The Effect of Temperature on the Rates of Photosynthesis, Respiration and the Activity of RuDP Carboxylase in Barley, Rice and Maize Leaves*

Ryuichi ISHII, Ryuh OHSUGI** and Yoshio MURATA
(Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo 113)

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Apparent photosynthesis of a plant shows the temperature response peculiar to each species. It has been reported that the optimum temperature for apparent photosynthesis varies with species and preceding growth temperatures^{10,11,12}. However, it has not been clarified by this time how the relationship between temperature and apparent photosynthesis is determined.

The rate of apparent photosynthesis is determined by the rates of true photosynthesis and respiration. Before the discovery of photorespiration, the rate of apparent photosynthesis was defined as the difference between the rate of true photosynthesis and that of dark respiration. Recently, however, the rate of apparent photosynthesis has been considered as the difference between the rate of true photosynthesis and that of photorespiration. Therefore, it may be considered that the response to temperature of photorespiration and/or the CO₂ fixing enzyme are responsible for the relationship between temperature and apparent photosynthesis in various species.

JOLLIFFE and TREGUNNA reported that the optimum temperature for the apparent

photosynthesis of wheat tended to decrease with increasing O₂ concentration, showing that photorespiration was responsible for the determination of the optimum temperature for apparent photosynthesis⁹. However, in their experiment, no significant difference in the optimum temperature could be observed between 20.8% and 1.8% O₂. HEW *et al* also showed the same results⁶.

As to the effect of temperature on the CO₂ fixing enzyme activity, it was reported by TREHARNE and COOPER that the optimum temperature for the activity of ribulose-1,5-diphosphate (RuDP) carboxylase was higher in the tropical grasses than in temperate grasses¹³. However, WEIDNER and SALISBURY found that the Arrhenius plot for the RuDP carboxylase of wheat was straight at temperatures between 2.5°C and 40°C, showing that in these temperature range, there was no optimum temperature for the activity of RuDP carboxylase¹⁴.

In this paper, it was tried to approach the mechanism determining the relationship between temperature and apparent photosynthesis, by comparing the responses to temperature of dark respiration, photorespiration and the CO₂ fixing enzyme activity in these plant species.

Materials and Methods

Species and varieties used were barley (*Hordeum vulgare* L. cv. Musashino-mugi), rice (*Oryza sativa* L. cv. Nihonbare) and maize (*Zea mays* L. cv. Okuzuruwase).

Culturing conditions : Each species was sown at the end of October in a plastic container (30×20×10 cm) with soil and the compound fertilizer containing 1.2 g, 1.8 g and 1.2 g of N, P₂O₅ and K₂O, re-

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** Present address: National Grassland Institute, Nishinasuno, Tochigi, 329-27.

Abbreviations: AP₂₀, rate of apparent photosynthesis in the mixed gas containing 20% O₂; AP₂, rate of apparent photosynthesis in the mixed gas containing 2% O₂; CCP, CO₂ compensation point.

spectively. Rice and maize were grown in a phytotron of natural light. The temperature was controlled at 25°C in daytime and 20°C at night. Barley was grown in a glass house up to the 4-leaf stage, and thereafter outdoors.

Method for measuring the rate of photosynthesis: The measurement of photosynthesis was carried out in December, when six leaves in rice, and about eight leaves in barley and maize had emerged. The youngest fully expanded leaves were excised and used for the measurement. The system for measuring photosynthesis could be changed alternately to an open and a closed one spontaneously with a magnetic valve. After the leaves were preilluminated for about 30 minutes in the normal air with the open system, 16 liter of the mixed gas packed in a gas storing bag was introduced into the system at a flow rate of 3.5 l/min. The mixed gas was obtained from the mixture of pure nitrogen gas, pure oxygen gas and the nitrogen gas containing 3% carbon dioxide. The CO₂ concentration was 700 ppm in all kinds of the mixed gas. After the system was completely replaced with the mixed gas, it was switched over to a closed one. Immediately after switching over, the CO₂ concentration in the system gradually decreased, approaching the CO₂ compensation point. The rate of apparent photosynthesis at different CO₂ concentrations could be calculated from the slope at the point crossing the level of each CO₂ concentration. The CO₂ concentration in the gas was measured with an infrared CO₂ gas analyzer (Beckman Model 864). The light intensity was 50 klx throughout all the experiments.

The assay of the activity of RuDP carboxylase: The youngest, fully expanded leaves of rice and barley were harvested at the same time for measuring photosynthesis. RuDP carboxylase was extracted and its activity was assayed by the method previously reported⁹⁾. Prior to initiation of the reaction, the extracted enzyme was pre-incubated at each required temperature for 5 minutes. As the enzyme activity was proportional to the incubation time at least

up to 5 minutes, we adopted 2.5 minutes as the incubation time.

