Studies on the Differences of Photosynthesis among Species

I. Differences in the response of photosynthesis among species in normal oxygen concentration as influenced by some environmental factors*

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Recent progress in research on the cause of differences in photosynthesis among species has been very rapid, since it was found that photosynthetic rate per unit leaf area varied many fold among species^{11,19}. Two important factors which may be concerned with the cause of such differences have been clarified by recent investigations: The one is "light respiration" —carbon dioxide liberation in the light²⁶— and the other is a new carboxylation pathway discovered by Kortschak et al¹⁶, and ascertained by Hatch et al^{9,10}. Maize, sugarcane, amaranthus and some tropical grasses do not liberate CO₂ in the light^{18,25}, i. e. their "CO₂ compensation point" (CCP) is near zero, while rice, sunflower, tobacco, soybean and most temperate grasses show high CCP. This implies the presence of "light respiration" pathway in the latter group.

Many evidences have shown the possibility of the former group to have the C-4 pathway in carboxylation^{9,10,23}. Viewed from these discoveries, it will be quite natural to suppose that such differences in dark reactions may be closely associated with the different responses of photosynthesis to environmental factors.

It was attempted to clarify the responses of photosynthesis to some influential environmental factors for the better understanding of differences of photosynthesis among species.

The response of photosynthesis to CO₂ concentration is quite indicative of the differences in carbon reduction cycle among species. There are many investigations on the "carbon dioxide curves"²⁰, but very few investigations have been

conducted with the aim to clarify species differences in photosynthesis except a series of reports by Hesketh et al^{11,12}. They obtained "carbon dioxide curves" of some species different in photosynthetic activity under a strong light intensity and found out differences in CO₂ liberation rate in CO₂-free air in the light. They showed that the photosynthetic rate of tobacco under a CO₂ concentration higher than 1,000 ppm surpassed that of maize.

Also many reports have been done to clarify the physiological significance of CCP. However, the CCP simply shows the intercellular CO₂ concentration in the light and does not seem to have any more physiological significance.

Hesketh et al^{11,12} also examined the differences of "light curves" among plants, and reported that the "light curves" of maize and sugar cane did not reach clear saturation up to a light intensity of about 100 klux.

As to the effect of temperature on photosynthesis among species, Murata et al¹⁹, reported that grasses could be classified into five different groups in terms of response of apparent photosynthesis to temperature.

However, the response of photosynthesis to a certain environmental factor is not independent from others, that is, photosynthesis is a function of many variables, CO₂ concentration, light intensity, temperature and O₂ concentration. It must, therefore, be investigated more collectively. We have conducted some experiments to clarify the differences among species in the photosynthetic response under their mutual interdependence to CO₂ concentration, light intensity and

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temperature. In other words, the function P (CO₂, light intensity, temperature) has been investigated in this experiment (Experiment-I).

On the other hand, it is natural to suppose that differences in physiological response of photosynthesis to environmental factors would be accompanied by morphological differences in photosynthesizing tissues. However, very few workers who investigated on the morphology of photosynthesizing tissues were aware of such great differences in photosynthetic rate per unit leaf area and in dark reaction of photosynthesis. According to Koriba¹⁵⁾, photosynthesizing tissues are well-developed around the vascular bundle of some Monocotyledoneae, some species of Gramineae and Cyperaceae. It was called "Chlorophyll Sheide" and he also commented on its functions.

Esau⁸⁾ reported on the structure of photosynthesizing tissue, pointing out the special starch-rich cell-arrangement with dense green color around the vascular bundle. She named it "Parenchymatous bundle sheath" and also commented on its probable function. According to her, this tissue plays an important role in connecting the mesophyll tissue and vascular bundle.

According to Maximov¹⁷⁾, those plants which had such a tissue were more resistant to drought and dry matter production per required water was higher than those which did not have such tissues.

Silaeva²²⁾ and many other Russian workers tried to clarify the function of this tissue. They found the same tissue in the stem of pumpkin and in the leaf petiole of beet too. As to the function, there are contradictory opinions. Some workers maintain from isotopic observation that these tissues play more important role in photosynthesis than the mesophyll tissues, and others insist that the tissues play an important role in translocating photosynthetic products to other organs because of its position, that is, usually it lies in the innermost part of leaf or stem so that it is inconvenient for utilization of light energy.

Silaeva²²⁾ made an electrosopic observation of that tissue and found out that the shape of chloroplasts in that tissue was clearly different from that in mesophyll tissues.

Stebbins²⁴⁾ tried to classify Gramineae from cytological point of view and reported that Panicoideae and Chloridoideae had this tissue and Al-Aish⁸⁾ found out those plants with this tissue were resistant to herbicide isopropyl-N-phenyl carbamate (IPC).

