Tohoku Journal of Agricultural Research
Vol. 23, No. 1, April 1972
*Printed in Japan*

# Relationship of Chlorophyll Content, Chloroplast Area Index and Leaf Photosynthesis Rate in *Brassica*

Kunio KARIYA and Shigesaburo TSUNODA

*Department of Agronomy, Faculty of Agriculture, Tohoku University, Sendai, Japan*

(Received December 27, 1971)

## Summary

An attempt was made in this paper to analyse the chlorophyll content of leaves into two components i.e., the chlorophyll content of a single chloroplast and the chloroplast number, in relation to the rate of leaf photosynthesis. Among nine strains of *Brassica campestris*, *oleracea*, and *Napus* as well as among $F_1$ and $F_2$ plants derived from a cross between dark green and pale green strains of *campestris*, close positive correlations were observed between the number of chloroplast and the rate of photosynthesis at 50,000 lux. While, an increase in the chlorophyll content of a single chloroplast was not necessarily associated with an increase in the photosynthetic rate determined at 50,000 lux.

The relationship between the rate of photosynthesis and the chloroplast area index (CAI) was still more distinct than that between the rate and the chloroplast number. The CAI is a new parameter introduced in this paper to express total sizes of chloroplast-area relative to a unit of leaf-area. This CAI is comparable with the leaf area index (LAI) which has been adopted in leaf-canopy analysis. It may be possible to increase the photosynthetic rate by breeding a strain with a high CAI.

The relationship between photosynthesis rate and chlorophyll content has received attention since the late 19th century. It has been confirmed that the content sharply affects the rate under light-limited conditions (1, 2). However, there is a discrepancy between the results obtained under light-saturated conditions. With some plant materials a positive association was still observed between the content and light-saturated photosynthesis, while with other materials no positive association was found between them.

A variation in the chlorophyll content per unit leaf-area may be brought about through a change either in the chlorophyll content in a single chloroplast or in the chloroplast number per unit leaf-area. An attempt is made in this paper to analyse the chlorophyll content of leaves into these two components. The results clearly show that the chlorophyll content of a single chloroplast is not necessarily positively related to the rate of photosynthesis in high light intensity, while an increase in the chloroplast number is associated with an increase in the rate.

In the early stages of investigation on leaf photosynthesis, Haberlandt (3) and Griffon (4) pointed out a possible relationship between the number and size of the chloroplasts and the photosynthetic rate. Thereafter, so far as we know, few investigations have been made to elucidate the exact relationship between them. The work reported here is specifically designed to make this point clear.

We introduce in this paper a new parameter termed the chloroplast area index (CAI) to express total chloroplast-area per unit leaf-area. This is a product of chloroplast number and chloroplast size, and is comparable with the leaf area index (LAI) which has been adopted in leaf-canopy analysis. The relationship between the rate of photosynthesis and the CAI was still more distinct than that observed between the rate and the chloroplast number.

## Materials and Methods

### Experiment 1.

Nine strains of three cultivated species of *Brassica* were used (Table 1). These were chosen from genetic stocks maintained at the Faculty of Agriculture, Tohoku University.

TABLE 1., *Materials used.*

| Strain | | Species | Genome |
|---|---|---|---|
| C101* | Hatana-4 turnip rape | *Brassica campestris* | AA |
| C220 | Matsushima-2 Chinese cabbage | 〃 | 〃 |
| C333 | Yukina turnip green | 〃 | 〃 |
| C334 | Mana turnip green | 〃 | 〃 |
| 0 5 | Yayoi cabbage | *Brassica oleracea* | CC |
| 0 8 | Yoshin tropical cabbage | 〃 | 〃 |
| N101 | Yokkaichi-kurodane rape | *Brassica Napus* | AACC |
| N264 | Sensation swede | 〃 | 〃 |
| N343 | Early giant fodder rape | 〃 | 〃 |

*: Registered strain number at Tohoku University.

Plants, planted on 9 September 1968, were grown outdoors for about one and a half months. A random block design with three replications was adopted. One block was formed by a concrete frame (120 cm×300 cm) filled with sieved loam soil mixed with ammonium sulfate, super phosphate, and potassium chloride containing 75 g nitrogen, 60 g $P_2O_5$ and 75 g $K_2O$, respectively.

Leaf disks (1.13 $cm^2$), punched out from the middle portion of fully expanded leaves of six to eight plants of each strain, were used for determinations of photosynthetic rates, chlorophyll contents, and cell numbers per unit leaf-area.

Photosynthetic rates were measured manometrically on single leaf disks as the amount of $O_2$ evolution under a light intensity of 50,000 lux at 30°C using the same photosynthetic respirator and the $CO_2$ buffer reported by Sasahara (5).

Measurements were replicated from six to eight times per strain.

The chlorophyll contents were determined according to Arnon's method (6). Two or three replications were made to get an average value, using four to six disks for each determination.

To count the cell number, leaf disks were fixed in FAA, washed in water, and macerated in 5% chromic acid at 30°C for 12–24 hours. Cells were separated with a magnetic stirrer, and the cell numbers were counted with a hemocytemeter. Then, the cells were stained with first green FCF (Merck) and the chloroplast numbers in single cells were counted under a light microscope. Measurements were repeated three times per strain.

To estimate the chloroplast size, leaf blades were homogenized with ice-cold 1/2 M sucrose solution (1/15 M phosphate buffer, PH 7.0). The debris was removed through eight layers of gauze and the homogenate was centrifuzed at 1,000 $\times$g for 5 minutes. Photographs of the chloroplasts in the precipitate were taken immediately, and the long and short diameters were measured, projecting the film with an enlarger. Areas of single chloroplasts were calculated as $\pi$ab, where 2a and 2b were the long and short diameter of the chloroplast, respectively.

