15$). Hence $A=3.50$, $B=0.65$, $C=1.04 \times 10^{-5} \text{ mgCO}_2$, whose percentages were as follows in order 67.5, 12.5, 20.1. This means that 67.4% of the captured light energy in 42 ms light period was consumed in the same light period, 12.5% in the first 42 ms dark period and the remaining 20.1% in the following $4 \times 42 \text{ ms}$ dark periods. The same kind of calculations were carried out also for the other varieties as is shown in fig. 8. This figure is valid for the estimation of the balance between the two reaction activities. In case of the variety having larger ratio of photochemical activities/dark reaction activities, larger percentage of the captured light energy is consumed in the dark periods than otherwise. This is the case with Shinanomejiro. On the contrary, in case of the variety having smaller ratio, smaller percentage of the captured light energy is consumed in the dark periods than otherwise. This is the case with YL-2-1. The other four varieties, Harosoy, Wasekogane, Shiromeyutaka and Norin NO. 1, had almost the same trend and the balance between the two reaction activities was almost the same. Thus, as to the varietal differences in the balance between the two reaction activities, I got almost the same conclusion from the two kinds of viewpoint.

Regarding the aging effect on the balance, no effect was observed as is shown in table 2. This means that both activities changed keeping pace with each other within the range of aging examined; from leaf developing stage to about 10 days after the

Fig. 7 Schematic illustration of consumption pattern of light energy captured during a 42 ms flash. Variety Harosoy (see text).

completion of development. The stars on the F values at eight and zero percent incident light means that the ratio of respiratory rate/photosynthetic rate is larger with the leaves under development than leaves already developed.

4. Light-photosynthesis curve

Varietal light-photosynthesis curves are shown in fig. 9. Varietal curves rarely crossed each other and as a whole, the variety having larger light-saturated photosynthetic rate had also larger light-unsaturated one. Varietal order of photosynthetic rate of Shinanomejiro, however, came down as the light intensity increased. The slopes of the straight lines drawn between respiratory rate and photosynthetic rate at 8.9 klux light intensity were correlative with light-saturated photosynthetic rates

Fig. 8 Varietal consumption patterns of light energy captured during a flash. Varietal symbols are the same as in fig. 3.

Fig. 9 Varietal light-photosynthesis curve. Varietal symbols are the same as in fig. 3.

Table 3 Chlorophyll concentration of the leaves used in photosynthesis measurement. mg chlorophyll (a+b)/dm²

Leaf position on the stem counted upward	3rd	4th	5th	6th	7th	8th	Mean
Age ^{a)}	25-26	22-23	19-20	16-17	13-14	6-7	
Variety							
Harosoy	2.32	4.93	4.89	4.63	4.44	4.30	4.17
Wasekogane	2.69	4.36	4.95	4.87	5.62	5.24	4.45
Shinanomejiro	2.83	3.74	4.46	3.03	4.85	3.86	3.67
Shiromeyutaka	1.59	2.92	3.27	3.84	4.00	3.69	3.15
Norin No. 1	1.60	2.44	3.27	3.26	3.66	2.89	2.78
YL-2-1	0.81	1.19	1.50	1.51	1.54	1.58	1.29

^{a)} Days from the start of leaf development.

Fig. 10 Comparison of relative photosynthetic rate under strong intermittent light and that under weak continuous light. ○ Intermittent light. ● Continuous light. Each dot represents mean value of five normal varieties. × The ratio, gross photosynthetic rate under continuous light/that under intermittent light.

($r=0.899^*$). The slopes were correlative also with max.f.y.s. ($r=0.990^{**}$).

5. Comparison of photosynthetic rates under strong intermittent light and weak continuous light

Utilization efficiency of incident light energy was dependent on the way how light energy was given to a leaf. The values of normal five varieties in fig. 6 and fig. 9 were averaged in fig. 10. The ratio of gross photosynthetic rate under continuous light/gross photosynthetic rate under intermittent light, was shown with the right hand scale. Even if the

mean incident light quantity per unit time was the same, weak continuous light was always more efficiently used than short strong flashes followed by dark periods. The ratio above mentioned increased as the mean incident light quantity decreased. The ratios themselves are variable depending on the experimental conditions; for instance saturating light intensity used or the shortest light period. But it may be concluded that the weak light which is given continuously was far more efficiently utilized than strong light which is given intermittently. The result is interesting in connection with the photosynthesis of lower leaves in leaf canopy.

6. Chlorophyll concentration

Chlorophyll concentration of the leaves used for photosynthesis measurements were listed in table 3. As stated earlier, leaves were analyzed on the day succeeding the last photosynthesis measurement, which made it impossible to refer the result in connection with photosynthetic rates. Concentration of YL-2-1 was about one-third of those of normal varieties.