After the great differences in photosynthetic rate per unit leaf area had been discovered, El-Sharkawy et al⁷⁾ tried to clarify the causes for such differences from morphological point of view. They failed to find out definite morphological causes but they pointed out that most tropical grasses which showed high photosynthetic rate had the unique cell arrangement around the vascular bundles. On the other hand, Hatch et al¹⁰⁾ have recently reported that some tropical grasses belonging to Gramineae and Cyperaceae have the C-4 pathway and Hesketh¹⁴⁾ reported that the photosynthetic rate of most tropical grasses was not enhanced in the oxygen-free air in contrast to most temperate grasses.

Downton et al⁶⁾ have recently reported that all the plants mentioned above have the same characteristics in the morphology of leaf and physiological responses of photosynthesis, that is, they have exclusively chlorophyllous bundle sheath around the vascular bundles and show low carbon dioxide compensation point. Further, it has been suggested that they are lacking in the light respiration and have the C-4 pathway in carboxylation.

Experiments were conducted, therefore, to ascertain these points using more other species and also to make the survey on the distribution of this tissue among species (Experiment-II).

Materials and Methods

Materials used for Expt-I were *Paspalum dilatatum* (dallisgrass), *P. notatum* (bahiagrass), *Echinochola Crus-galli* var. *caudata* (barnyard-

grass), *S. vulgare*, *Dactylis glomerata* (orchardgrass), *Avena sativa* (oat), *Hordeum vulgare* (barley), *Triticum aestivum* (wheat) and *Oryza sativa* (rice var. Norin No. 29). They were soil-cultured in a one-liter beaker in a green house. One-month old seedlings about 20~25 cm tall were used. Some wild plants were also used to examine the types of "carbon dioxide curves".

To avoid the decrease of photosynthesis due to leaf water deficiency and others, intact seedlings with roots were used as material.

Apparatus used is shown in fig. 1. As a CO₂-generator we used a dark box which contained many intact plants. By combining a soda lime tower with this, we could freely adjust the CO₂ concentration to any required level ranging from zero to 500ppm. Illumination was made with incandescent lamps. In experiments to obtain the "light curves", an apparatus shown in fig. 1-A was used. A small fan was accommodated on the side wall of the chamber to stir the air in the assimilation chamber. Illumination was made exclusively from above. Temperature was kept constant automatically.

In experiments where light intensity was not changed, an apparatus shown in fig. 1-B was used. The chamber was illuminated from both sides of it with an equal light intensity. The material for these experiments was a few seedlings which were taken out carefully from the pot so as not to hurt the root and placed with the root dipped in water, in the assimilation chamber made of glass tube. Temperature was changed by regulating the temperature of surrounding air of the chamber. In both apparatuses, light scattering paraffin paper was inserted between water heat filter and assimilation chamber to equalize the distribution of light as well as to enhance the efficiency of its utilization by plants. Intensity of illumination was adjusted by changing the number of sheet of

Fig. 1 Apparatus to get the relationships of CO₂ concentration, temperature, and light intensity to photosynthesis of plants.

- A. Apparatus used to obtain "light curves".
B. Apparatus used to obtain "CO₂ curves".

the paper or the distance of the chamber from the light source. Light intensity was measured by an illumination meter (Toshiba No. 5). When illumination was made from both sides of the chamber, light measurement was done at one side of it.

Leaf temperature was measured by thermocouples attached to the leaves by cellophane tape. Temperature was changed from low high in ordinary cases.

Light intensity was changed from low to high in ordinary cases. However, light intensity was not increased beyond 40 klux because the photosynthetic rate under a high light intensity was not stable. It was probably due to rapid water loss from leaves. Air flow rate was regulated to 100~150 l/hr. The effect of air flow rate on photosynthesis was checked. However, the effect was proved to be little. All the experiments were carried out under the same oxygen concentration as that of ordinary air. The mathematical mean of the CO₂ concentration at the

Table 1. Materials used for microscopic observation and measuring carbon dioxide compensation point.