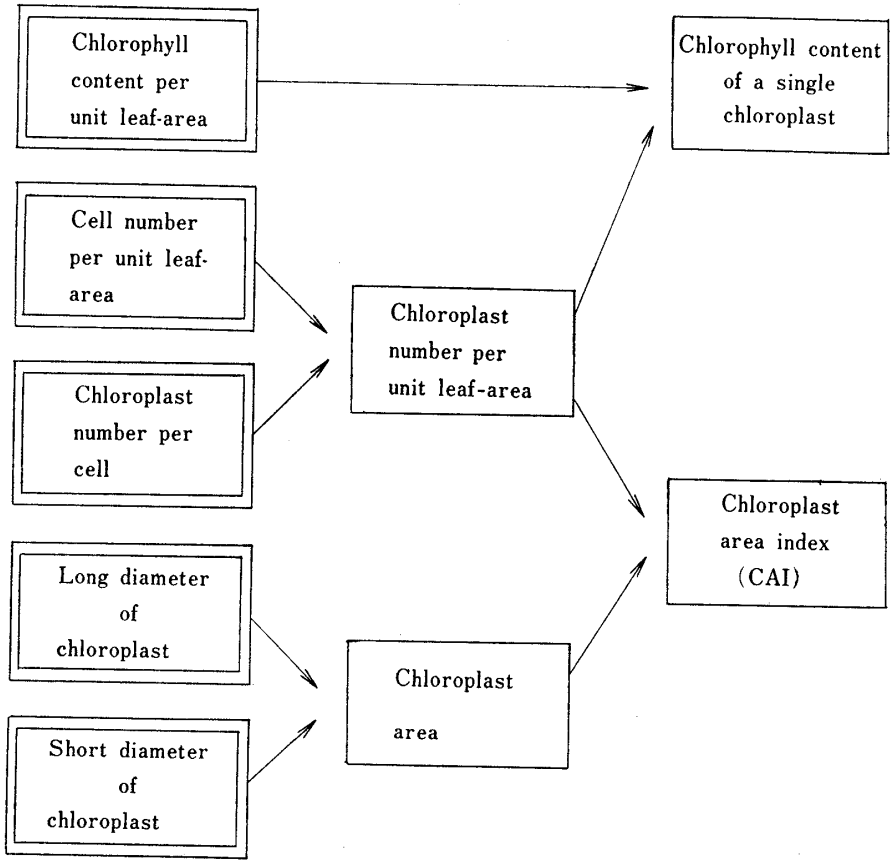


FIG. 1. Procedure of evaluation
▣ measured □ calculated

Based on these data, values for some other quantitative characteristics such as chlorophyll content per chloroplast, chloroplast number per unit leaf-area, and chloroplast area index (CAI, the ratio of total chloroplast-area to leaf-area) were computed according to the procedure shown in Fig. 1.

**Experiment 2.**

$F_1$ and $F_2$ progenies derived from the reciprocal hybridization between two strains of *campestris*, i.e., C333 Yukina with dark green leaves and C334 Mana with pale green leaves, were observed.

Plants from seed sown on 15 February 1970 were transplanted singly in pots (9 cm in dia., 20 cm in ht.) on 4 March, and grown in a greenhouse for about one and a half months at 30/25°C. N, $P_2O_5$ and $K_2O$ were fertilized at rates of 1.4 g, 1.8 g, and 1.4 g per pot, respectively. Plants were selected to cover a range in individual visual variation in each generation, and individual variations in leaf photosynthetic rate and other leaf characters were observed using the same methods as adopted in Experiment 1. Measurements were repeated twice per individual in most cases.

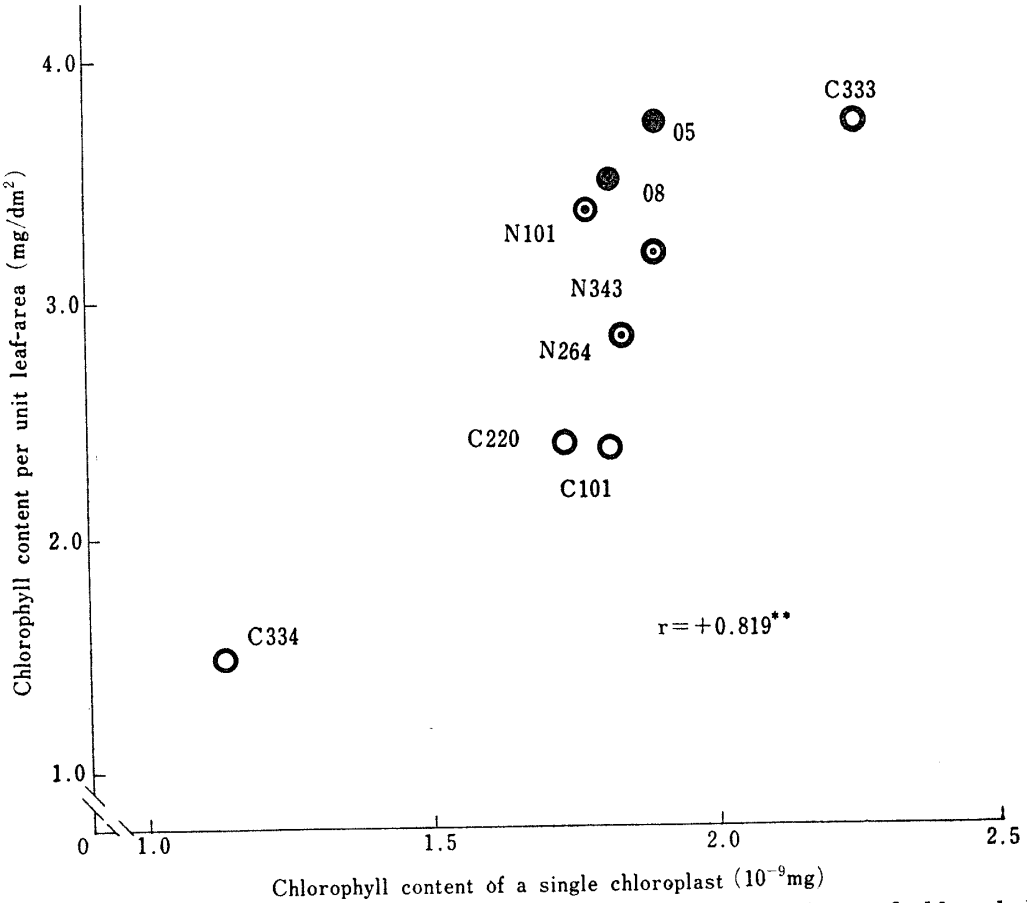


FIG. 2. Relationship between chlorophyll content of a single chloroplast and chlorophyll content per unit leaf-area (Exp. 1)
○ *B. campestris* ● *B. oleracea* ⊙ *B. Napus* **: significant at 1% level

## Results

### Experiment 1.

*Relationships of chlorophyll content per unit leaf-area to chlorophyll content of a single chloroplast and to chloroplast number per unit leaf-area*: The chlorophyll content per unit leaf-area can be analysed into two components; chlorophyll content of a single chloroplast and chloroplast number per unit leaf-area. As shown in Figs. 2 and 3, the chlorophyll content per unit leaf-area was positively correlated with the chlorophyll content of a single chloroplast as well as with the chloroplast number per unit leaf-area among strains observed.