Results

1. *The effect of temperature on AP₂₀ and AP₂:* The AP₂₀ and AP₂ of barley, rice and maize leaves were measured with the closed system under three levels of temperature. The original data of the experiments were shown in Fig. 1.

As can be seen in Fig. 1, the relationship between CO₂ concentration and AP₂₀ in barley was almost linear under every level of temperature, while in maize a hyperbolic tendency was found at 35°C, being more and more evident with decreasing temperature. In rice, the relationship was intermediate between barley and maize.

The AP₂ of barley and rice ran in nearly parallel with AP₂₀ under every level of temperature, but in maize, AP₂ coincided with AP₂₀.

Thus, the response to CO₂ concentration of apparent photosynthesis is different with species and temperature during the measurement.

Based on the results of Fig. 1, the effect of temperature on AP₂₀ and AP₂ under two levels of CO₂ concentration was analyzed as shown in Fig. 2. Under 300 ppm CO₂ which is the normal CO₂ concentration in the air, the maximum AP₂₀ was obtained at 15°C in barley, at 25–35°C in rice and at 35°C in maize. Under 600 ppm CO₂, the maximum AP₂₀ was observed at the higher temperature than under 300 ppm CO₂; 15–25°C in barley and 35°C in rice. In maize, the maximum AP₂₀ under 600 ppm CO₂ was observed at the same temperature as under 300 ppm CO₂.

As to AP₂, it became more positively sensitive to temperature than AP₂₀ in barley and rice particularly under 600 ppm CO₂. Therefore, the AP₂ under 600 ppm CO₂ of rice increased almost proportionally with temperature, showing the same trend as that of maize qualitatively. But, the AP₂ under 600 ppm CO₂ of barley dropped at 35°C though it increased more steeply than AP₂₀ up to 25°C.

Fig. 1. The effect of CO₂ concentration on the rates of apparent photosynthesis under three levels of temperature and two levels of O₂ concentration in the excised leaves of barley, rice and maize.

○—○ : in 2% O₂, □—□ : in 20% O₂

2. *The effect of temperature on the rates of photorespiration and dark respiration:* The rate of photorespiration was estimated by extrapolation of the CO₂ response curve for photosynthesis to zero CO₂, and the effect of temperature on photorespiration in barley, rice and maize was shown in Fig. 3. The rate of photorespiration was higher in barley than in rice, but the sensitivity to temperature was the same in both species. In maize, photorespiration was very low and showed no response to temperature.

Although the temperature response of the absolute rate of photorespiration was almost

the same in barley and rice, that of the relative magnitude of photorespiration revealed in the following two values was different in these two species; one is the degree of enhancement of apparent photosynthesis by lowering O₂ concentration and the other is the CO₂ compensation point.

The degree of enhancement of apparent photosynthesis by lowering O₂ concentration was calculated from AP₂₀ and AP₂ of barley and rice in Fig. 1, and shown in Fig. 4. It increased with decreasing CO₂ concentration and with increasing temperature showing that photorespiration is relative-

Fig. 2. The effect of temperature on the rates of apparent photosynthesis under two levels of each CO₂ and O₂ concentration in the excised leaves of barley, rice and maize.

○---○ : 300 ppm CO₂, 20% O₂ □---□ : 600 ppm CO₂, 20% O₂
 ●—● : 300 ppm CO₂, 2% O₂ ■—■ : 600 ppm CO₂, 2% O₂

Fig. 3. The effect of temperature on the rates of photorespiration in the excised leaves of barley, rice and maize estimated by extrapolation of the CO₂ response curves for photosynthesis to zero CO₂ in Fig. 1.

ly high in relation to photosynthesis under such conditions. Furthermore, the temperature response of the enhancement was higher in barley than in rice.

The CCP of barley and rice showed a tendency to increase with increasing temperature, while the CCP of maize remained constant at all temperatures as shown in Table 1. However, more precise comparison between barley and rice revealed out that the CCP in the former increased in the same way as in the latter up to 25°C, but increased far more steeply than in the latter at 35°C. Further, it was noticed that the CCP in barley showed a steep increase even in 2% O₂ between 25°C and 35°C, while that in rice showed the same value at 25°C and 35°C. It is known that in 2% O₂ photorespiration is inhibited, but dark respiration is not^{3,4}. Therefore, the CCP obtained here must show the concentration where photosynthesis and dark respiration (in the light) are in equilibrium. From these, it may be inferred that dark respiration in barley was markedly enhanced by high temperature as compared with rice.