Monocotyledoneae

Family, Tribe	Genus, Species	Common name English	Japanese
Gramineae			
Oryzeae	<i>Oryza sativa</i> <i>O. granulata</i> <i>O. officinalis</i> <i>O. eichingeri</i> <i>O. brachyantha</i> <i>O. latifolia</i> <i>O. Ridleyi</i> <i>O. minuta</i> <i>O. Sublata</i> <i>O. breviligulata</i> <i>O. punctata</i> <i>O. australiensis</i> <i>O. malampuzhaensis</i> <i>O. glandiglumis</i> <i>O. paraguayensis</i> <i>Zizania latifolia</i>	Rice	Ine
Festuceae	<i>Dactylis glomerata</i> <i>Lolium multiflorum</i> <i>L. perenne</i> <i>Poa pratensis</i> <i>P. annua</i> <i>Festuca ampla</i> <i>Bromus catharticus</i> <i>B. remotiflorus</i> <i>B. japonicus</i>	Orchard grass Italian ryegrass Perennial ryegrass Kentucky bluegrass Annual bluegrass Rescue grass	Makomo Kamogaya Nezumemugi Nagahagusa Suzumenokatabira Inumugi Kitsunegaya Suzumenochahiki
Triticeae	<i>Hordeum vulgare</i> <i>H. distichon</i> <i>Aegilops cylindrica</i> <i>A. perignina</i> <i>Agropyron ciliare</i> <i>A. transiens</i> <i>Triticum aestivum</i> <i>Secale cereale</i>	Barley Common barley Wheatgrass Wheat Rye	Oomugi Yabaneoomugi Aokamozigusa Kamozigusa Komugi Raimugi
Aveneae	<i>Avena fatua</i> <i>A. sativa</i>	 Oat	Karasumugi Enbaku
Agrostideae	<i>Agrostis alba</i> <i>Phleum alpinum</i> <i>P. paniculatum</i> <i>Alopecurus aequalis</i>	Red top Timothy Rough timothy	Konukagusa Miyama awagaeri Awagaeri Suzumenoteppou
Bambuseae	<i>Phragmites communis</i>	Reed	Yoshi
Arundineae	<i>Zoisa japonica</i>		Shiba
Chlorideae	<i>Cynodon dactylon</i>	Bermudagrass	Gyoungishiba
Eragrosteae	<i>Eleusine indica</i> <i>Eragrostis ferrugineae</i> <i>E. multicaulis</i>	Goosegrass Lovegrass	Ohishiba Kazekusa Niwahokori
Paniceae	<i>Digitaria adscendens</i> <i>Echinochloa Crus-galli</i> var. <i>caudata</i>	Fingergrass	Mehishiba Keinubie

Family, Tribe	Genus, Species	Common name English	Japanese
Andropogoneae	<i>E. Crus-galli</i> var. <i>hispidula</i>		Tainubie
	<i>Pennisetum alopecuroides</i>		Chikarashiba
	<i>Paspalum notatum</i>	Bahiagrass	
	<i>P. dilatatum</i>	Dallisgrass	Shimasuzumenohia
	<i>Setaria viridis</i>		Enokorogusa
	<i>Andropogon brevifolius</i>		Ushikusa
	<i>Sorghum sudanense</i>	Sudangrass	
	<i>S. vulgare</i>		
	<i>Imperata cylindrica</i>	Cogongrass	Chigaya
	<i>Miscanthus sinensis</i>	Eulakia	Susuki
	<i>M. sacchariflorus</i>		Ogi
	<i>Zea mays</i>		Toumorokoshi
Cyperaceae	<i>Coix lacryma-jobi</i>		Juzudama
	<i>Euchlaena mexicana</i>	Teosinte	
	<i>Cyperus iria</i>	Umbrella sedge	Kogomegayatsuri
	<i>C. rotundus</i>	Cocograss	Hamasuge
	<i>C. serotinus</i>		Mizugayatsuri
	<i>C. difformis</i>		Tamagayatsuri
	<i>Killingia brevifolia</i>		Himmekugu
	<i>Eleocharis acicularis</i>	Spike rush	Matsubai
	<i>E. kuroguwai</i>		Kuroguwai
	<i>Fimbristylis littoralis</i>		Hideriko
	<i>F. aestivalis</i>		Azetentsuki
	<i>Pycnus unioloides</i>		Mugigaragayatsuri
Dicotyledoneae	<i>Rhynchospora Faberi</i>		Itoinunohanahige
	<i>Carex Kobomugi</i>		Kouboumugi
Amaranthaceae	<i>Amaranthus lividis</i>	Wild amaranthus	Inubiyu
	<i>A. patulus</i>		Aobiyu
	<i>A. viridis</i>		Honagainubiyu
	<i>Celosia</i>	Celosia	Keito
Chenopodiaceae	<i>Achyranthes japonica</i>		Inokozuchi
	<i>Atriplex gmelinii</i>	Orach	Hamaakaza
	<i>Beta vulgaris</i>	Silver beet	
	<i>Chenopodium album</i>	Goose foot	Shiroza
Portulacaceae	<i>Spinacia oleracea</i>	Spinach	Hourensoo
	<i>Portulacaceae</i>	Pigweed	Suberihiyu
Polygoneaceae	<i>P. grandiflora</i>	Rose moss	Matsubabotan
	<i>Rumex acetosa</i>	Common sorrel	Suiba
Fabaceae	<i>Vicia sativa</i>	Common vetch	
	<i>Vicia faba</i>		Soramame
	<i>Medicago sativa</i>	Alfalfa	Murasakiumagoyashi
	<i>Glycine max</i>	Soybean	Daizu
Helianthaceae	<i>Trifolium pratense</i>	Red clover	
	<i>T. repens</i>	White clover	Shirotsumekusa
	<i>Helianthus annuus</i>	Sunflower	Himawari
Solanaceae	<i>Solanum lycopersicum</i>	Tomato	

inlet and outlet of the chamber was taken to show the average CO₂ concentration within the chamber.

The number of species used in Expt-II for microscopic observation was about 100 (table 1). Most crops and grasses were soil-cultured by

the ordinary method. Only fully developed leaves were used for experiments. The leaf of some weeds and wild plants was also used.

On the other hand, as an index for physiological characteristics of "carbon dioxide curves", CCP at 21 per cent O₂ under a light intensity of 10 klux was measured by the apparatus mentioned before.