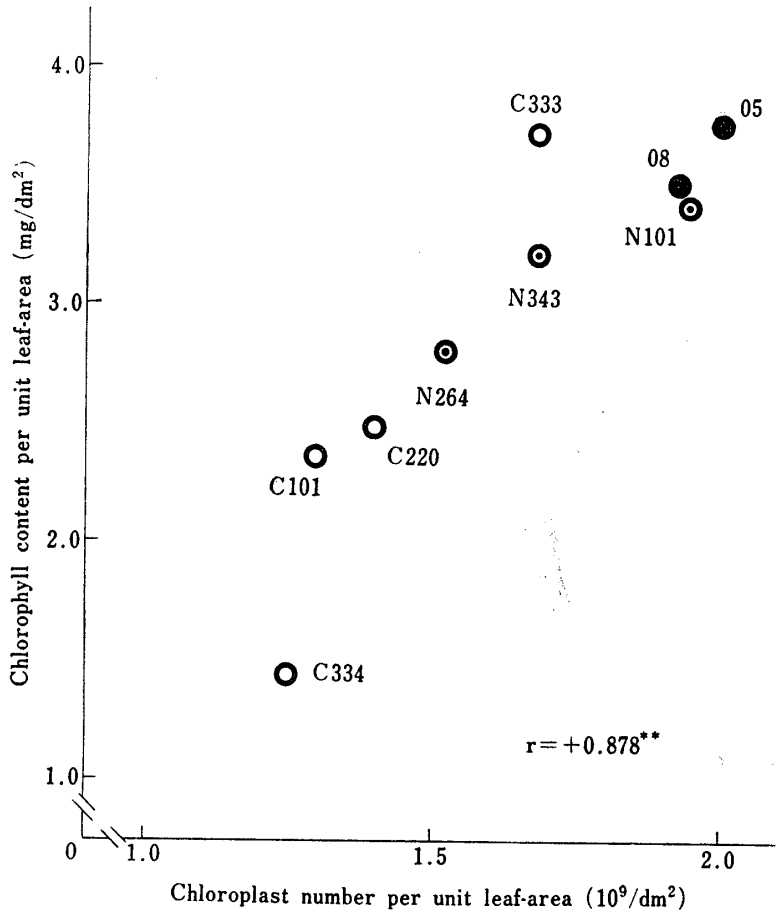


FIG. 3. Relationship between chloroplast number per unit leaf-area and chlorophyll content per unit leaf-area (Exp. 1)
**:significant at 1% level

*Relationship between photosynthetic rate per unit leaf-area and chlorophyll content per unit leaf-area*: The photosynthetic rate per unit leaf-area was positively related to the chlorophyll content per unit leaf-area, as shown in Fig. 4. However, among the *campestris* strains, C333 tended to show a relatively low rate for its high chlorophyll content, while C334 showed a relatively high rate for its low chlorophyll content.

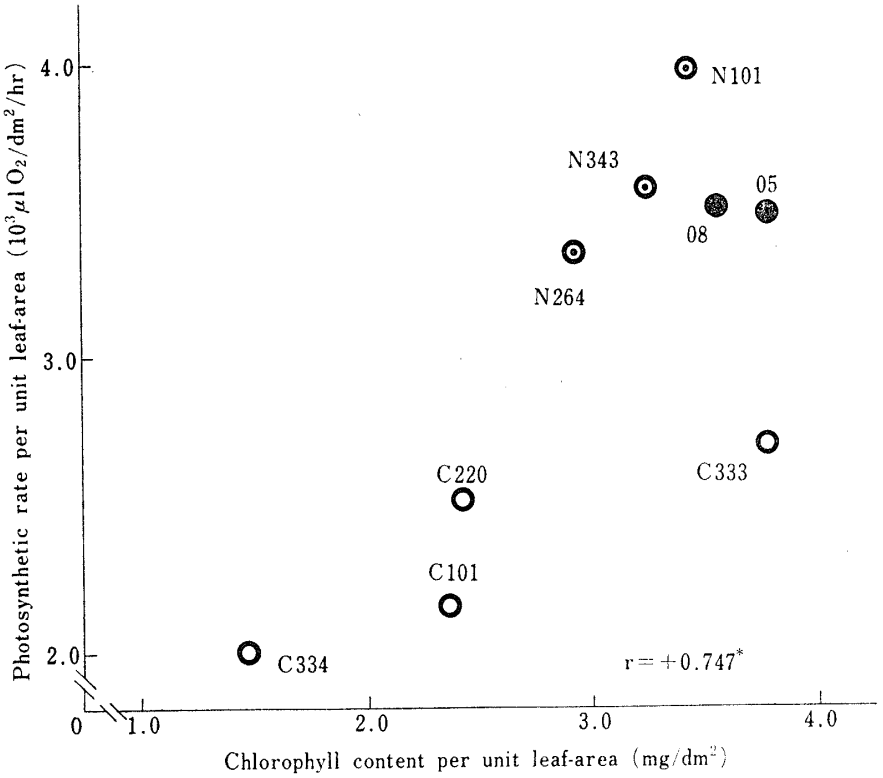


FIG. 4. Relationship between chlorophyll content per unit leaf-area and photosynthetic rate per unit leaf-area (Exp. 1)
*: significant at 5% level.

*Relationships of photosynthetic rate per unit leaf-area to chlorophyll content of a single chloroplast and to chloroplast number per unit leaf-area*: As shown in Fig. 5, the photosynthetic rate per unit leaf-area was not significantly correlated with the chlorophyll content of a single chloroplast. On the contrary, a highly significant positive correlation was observed between the rate and the chloroplast number per unit leaf-area, as shown in Fig. 6.