DISCUSSION

Intermittent or flash light technique has brought about much progress in the studies of photosynthesis since the first experiment by Brown and Escombe³⁾. Blackman¹⁾ and Blackman and Smith²⁾ suggested the existence of two kinds of reactions in photosynthesis: photochemical reactions and dark reactions. Characteristics of the two reactions were skilfully examined by Emerson and Arnold⁴⁾ using intermittent light. These studies made me search for the experi-

mental technique which enabled measurement of dark reaction activities using intact leaves, but in vain. It occurred to me however that both the balance between the two reaction activities and photochemical activities might be measured using this technique in the way explained in "materials and methods" and that the relationship between photosynthetic rate and dark reaction activities might be estimated. To make sure that the idea was correct, a virescent mutant line YL-2-1 was added to the varieties formerly used. The experimental results of this line were just as expected and the idea seemed to be correct.

The experimental results so far obtained have led me to the conclusive estimation that dark reaction activities were also correlative with light-saturated photosynthetic rate, which will be explained as follows.

1. Close positive correlation was observed between photochemical activities and light-saturated photosynthetic rate. As indicative characters of photochemical activities, Hill activity of chloroplasts per unit leaf area,⁹⁾ max.f.y. and slope of light-photosynthesis curve at weak light were used to get the same conclusion.

2. With normal varieties, there existed little varietal difference in the balance between photochemical activities and dark reaction activities. This means that the variety having higher photochemical activities had a tendency to have higher dark reaction activities.

3. These two observations suggest the existence of close positive correlation between dark reaction activities and light-saturated photosynthetic rate.

The observed correlation between photochemical activities and light-saturated photosynthetic rate is not easy to interpret. It is not clear so far whether the correlation is causal or apparent. High productivity of photochemical products such as adenosine triphosphate (ATP) or reduced nicotinate adenine dinucleotide phosphate (NADPH₂) may promote dark reactions, just as increase in CO₂ concentration promotes dark reactions. These problems should be solved in future experiments.

SUMMARY

Varietal photosynthetic rates of soybean leaves [*Glycine max* (L.) Merrill] were measured under intermittent light in order to confirm the relationship between photochemical activities and light-saturated photosynthetic rate and to examine varietal differences in the balance between photochemical activities and dark reaction activities. Main results were as follows.

1. Varietal photosynthetic rates under saturating and continuous light were in the order of Harosoy > Wasekogane > Shiromeyutaka > Shinanomejiro > Norin NO. 1 > YL-2-1. The order was almost the same as that of previous report⁹⁾.

2. A virescent mutant line YL-2-1 had smaller ratio of photochemical activities/dark reaction activities, than normal varieties.

3. Regarding the other normal varieties, varietal difference was not observed as a whole in the balance between the two reaction activities. Namely, the variety having higher photochemical activities was supposed to have higher dark reaction activities.

4. Maximum flash yields, one of the indicative characters of photochemical activities, were positively correlated with light-saturated photosynthetic rates. This result was in accordance with the previous report⁹⁾, where Hill activity of chloroplasts was measured.

5. A positive correlation was supposed between dark reaction activities and light-saturated photosynthetic rate.

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[和 文 摘 要]

大豆葉同化速度の品種間差異の生ずる機作

第2報 光化学活性と暗反応活性との相対関係の品種間差

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間けつ光下での大豆個葉の同化速度を測り、光化学活性と暗反応活性との相対関係の品種間差および飽和光下同化速度と光化学活性との関係を調べた。また暗反応活性と飽和光下同化速度との関係を推定した。その結果次に示す諸点が明らかにされた。

1. 連続飽和光下での同化速度品種間順位は、Harosoy>ワセコガネ>シロメユタカ>しなのめじろ>農林1号>YL-2-1であつた。この順位は前報の順位とはほぼ一致した。
2. クロロフィル突然変異系統の YL-2-1 では、光化学活性/暗反応活性の比が正常品種に比して明らかに小さかつた。
3. 正常品種では両活性の相対関係の品種間差はほとんど認められなかつた。すなわち、光化学活性の高い品種は、概してその暗反応活性もまた高い傾向があるものと推定された。
4. 光化学活性の指標の一つとして測定した maximum flash yield と飽和光下同化速度との間に正の相関関係が認められた。この結果は、光化学活性の指標の一つとして、クロロプラストの Hill 活性を測定した前報の結果と一致した。
5. 暗反応活性と飽和光下速度との間にも正の相関関係があることが推定された。