Intact preparations were obtained by using an electrofreezing microtome. In ordinary cases the preparations were observed without staining. In some cases, however, they were stained with K-KI solution to detect starch and to get clearer photographs.

Isotopic experiments were conducted to see the function of that tissue. After feeding ¹⁴CO₂ to maize and rice leaves for 10, 30, 60, 120 and 300 sec., they were fixed in the boiling alcohol. Preparations were obtained by the ordinary paraffin method. Then autoradiographic film (ET-2F, Fuji-photo-film Co.,) was set on the preparations. Time of exposure was about two months. The distribution of labelled compounds which turned insoluble to alcohol with time was investigated.

Fig. 2 Effect of CO₂ concentration on the apparent photosynthesis of various species in 15 klux (illuminated from both sides).

Fig. 3 Examination on the fitness of "CO₂ curve" to quadratic equation.

Results and Discussions

Experiment-I

In the following description, "CO₂ curve" shows photosynthesis at various CO₂ concentration in constant light intensity and at constant temperature. In other expression, it shows a function P (var., const., const.). "Light curve" corresponds to the function P (const., var., const.). Likewise, the photosynthetic response under mutual interdependence to CO₂ concentration and light intensity corresponds to the function P (var., var., const.).

1) Effect of CO₂ concentration on apparent photosynthesis of various species under comparatively low CO₂ concentration and normal oxygen concentration.

Two clearly different types of "CO₂ curves" were found among species (fig. 2). Great differences were found in the CO₂ compensation point (CCP), in the rate of CO₂ liberation in CO₂-free air in the light, and in the saturating CO₂ concentration. One group, to which sudangrass, dallisgrass, barnyardgrass, new-sorgo, teosinte, bahiagrass, etc. are belonging, is characterized by "CO₂ curves" with, low CCP (near zero), low CO₂ liberation rate in CO₂-free air in the light, and abrupt saturation under the conditions mentioned in the figure. The other group to which rice, barley, wheat, orchar-

grass, oat, etc. are belonging, possesses "CO₂ curves" with characteristics just reverse to those of former group, i.e., slow and gradual saturating curves with different origins and high CCP. "CO₂ curves" of those species belonging to the former type seem to fit a certain mathematical expression.

We adopted the following quadratic expression:

$$P : KP_{\max}(\text{CO}_2)^2/[1+K(\text{CO}_2)^2] \dots\dots\dots (1)$$

K : constant determined by the half-saturating CO₂ concentration,

P_{max} : photosynthetic rate at the saturating CO₂ concentration

By dividing each side with P_{max}, equation (1), can be rewritten into :

$$P_r = P/P_{\max} = K(\text{CO}_2)^2/[1+K(\text{CO}_2)^2] \dots\dots\dots (2)$$

Examination of the fitness of our data to equation (2) has proved (fig. 3) that, in nearly all the cases, the above-mentioned expression is applicable to the "CO₂ curves" of any species belonging to the former group examined. Differences seem to lie in the constants K and P_{max}.

In contrast to the clear relationship found in these species, the pattern of "CO₂ curves" of rice and some temperate grasses was rather complex: "CO₂ curves" of the latter did not reach saturation within the range of CO₂ concentration tested. Moreover, the origin of the curve was different from species to species. It was proved to be difficult to apply a single, common expression to them. So far it has been thought that photosynthetic rate is proportional to CO₂ concentration under comparatively low levels. However, it has been proved that this is no longer applicable to the former group even in a CO₂ concentration range lower than 300 ppm. Hesketh¹²⁾ also reported on the "CO₂ curves" of some plants under a high light intensity.

However, according to his data on maize which belongs to the former group, such a clear saturation as ours is not found up to 1,000 ppm in CO₂ concentration. It is probably due to the stronger light intensity than ours. The cause of such differences will be discussed later in this

Fig. 4 Effect of temperature, CO₂ concentration on apparent photosynthesis (illuminated from both sides).

Fig. 5 Effect of temperature, CO₂ concentration on apparent photosynthesis of rice.

paper.

2) Effect of temperature on "CO₂ curves".

The effect of temperature on "CO₂ curves" of some plants, when other environmental conditions were kept at the same level is shown in figs 4-7. The CCP of barnyardgrass and dallisgrass, which are the representatives of the "abrupt saturation" group, were little affected by the temperature, while those of rice and orchardgrass, which are the representatives of

Fig. 6 Effect of temperature, CO_2 concentration on apparent photosynthesis of rice.

Fig. 7 Effect of CO_2 concentration, temperature on apparent photosynthesis in 15 klux (illuminated from both sides).

Fig. 8 Effect of temperature on CO_2 compensation point in 15 klux.

the "gradual saturation" group, became higher with increasing temperature (fig. 8). In some

Fig. 9 Effect of temperature on " CO_2 curves" of dallisgrass.