*Relationship of photosynthetic rate per unit leaf-area to chloroplast area index (CAI)*: As shown in Fig. 7, a highly significant positive correlation was observed between the photosynthetic rate per unit leaf-area and the CAI i.e., the ratio of total chloroplast area to leaf-area. In this case, the total chloroplast area was computed as a product of the number of chloroplast per unit leaf-area and the mean area of a single chloroplast computed as $\pi ab$. The correlation coefficient was as high as +0.938.

**Experiment 2.**

*Relationships of chlorophyll content per unit leaf-area to chlorophyll content of a single chloroplast and to chloroplast number per unit leaf-area*: Among $F_1$ as well as among $F_2$ plants, highly significant positive correlations were found between the

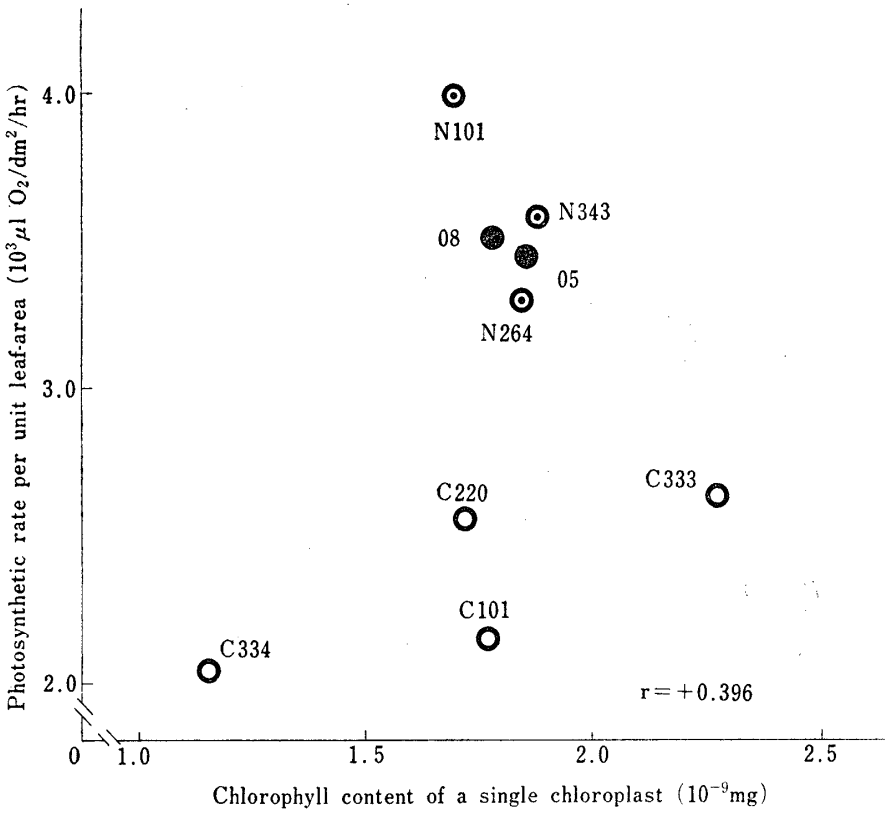


FIG. 5. Relationship between chlorophyll content of a single chloroplast and photosynthetic rate per unit leaf-area (Exp. 1)

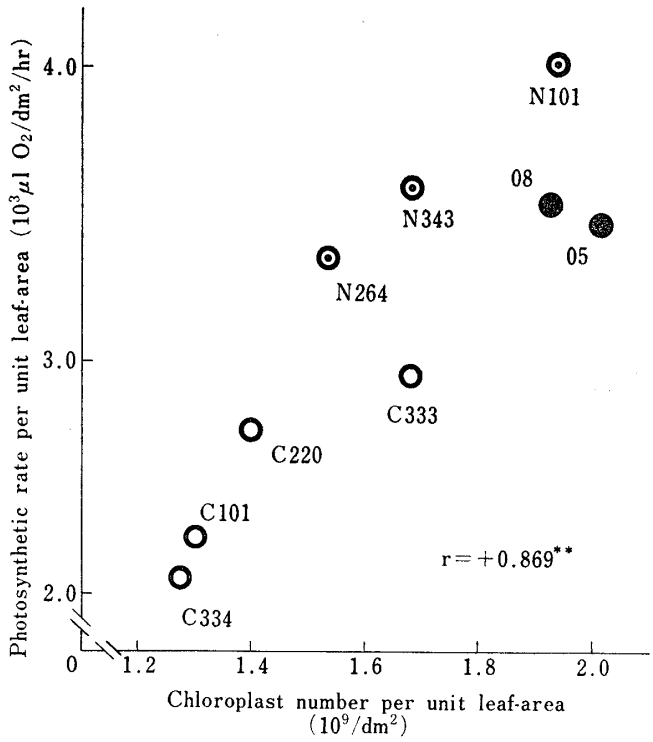


FIG. 6. Relationship between chloroplast number per unit leaf-area and photosynthetic rate per unit leaf-area (Exp. 1)
**: significant at 1% level.