3. *The effect of temperature on the activity of RuDP carboxylase in vitro:* As shown in Fig. 5, the activities of RuDP carboxylase both in barley and rice increased with increasing temperature at least up to 40°C. However, RuDP carboxylase in rice was more sensitive to temperature than in barley,

Fig. 4. The effects of temperature and CO₂ concentration on the degree of enhancement of apparent photosynthesis by lowering O₂ concentration of the air to 2% in the excised leaves of barley and rice.

Table 1. The effects of temperature and O₂ concentration on the CO₂ compensation points in the excised leaves of barley, rice and maize.

	Barley			Rice			Maize		
	2% O ₂	20% O ₂	50% O ₂	2% O ₂	20% O ₂	50% O ₂	2% O ₂	20% O ₂	50% O ₂
15°C	18	40	80	15	40	84	8	8	8
25°C	24	64	108	22	56	109	8	8	8
35°C	42	98	162	22	64	145	8	8	8

The CO₂ compensation points are expressed in ppm.

Fig. 5. The effect of temperature on RuDP carboxylase activity in the leaves of barley and rice.

showing that potential CO₂ fixation is more active in rice than in barley at high temperatures. On the other hand, photorespiration was higher in barley than in rice as shown in Fig. 5. Therefore, it can be inferred that the high CCP or the great enhancement of apparent photosynthesis by lowering O₂ concentration under the high temperature observed in barley, may be due to all of the three factors, the low CO₂ fixating ability, the high photorespiration and the high dark respiration, under the high temperature.

Discussion

In our experiments, the apparent photosynthesis under the normal CO₂ concentration showed its maximum value at 15°C in barley, 25–35°C in rice, and 35°C in maize. On the other hand, as shown in Fig. 5, the RuDP carboxylase activities of barley and rice increased with increasing temperature, and the optimum temperature for their activity could not be found. At the same

time, it was shown that the sensitivity to temperature of RuDP carboxylase activity was higher in rice than in barley.

Assuming that the temperature response of true photosynthesis or CO_2 fixation is represented by that of RuDP carboxylase activity, the true photosynthesis of barley and rice cannot have a optimum temperature up to 40°C , and the sensitivity to temperature of true photosynthesis was higher in rice than in barley.

Our concern in this experiment was whether we could explain the difference between the temperature response of the true photosynthesis and that of AP_{20} under the normal CO_2 concentration, by the temperature response of some factors such as CO_2 diffusion resistance, photorespiration and dark respiration.

The effect of temperature on photosynthesis is considered to work mainly through the chemical processes of CO_2 fixation and respiration. Therefore, when the rate of apparent photosynthesis is free from the limitation in the physical processes of CO_2 diffusion, it will be affected more strongly by temperature⁵⁾. In our results, too, AP_{20} under 600 ppm CO_2 became more sensitive to temperature and its optimum temperature shifted to a value higher than under 300 ppm CO_2 . If the CO_2 concentration is raised further above 600 ppm, the temperature response will become more evident. In this sense, the CO_2 diffusion resistance may be considered as the first factor determining the relationship between temperature and apparent photosynthesis.

As the second factor, photorespiration may be listed. The temperature for the maximum AP_2 was higher than that for the maximum AP_{20} in rice and barley. Especially in rice, AP_2 under 600 ppm CO_2 increased almost proportionally with increasing temperature, showing that photorespiration was responsible for the decrease of AP_{20} at high temperatures. Furthermore, it was shown that the temperature response of AP_2 of rice under 600 ppm CO_2 was similar qualitatively to that of true photosynthesis. However, AP_2 of barley under 600 ppm CO_2 increased with increasing

temperature up to 25°C and dropped at 35°C . Therefore, in barley, the temperature response of AP_2 could not be considered as the one approaching to true photosynthesis. Thus, the temperature response of dark respiration was supposed as the third factor.

It is considered that dark respiration continues in the light at the same velocity as in the dark¹⁾. Furthermore, it was reported that the rate of dark respiration increased with increasing temperature up to 45°C , while the rate of photorespiration saturated at about 30°C ^{6,7)}. In our experiment, the CCP of barley in 2% O_2 increased steeply at 35°C . This phenomenon may be considered as indicating that at high temperature dark respiration is most heavily responsible for the relationship between temperature and apparent photosynthesis, particularly in barley. Therefore, if the rate of dark respiration measured at each temperature was further added to AP_2 , its response to temperature would become more sensitive and its optimum temperature would shift to even higher temperature. As we actually did not measure the rate of dark respiration, we cannot help supposing the contribution of dark respiration from the temperature response of CCP under 2% O_2 .