Fig. 10 Effect of temperature on " CO_2 curves" of rice.

tropical grasses tested, the effect of temperature on apparent photosynthesis under a constant light intensity seemed to cause changes of constants K and $P_{\max}$ of the " CO_2 curves". The $P_{\max}$ for apparent photosynthetic rate of dallisgrass continued to increase up to the optimum temperature, 35°C (fig. 9). It was also true for other tropical grasses. The optimum temperature:

showed little change under different CO_2 concentrations.

On the other hand, the relationships of CO_2 curves of rice and orchardgrass to temperature were more complicated (figs. 5, 6, 7). The optimum temperature for apparent photosynthesis of rice in 12 klux light at 300 ppm CO_2 , illuminated exclusively from above, was as low as 15°C (fig. 6). On the other hand, the value rose up to 25°C in 10 klux light at 300 ppm CO_2 , illuminated from both sides of the chamber, and absorbed energy by plants was higher than the former one (fig. 5).

It may be due to the difference in absorbed energy by the plants, that is, the difference of the balance of photosynthesis and respiration. The optimum temperature is determined by the balance of true photosynthesis and respiration, including light respiration. The phenomenon, shift of optimum temperature for apparent photosynthesis to lower side under weaker light intensity, also occurs in tropical grasses. However, in the case of dallisgrass, the phenomenon is comparatively limited in the weaker light intensity.

It seems to indicate that the optimum temperature for apparent photosynthesis of tem-

perate grasses is greatly influenced by light intensity and CO_2 concentration compared with the case of tropical grasses.

A three-dimensional expression is adopted in the case of rice plants for an easier and more collective understanding of the situation (fig. 10).

3) Effect of light intensity on " CO_2 curves"

Sets of " CO_2 curves" which were obtained with light intensity as a parameter and keeping the other environmental conditions at the same level are shown in figs. 11, 12. In the following "light curves", the rate of photosynthesis is plotted as a function of light intensity. However, we could not increase light intensity beyond 40 klux, because photosynthesis in stronger light intensity was proved to be unstable and less reversible. Transpiration rate must have been considerably accelerated under such conditions, bringing about water deficiency in the leaf. If photosynthesis were measured under a low air-circulation to diminish the water vapour concentration gradient between leaf and surrounding air, photosynthesis would steadily increase up to such a strong

Fig. 11 Effect of light intensity on " CO_2 curves" of dallisgrass at 25°C

Fig. 12 Effect of light intensity on " CO_2 curves" of barley at 25°C .

Fig. 13 "Light curves" of dallisgrass.

light intensity as would cause photo-oxidation.

In most tropical grasses, "CO₂ curves" rose linearly up to a certain CO₂ concentration and at close to saturation they suddenly turned horizontal as mentioned before. Curves corresponding to different light intensities diverge from the beginning. P_{max} is elevated as the light intensity increases (fig. 11). However, the increasing rate of the former does not converge as the latter increases. The constant K becomes smaller (figure is omitted). Consequently, light curves of these tropical plants show unsaturated type curves, independent of the CO₂ concentration (fig. 13). Such unsaturated type light curves as reported by Hesketh et al¹¹⁾ are recognized again in dallisgrass. Judging from the shape of the curves, it may be more appropriate to consider that two different reactions, corresponding to the initial part and to that at higher light intensities than 10 klux, are concerned here.

On the other hand, curves of rice and some temperate grasses also rise up linearly and saturation is reached very gradually, and in addition the saturating CO₂ concentration is higher than that of the former group.

"CO₂ curves" were more complex and CCP differs with each other according to the differences of light intensity. The initial slope is not, however, proportional to the light intensity and the increasing rate of initial slope becomes

Fig. 14 "Light curves" of barley.

Fig. 15 Examination on the fitness of light-photosynthesis curves to quadratic expression.

smaller and smaller, and it seems to converge to a certain value as the light intensity increases. The convergence is reached earlier under low CO₂ concentration (fig. 12). Therefore, "light curves" of these plants seem to saturate somewhat in wider range at any CO₂ concentration level. In normal CO₂ concentration, 300 ppm, light curves reach saturation in 50 klux (fig. 14), as was reported by many investigators. The quadratic expression reported by us on rice leaves¹⁾:

$$P = KP_{max}I^2/[1+KI^2] \dots \dots \dots (4)$$

shows satisfactory fitness to the photosynthesis, P , when it is related to a wider range of light intensity, I , in 300 ppm CO₂-concentration on apparent photosynthesis under a constant tem-

perature (figs. 16, 17).

Here the relationships mentioned before may be more clearly understood.

The "roof" in such expressions corresponds to the photosynthesis affected by the two factors. The "roof" changes with rising temperature, reaching and passing its highest position and thus, more clearly showing the characteristics of light curves.

In ordinary experiments to get light curves, temperature will be maintained around room temperature. It is, however, difficult to maintain leaf temperature strictly constant under varying light intensity. Leaf temperature under a high light intensity is apt to become higher than the room temperature. On the other hand, the optimum temperature for photosynthesis of tropical grasses are usually higher than the room temperature and that of temperate grasses are usually lower than room temperature. Photosynthesis of tropical grasses may increase under a strong light intensity because of the increased leaf temperature. In contrast to this, photosynthesis of temperate grasses will be lowered under a high light intensity. Therefore, the difference of "light curves" between the two types of plants becomes clearer.