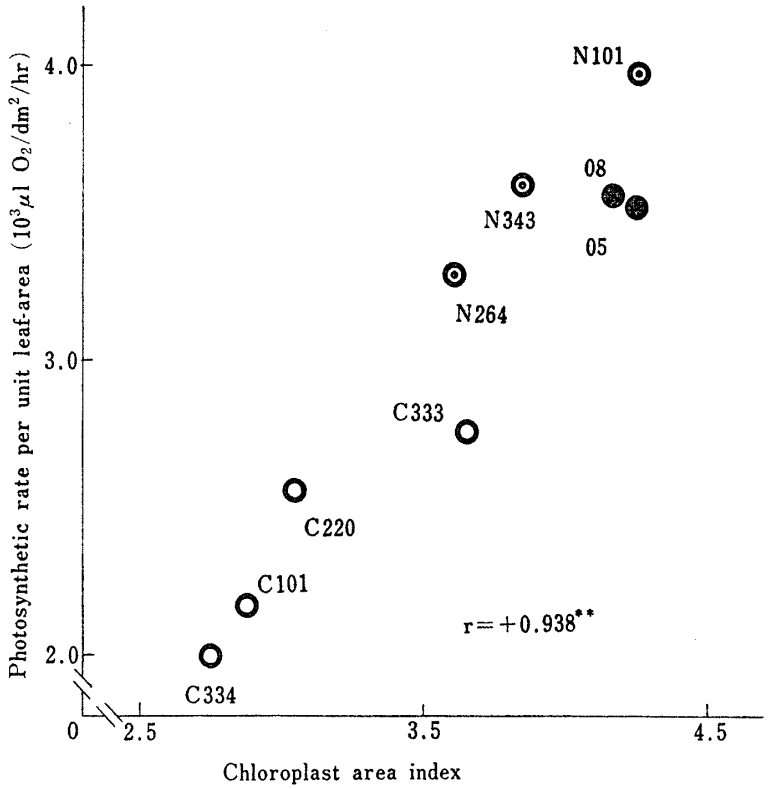


FIG. 7. Relationship between chloroplast area index and photosynthetic rate per unit leaf-area (Exp. 1)
**: significant at 1% level

chlorophyll content per unit leaf-area and the chlorophyll content of a single chloroplast, as shown in Fig. 8. The correlation between the chlorophyll content per unit leaf-area and the chloroplast number per unit leaf-area was not significant at the 0.05 level both in $F_1$ and $F_2$, as shown in Fig. 9.

*Relationship between photosynthetic rate per unit leaf-area and chlorophyll content per unit leaf-area*: As shown in Fig. 10, the photosynthetic rate per unit leaf-area was not correlated with the chlorophyll content per unit leaf-area among $F_1$ plants nor among $F_2$ plants.

*Relationships of photosynthetic rate per unit leaf-area to chlorophyll content of a single chloroplast and to chloroplast number per unit leaf-area*: As shown in Fig. 11, the relation between the photosynthetic rate per unit leaf-area and the chlorophyll content of a single chloroplast was rather negative among $F_1$ and $F_2$ plants, while the rate was positively correlated with the chloroplast number per unit leaf-area as shown in Fig. 12.

*Relationship of photosynthetic rate per unit leaf-area to chloroplast area index (CAI)*: As shown in Fig. 13, a clear positive correlation was found between the photosynthetic rate and the CAI. The correlation coefficient was higher than that obtained between the rate and the number of chloroplasts per unit leaf-area.

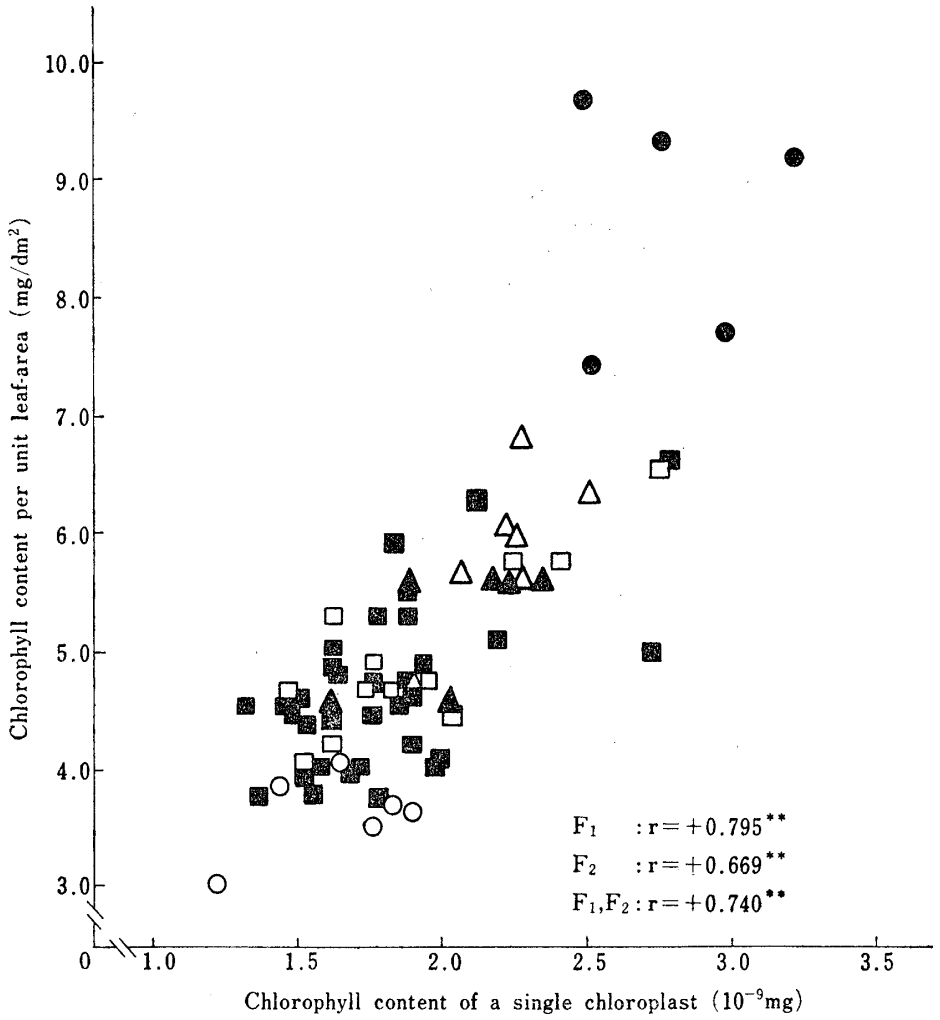


FIG. 8 Relationship between chlorophyll content of a single chloroplast and chlorophyll content per unit leaf-area (Exp. 2)
● C333 ○ C334 ▲ $F_1$ (333×334) △ $F_1$ (334×333) ■ $F_2$ (333×334)
□ $F_2$ (334×333) **: significant at 1% level

## Discussion

As mentioned in the introduction, it is known that the rate of photosynthesis is not necessarily related to the chlorophyll content, at least under high light intensities.