As a result, it can be inferred that the relationship between temperature and apparent photosynthesis is determined by the temperature response of all the three components, the CO_2 fixing enzyme activity, photorespiration and dark respiration. In the case of barley, the decrease of AP_{20} at high temperature could be due to the greater enhancement of dark respiration or photorespiration and the smaller enhancement of CO_2 fixation, by high temperatures. CHARLES-EDWARDS also obtained a similar result in *lolium* species²⁾.

However, it is impossible at present to explain the relationship between temperature and apparent photosynthesis only by the three factors mentioned above.

Summary

It was tried to approach the mechanism

by which the relationship between temperature and apparent photosynthesis are determined in barley, rice and maize leaves.

The maximum rate of apparent photosynthesis was obtained at 15°C in barley, at 25–35°C in rice and 35°C maize under the normal condition (300 ppm CO₂, 20% O₂). On the other hand, the activity of RuDP carboxylase *in vitro* increased with increasing temperature up to 40°C, suggesting that the CO₂ fixation or true photosynthesis had no optimum temperature below 40°C.

The main concern of this experiment was laid on what factors can qualitatively compensate the gap between the temperature response of the apparent photosynthesis under the normal condition and that of the RuDP carboxylase activity.

In maize and rice, the apparent photosynthesis under 600 ppm CO₂ and 2% O₂ showed an almost linear increase with increasing temperature up to 35°C. Therefore, in these species, CO₂ diffusion resistance and photorespiration could be considered to compensate the gap qualitatively. However, in barley the apparent photosynthesis under 600 ppm CO₂ and 2% O₂ dropped at 35°C, suggesting that dark respiration in the light was heavily responsible to compensate the gap particularly in barley. The temperature response of dark respiration in the light was estimated from that of the CO₂ compensation point under 2% O₂ where photorespiration was inhibited. This value showed a steep increase at 35°C in barley, while it was the same between 25°C and 35°C in rice, suggesting that dark respiration was greatly enhanced at high temperature in barley comparing with rice.

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〔和 文 摘 要〕

オオムギ, イネ, トウモロコシの光合成速度, 呼吸速度, RuDP カルボキシ
ラーゼ活性に及ぼす温度の影響

石 井 龍 一・大 杉 立・村 田 吉 男

(東京大学農学部)

みかけの光合成の温度反応が異なるオオムギ, イネ, トウモロコシを用い, 温度とみかけの光合成の関係が決定される機構を明らかにすることを目的として実験を行なった. ガス代謝の測定は切断葉を用い, 閉鎖系の光合成測定装置により 50 klx の照度の下で行なった.

通常の空気 (300 ppm CO_2 , 20% O_2) の下でのみかけの光合成の最大値はオオムギで 15°C に, イネでは 25~35°C に, トウモロコシでは 35°C に認められた. 一方, 光合成における CO_2 固定の key enzyme である RuDP カルボキシラーゼ活性は, 40°C まで温度の上昇にともなって上昇し, 適温は認められなかった. ここで著者らは, RuDP カルボキシラーゼ活性の温度反応を真の光合成の温度反応と定性的にはみることができるという仮定の下に, どのような影響を除去するとみかけの光合成の温度反応が, 真の光合成の温度反応に近づくかを検討した.

トウモロコシ, イネでは, 600 ppm CO_2 , 2% O_2 の下でのみかけの光合成は 35°C までほぼ直線的に増加した. したがって, 少なくとも CO_2 拡散抵抗, 光呼吸の影響を弱めるか, 除去するとトウモロコシ, イネの場合には, みかけの光合成の温度反応が真の光合成の温度反応に定性的には近づくことが明らかになった. しかしオオムギでは, 600 ppm CO_2 , 2% O_2 の下でのみかけの光合成は, 25°C で最大値をとり, 35°C では低下する.

一方, 2% O_2 の下で CO_2 補償点を測定したところ, オオムギではトウモロコシ, イネと異なり, 25°C から 35°C にかけて急激に上昇した. 2% O_2 の下では光呼吸は阻害され, 暗呼吸のみが行なわれていると考えられるため, 特にオオムギでは 25°C 以上の高温で, 明中の暗呼吸が急激に上昇することが考えられた. オオムギの 600 ppm CO_2 , 2% O_2 下でのみかけの光合成は, こうした高温による明中での暗呼吸の促進により 35°C で低下したと考えられる.

なお, RuDP カルボキシラーゼの温度反応はイネの方がオオムギより敏感であった. したがって, イネの場合にも明中の暗呼吸の影響を何らかの方法で除去するか, あるいは定量化し, 高 CO_2 , 低 O_2 下のみかけの光合成に加えれば, その値はオオムギより高い温度反応を示し, RuDP カルボキシラーゼ活性の温度反応におけるイネとオオムギのちがいに对应すると考えられた.