4) Effect of light intensity and temperature on apparent photosynthesis.

The effect of temperature and light-intensity on apparent photosynthesis is shown in figs. 18, 19.

The "roof" of these figures corresponds to the apparent rate of photosynthesis affected by temperature and light intensity. There are clear differences between the two types of plants in the shape of the "roof", i.e., in their response of apparent photosynthesis to temperature and light intensity. Under natural conditions, especially during the daytime, fluctuations of CO_2 -concentration are small. In contrast to this, the change of temperature and light intensity is

Fig. 16 Changes in "light curves" and " CO_2 curves" of dallisgrass.

Fig. 17 Changes in "light curves" and " CO_2 curves" of barley

very large. The relationship of apparent rate of photosynthesis to light intensity and temperature is, therefore, more important than to CO_2 concentration in analyzing dry matter production under natural conditions. It is natural to conclude from figs. 18, 19, that the "light curves" differ greatly according to the change of temperature in a day as reported by Chmora⁵⁾.

Now let us try to explain the differences in the relationship of apparent photosynthesis to light intensity between barley and dallisgrass on a typical summer day, assuming that they are illuminated from the same direction. Apparent photosynthesis will change in such a manner as is plotted on the "roof" in figs. 18, 19. If we want to get a plain expression of the relation between apparent photosynthesis and light intensity, the only thing we must do is

Fig. 18 Effect of light intensity and temperature on apparent photosynthesis of dallisgrass at 300 ppm CO_2 concentration.

Fig. 19 Effect of light intensity and temperature on apparent photosynthesis of barley at 300 ppm CO_2 .

Fig. 20 Apparent photosynthesis vs. light intensity.

to light the "roof" from the direction shown by the arrow. It will be projected on the screen behind the opposite side of the "roof". In barley, apparent photosynthetic rate in the morning is higher than that in the afternoon. In dallis-

grass, however, the relationship is reversed (fig. 20).

We have reported that the relationship of apparent photosynthesis of a rice population to light intensity differs between morning and afternoon, when the temperature is not symmetric between the two²⁾. Such phenomena can be found frequently in the data of other workers. In rice, sunflower and probably most temperate grasses, apparent photosynthetic rate in the morning is higher than in the afternoon. In maize and probably in most other tropical grasses, however, the relation is often reversed⁴⁾, because under natural conditions on a clear day, temperature in the afternoon is usually higher than that in the morning.

The explanation given above may be far from complete for explaining all of such phenomena occurring in the natural plant communities, because many other factors such as the change

Fig. 21 Bundle sheath of rice ($\times 600$). Fig. 22 Transection of rice leaf ($\times 300$).
 Fig. 23 Transection of dallisgrass leaf. Fig. 24 Longitudinal section of dallisgrass leaf.
 Fig. 25 Transection of *Miscanthus sinensis* leaf ($\times 300$).
 Fig. 26 Transection of bahiagrass leaf ($\times 300$).
 Fig. 27 Transection of barnyardgrass leaf ($\times 300$).
 Fig. 28 Longitudinal section of maize leaf ($\times 300$). Maize leaf was supplied with $^{14}\text{CO}_2$ for 5 min. in 40 Klux.

CPBS: chlorophyllous parenchymatous bundle sheath, OPBS: ordinary parenchymatous bundle sheath, EOPBS: extension of ordinary parenchymatous bundle sheath, M: mesophyll tissue, Mo: mortar cells, E: epidermis, X: xylem, S: schlerenchyma, VB: vascular bundle, P: phloem, IBS: inner bundle sheath.

Table 2. Classification of plants with reference to their morphological characteristics of bundle sheath and physiological response of photosynthesis.

	"Plants with CPBS"	"Plants without CPBS"
	Amaranthaceae <i>Amaranthus</i> ^m Cyperaceae <i>Cyperus</i> ^k Cyperaceae <i>Killinga</i> Cyperaceae <i>Fimbistylis</i> Cyperaceae <i>Pycnus</i> Gramineae <i>Andropogoneae</i> ^s Gramineae <i>Eragrostae</i> ^s Gramineae <i>Panicaceae</i> ^s Gramineae <i>Chlorideae</i> ^s Chenopodiaceae <i>Atriplex</i> ^m Chenopodiaceae <i>Kochia</i> ^t Zygophyllaceae <i>Tribulus</i> ^m Portulacaceae <i>Portulaca</i> ^t (contains some exclusive cases)	Almost all plants other than described in the left
CO ₂ -curve	abrupt saturation	gradual saturation
carbon dioxide compensation point	near zero	high
light respiration	low or absent	present
carboxylating type	C-4 pathway	Calvin type
optimum temperature for app. photosynthesis	high (30~35°C)	comparatively low
	(changes with light intensity and CO ₂ conc.)	