In the present experiments, the rate was observed under a relatively high light intensity of 50,000 lux. In Experiment 1, a positive correlation was still found between the rate and the content (Fig. 4). In this case, however, the chlorophyll content per unit leaf-area was strongly affected by change in chloroplast number (Fig. 3), and the correlation between the rate and the chloroplast number was more distinct than that between the rate and the chlorophyll content (Fig. 6). In Experiment 2, among hybrid progenies derived from a cross between

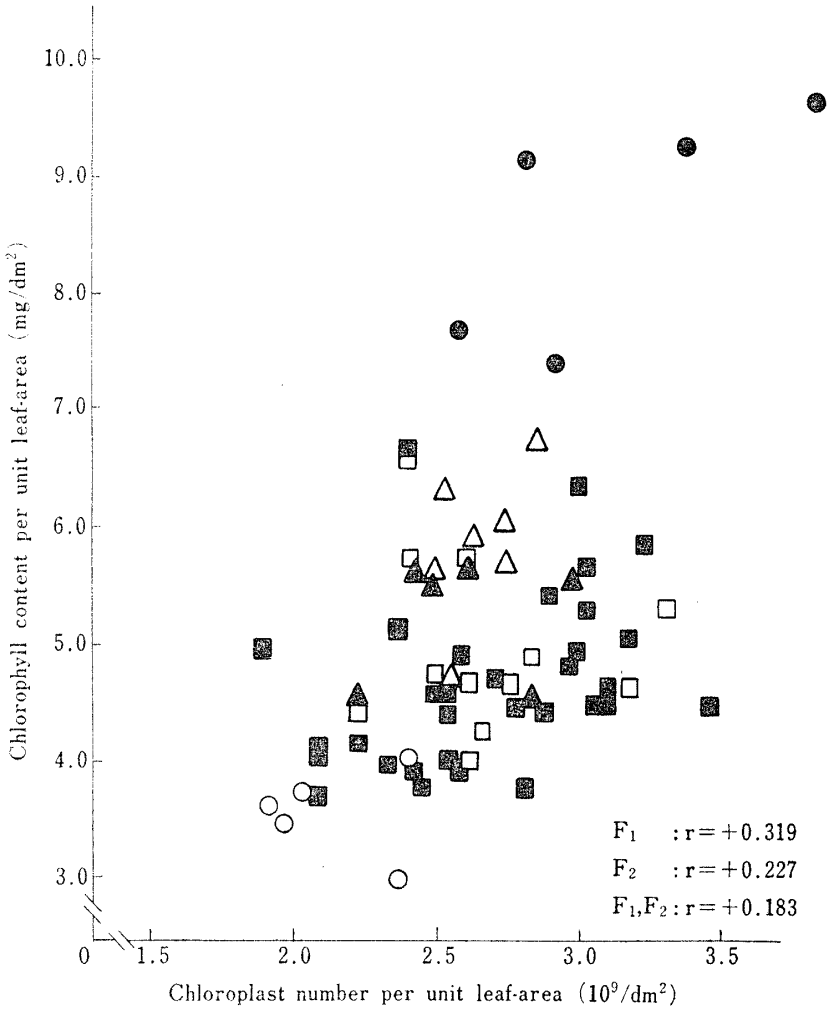


FIG. 9 Relationship between chloroplast numbr per unit leaf-area and chlorophyll content per unit leaf-area (Exp. 2)

dark green and pale green strains, the rate was not correlated with the content at all, though a considerable variation was found in the content among plants tested (Fig. 10). In this case, the chlorophyll content per unit leaf-area was mainly affected by a variation in chlorophyll content of a single chloroplast (Fig. 8), and not by a change in the chloroplast number per unit leaf-area (Fig. 9).

These results suggest that an increase in the chlorophyll content does not bring about an increase in the photosynthetic rate at least in high light intensity, unless the high content is associated with an increase in chloroplast number.

On the other hand, highly significant correlations were observed between the rate and the number of chloroplasts per unit leaf-area in Experiment 1 (Fig. 6) as well as in Experiment 2 (Fig. 12).

The correlation between the rate and the chloroplast area index (CAI) was still more distinct than that between the rate and the chloroplast number in Experiment

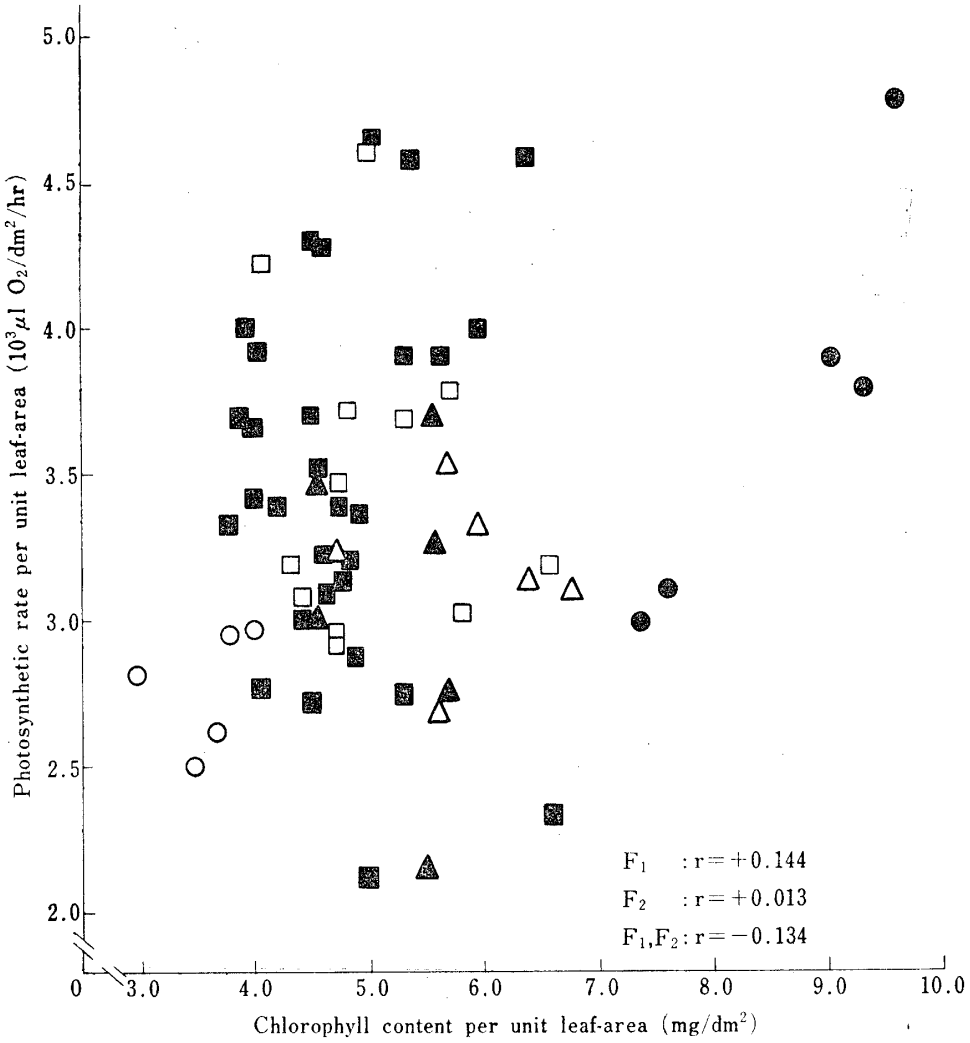


FIG. 10 Relationship between chlorophyll content per unit leaf-area and photosynthetic rate per unit leaf-area (Exp. 2)

1 (Fig. 7) as well as in Experiment 2 (Fig. 13). CAI is a product of chloroplast number and chloroplast size, and may be useful as an index to express the total size of chloroplast-area relative to a unit of leaf-area.

In leaf canopy photosynthesis, an increase in the leaf area index (LAI) tends to be associated with an increase in the photosynthetic rate per unit land-area, until it reaches a critical value termed the optical leaf area index. The chloroplast area index (CAI) which we introduced here, showed a close positive relation to the photosynthetic rate per unit leaf-area, at least in the range of 2.5 to 4.5 in Experiment 1 (Fig. 7) and in the range of 4.0 to 8.0 in Experiment 2 (Fig. 13). These results suggest that we can increase the rate of photosynthesis by breeding a strain with a higher CAI value.

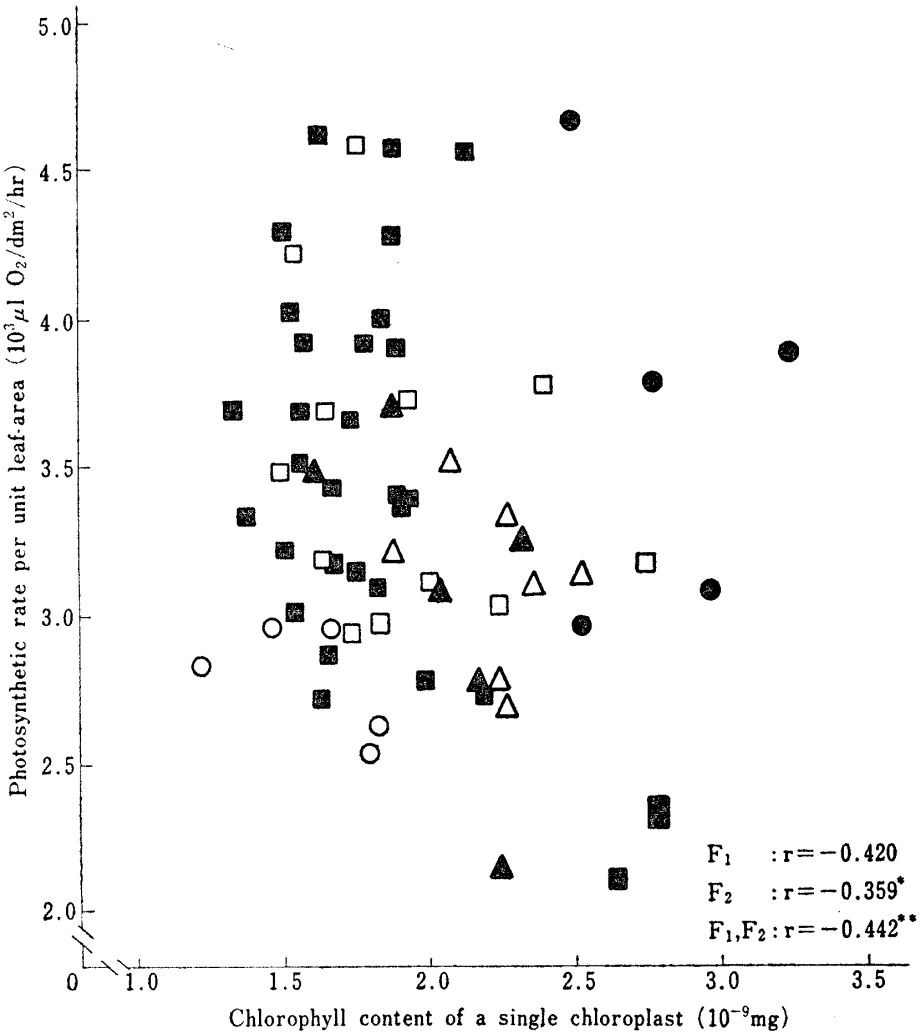


FIG. 11 Relationship between chlorophyll content of a single chloroplast and photosynthetic rate per unit leaf-area (Exp. 2)
*: significant at 5% level **: significant at 1% level