^m: Derived from results of Maximov.^s: Derive from results of Stebbins.^k: Derived from results of Koriba.^t: Derive from results of Tregunna et al.

of proportion of the energy effective to photosynthesis to the total solar radiation, water content, geometric distribution of constituent leaves, etc., will be concerned. This explanation, however, will be a great help for a better understanding of the diurnal change in photosynthesis under natural conditions.

Experiment-II

The name of the particular cell arrangement around the vascular bundle is different according to investigators. For example, "bundle sheath"²²⁾, "unique cell arrangement around the vascular bundles"⁷⁾, "parenchymatous bundle sheath"⁸⁾, "Chlorophyll Sheide"¹⁵⁾ etc.

It is desirable to give an appropriate name which will correctly express the morphology of that tissue. According to the accurate observation made by Esau⁷⁾, in those which lack in such a chlorophyllous bundle sheath, an ordinary bundle sheath was found, and there were two types of

bundle sheath: one was a thin-walled tissue with chloroplasts and of parenchymatous origin and the other was a thick-walled tissue lying inside the former. In Dicotyledoneae only the former was present and Gramineae was divided into two groups with respect to the occurrence of sheath: the one always has the single thin-walled bundle sheath which is of parenchymatous origin. The other has double sheaths: the outer one originated from parenchyma and the inner one originated from vascular bundle.

Our results (figs. 21~24) agreed well with these concepts. There are, however, two types of parenchymatous bundle sheath, which is clearly distinguishable with respect to its chlorophyll content as was reported by Stebbins²⁴⁾ of Gramineae and by many other investigators of Cyperaceae and some species of dicotyledoneae as mentioned above. It is considered that this very difference will be responsible for the physiological differ-

ences. Therefore, these two types must be strictly distinguished from each other.

From these observations and discussions it is proposed to call these chlorophyll-rich, parenchymatous tissue around the vacular bundle as "chlorophyllous parenchymatous bundle sheath" (CPBS), and the other parenchymatous bundle sheath as "ordinary parenchymatous bundle sheath".

Diagrammatic expression of longitudinal section of dallisgrass is shown in fig. 24. Cells of CPBS make regular rectangular, one-layer-arrangement around the vascular bundles. When CPBS is stained with K-KI solution, it turns into dark violet in contrast to the mesophyll cells because of its high content of starch as pointed out by many workers^{15,21}. It is very interesting from the evolutionary point of view that there are two different types of tissues which are distinctively different in their chloroplast content in spite of their being the same parenchymatous origin and that no intermediate type is found to exist.

The CPBS was recognized in the following species examined: all the species of Amaranthaceae *Amaranthus*, Portulacaceae *Portulaca*, Gramineae Paniceae, *G. Chlorideae*, *G. Eragrosteae*, *G. Andropogoneae*, Cyperaceae *Kilinga*, *C. Fimbristylis*, *C. Pycneus* and most species of *C. Cyperus* and *C. Eleocharis*.

Many species with CPBS were found including those species reported by previous investigators. However, we could not find out new genus which contains species with CPBS within the 100 species tested.

Those species with CPBS are exclusively related with low CCP, while those without CPBS show a high correlation with comparatively high CCP.

Further, it was examined on whether the presence of CPBS is a characteristic proper to a genus without any variation among species within the genus. If it varied among species within the same genus, the possibility to introduce such

tissue into other species lacking in it will be left.

However, there could be found little variation, within a genus except for very few cases. As one of such cases, *Cyperus difformis* can be mentioned: it has no CPBS in contrast to other species in the same genus, *Cyperus*. It has gradually-saturating "carbon dioxide curves".

This fact means that physiological characteristics can be different from each other in the same genus. According to Maximov, some species of *Atriplex* with CPBS showed a higher dry matter production rate than those without it. This implies a close relationship between the morphology of the bundle sheath and physiological characteristics. It also implies that such a relationship is sometimes seen beyond the ordinary classification made from morphological differences.

Such a case, however, is rather rare and in most cases the presence of CPBS, the pattern of "carbon dioxide curve", and carboxylation pathway seem to be closely related with each other without variation within tribe in Gramineae and a genus in other families (table 2). Thus the important role of CPBS in photosynthesis has been suggested again. Its true function must be clarified. Fig. 28 shows the microautoradiographic preparations of maize leaf 300 seconds after feeding $^{14}\text{CO}_2$ in 40 klux, at 30°C. Labelled starch, accurately speaking, substances insoluble to alcohol, appeared only in the CPBS and not detectable in mesophyll tissues. On account of mischievous treatment, those preparations 120 seconds after feeding $^{14}\text{CO}_2$ could not be obtained.

It was impossible to know the precise time when the labelled substances first appeared in maize. Rice did not accumulate radio-active substances insoluble to alcohol even 10 minutes after feeding $^{14}\text{CO}_2$. The CPBS seems to play an important role in getting rid of accumulated sugars from photosynthetic cycle by transforming them into chemically inactive substance as pro-

posed by some workers. It is not, however, a complete explanation for the function of CPBS. The CPBS lies at the most unfavorable position for utilizing the solar energy.