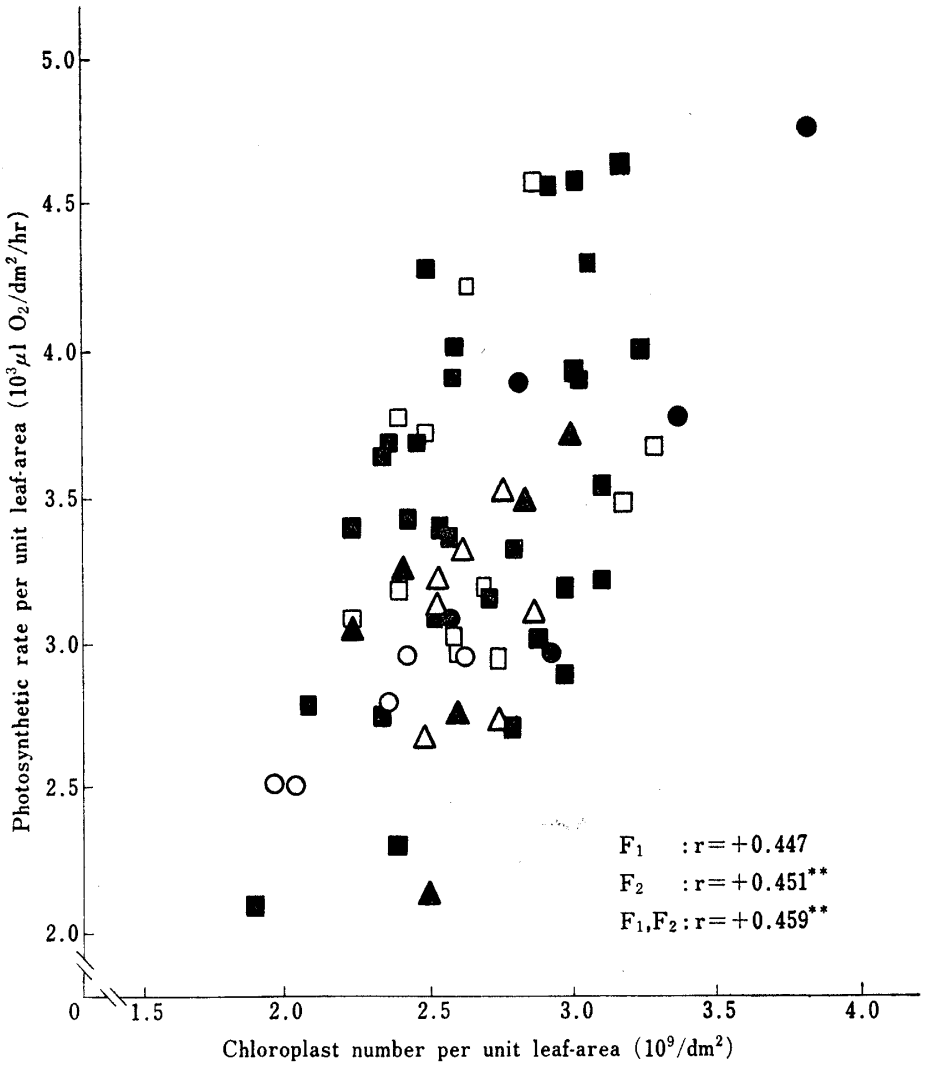


FIG. 12 Relationship between chloroplast number per unit leaf-area and photosynthetic rate per unit leaf-area (Exp. 2)
**: significant at 1% level.

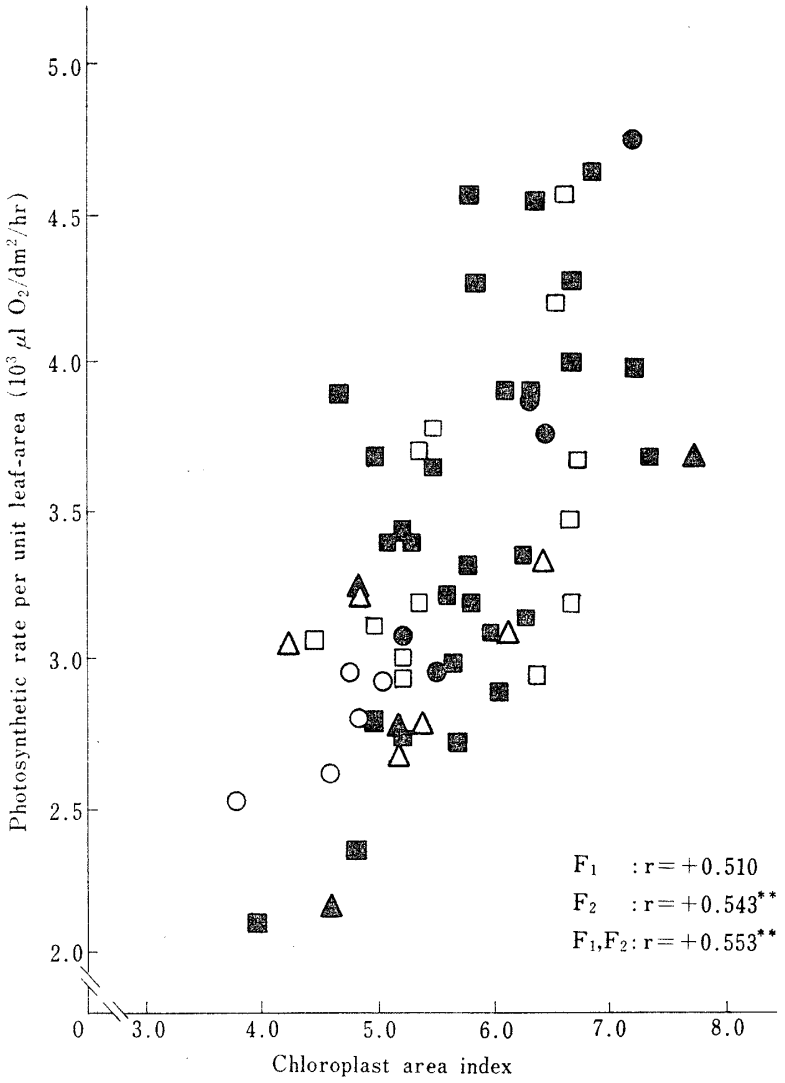


FIG. 13 Relationship between chloroplast area index and photosynthetic rate per unit leaf-area (Exp. 2)
**: significant at 1% level

## Acknowledgement

The authors are heartily grateful to Mr. Takeo Sasahara for his valuable suggestions and co-operation in these experiments.

## References

1) Willstätter, R., and Stoll, A., "Untersuchungen über die Assimilation der Kohlensäure", Springer, Berlin (1918)
2) Gabrielsen, E.K., *Physiol. Plantarum*, **1**, 5 (1948)
3) Haberlandt, G., "Physiological Plant Anatomy", translated from the 4th German edition by Drummond, M., Macmillan, London (1914)
4) Griffon, E., *Ann. Sci. Nat. Bot.* (Paris), **10**, 1 (1899), cited in Gabrielsen (1948)
5) Sasahara, T., *Japan. J. Breeding* **21**, 61 (1971)
6) Arnon, D.I., *Plant Physiol.*, **24**, 1 (1949)