Judging from the absence of respiration in species with CPBS, CPBS may also be associated with such a mechanism as reducing the oxidation rate of some intermediates in photosynthetic cycle. It has been clarified in the present experiments that there are major differences in the response of photosynthesis to environmental factors between the two types of plants: "plants with CPBS" and "plants without CPBS". Further investigation is needed to clarify the inner cause responsible for all such differential responses, especially about the definite function of CPBS.

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Summary

The difference of the photosynthetic response to some environmental factors among species was investigated and the results were examined in relation to the leaf structure. Following results were obtained.

1. The "CO₂ curve", in 21% O₂, of most tropical grasses reached saturation at comparatively lower CO₂ concentration than that of the temperate grasses.

2. Optimum temperature for apparent photosynthesis of most temperate grasses was greatly changed with CO₂ concentration and light intensity in comparison with the case of tropical grasses.

3. The "light curve" of dallisgrass, one of

tropical grasses, showed the "unsaturated type" in higher CO₂ concentration. However, in the lower CO₂ concentration, it became "saturated type".

4. The photosynthetic response in higher plants could be clearly divided into two types with respect to the chlorophyllous parenchymatous bundle sheath. And no intermediate type was found within the 100 species tested.

5. The photosynthetic response to some environmental factors were discussed collectively.

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〔和 文 摘 要〕

光 合 成 の 種 間 差 に 関 す る 研 究

第 1 報 主要な環境要因の変化に対する光合成の反応および維管束鞘の
形態からみた種間差について

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著者らは、数種の高等植物を用い、温度、CO₂ 濃度、光の強さなどの環境要因の変化が光合成に及ぼす影響について、より総合的に検討し、また、この種間差と葉身の内部形態との関係を明らかにしようとして、次のような結果を得た。

1. CO₂ 濃度以外の環境条件を一定にした場合の CO₂ 濃度の変化と光合成の関係すなわち“CO₂ カーブ”は種により大きく二つのパターンに分類された。一つは“CO₂ カーブ”の立上りが早く比較的低い CO₂ 濃度で飽和に達するもので、ほとんどの南方型牧草がこれに属し、他は対照的に“CO₂ カーブ”の立上りが遅く、比較的高い CO₂ 濃度で飽和に達するもので、北方型牧草およびイネなどがこれに属する。

2. 南方型牧草類の“CO₂ カーブ”の形は

$$P=KP_{\max}(\text{CO}_2)^2/1+K(\text{CO}_2)^2$$

という式により比較的良好に近似できることが示された。

3. 温度に対するみかけの光合成の反応は種により大きく 2 つのグループにわけられ、南方型牧草の光合成適温は 35°C 近辺であつた。これに対し北方型牧草およびイネなどのそれは比較的低く CO₂ 濃度、光の強さなどにより大きく変動することが示された。CO₂ 濃度が 300 ppm の場合には一般に南方型牧草より低い値を示し、この値は光の強さによつても変動し、光が強くなるにつれて、その値は高くなることが示された。以上の結果に基づき、みかけの光合成の日変化の種による違いについて考察がなされた。

4. 光に対するみかけの光合成の反応は種により大きく 2 つのタイプに分けられ、北方型牧草類は CO₂ 濃度にかかわらず 40 klux 以上ではほぼ飽和するような“光-光合成カーブ”を示す。一方南方型牧草類の多くは、CO₂ 濃度が高くなるほど光によるカーブのパターンの変化が小さくなり、少なくとも 40 klux では飽和に達しないような“光-光合成カーブ”を示した。

5. 葉緑維管束鞘 (chlorophyllous parenchymatous bundle sheath 以下 CPBS と略記する) の存在と CO₂ 補償点 (CCP) との関係が Downton らにより明らかにされたが、この点についてさらに多くの植物について検討した結果、CPBS を有する植物は例外なく低い CCP の値を示し、光合成の主要な環境要因に対する反応の種による違いと CPBS が密接な関係を有することが確認された。

6. CPBS の有無は分類学上の属のレベルで分類可能であつた。イネ科では亜科のレベルでの分類と平行しているが、例外もみられ、カヤツリグサ科では同一族内の植物であるにもかかわらず、CPBS の存在する種としない種 (タマガヤツリ) が認められた。しかし、CPBS の存在と光合成の生理反応の関係は非常に密接であつて、CPBS の存在と光合成の生理反応の関係における例外はみられなかつた。

7. 本実験の範囲では、CPBS を有する植物と有しない植物との 2 群に明らかに区別ができ、両者の中間型のものは認められなかつた。

8. ¹⁴CO₂ を与え、光合成を行なわせた後、一定の時間ごとにアルコールで固定して得られた葉について、ミクロオートラジオグラフィーにより、光合成産物の集積場所を観察した結果、¹⁴CO₂ を与えて遅くとも 5 分後には CPBS のみにアルコール不溶性の光合成産物が蓄積するのが観察された。