Effect of Light on the CO₂ Evolution of C₃ and C₄ Plant in Relation to the Kok Effect

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We reported in the previous papers that the Kok effect was observed in C₃ plants while it was not in C₄ plants¹⁹,²⁰. The Kok effect is known as the phenomenon that the slope of the line showing the relationship between light intensity and apparent photosynthesis is steep below the light compensation point (LCP) and gentle above that, showing the break of the line around LCP²¹. Kok and some workers suggested that the steep increase of apparent photosynthesis below LCP was caused by the inhibition of dark respiration by weak light¹⁷,²¹. It could be inferred, however, by our results that photosynthesis which apparently began around LCP suppressed apparent photosynthesis above LCP, being responsible for the Kok effect, because the Kok effect could not be observed in C₄ plants and not in C₃ plants subjected to low O₂ concentration¹⁹,²⁰. In these situations, it is necessary to examine the effect of light intensity on the rates of photosynthesis, photorespiration and dark respiration for pursuing the cause of the Kok effect.

It can be considered that photosynthesis and photorespiration increase with increasing light intensity. As for the dark respiration, however, there have been two contrary opinions. One is that dark respiration in the light is equal to that in the dark³,⁴,⁵,⁷,⁹,¹³,¹⁴,¹⁵,²²,²⁴, and the other is that dark respiration is inhibited by weak light¹,⁸. These contrary results are presumably attributable to the technical difficulty to separate respiration from photosynthetic CO₂ fixation because these two reactions proceed at the same time in light. In this work, we measured the CO₂ evolution into CO₂-free air for the estimation of dark respiration and photorespiration. In this method too, the problem of photosynthetic re-fixation of respiratory CO₂ cannot be avoided. In the results previously obtained by some workers, we can find the opposite results in the respect of CO₂ evolution rates in light and darkness²⁵,²⁶,²⁷,²⁸,²⁹.

Materials and Methods

The species and varieties used were rice (Oryza sativa L. cv. Nihonbare) as a C₃ plant and maize (Zea mays L. cv. Okuzuruwase) as a C₄ plant. They were grown in the experimental field of the University during summer season, according to the cultivating methods ordinarily followed in Japan.

The apparatus for measuring the rate of CO₂ evolution into CO₂-free air (CE) consisted of a gas storing balloon, an air pump, a regulator for gas flow rate, a humidifying bottle, an acrylic plastic leaf chamber (10×15×0.6 cm, 90 ml), a desiccating tube buried in ice and an infrared CO₂ gas analyzer (Beckman, Model 315). The humidifying bottle and the leaf chamber were covered with an acrylic plastic chamber.
Fig. 1. CO₂ evolution of rice leaves in response to the successive change of light intensity at 15°, 25° and 35°C. Time on the horizontal line indicates the time after CO₂ evolution in dark leveled off. Arrows indicate the time when light intensity was changed to 28.4 (L1), 16.2 (L2), 9.9 (L3), 5.0 (L4), 1.9 (L5), 0.8 (L6), 0.4 (L7) and 0 (D) klx.

(40×40×40 cm) in which the temperature was regulated. A metal halide lamp (Toshiba, 400W) was used for the light source.

Five, in case of rice, and two, in case of maize, youngest and fully expanded leaves were cut from the adult plants and set in the leaf chamber. The leaves were kept in dark and the CO₂-free air was introduced at a rate of 950 ml/min equivalent to about 10 volumes of the leaf chamber per minute. After CE in dark levelled off, the light was switched on and CE in light was determined at the stable level. The light intensity was changed by inserting a paper before the lamp. 

CO₂-free air was obtained by passing the air through the tube containing soda lime, and that of low O₂ concentration (2% O₂) was obtained by mixing the pure nitrogen and oxygen gases.

Results

Fig. 1 shows the time course of CE of rice leaves in response to the successive change of light intensity. CE decreased steeply immediately after the illumination of high light intensity was projected, dropping to nearly zero, and continued to stay at this low level. After 10 or 30 minutes, depending on the temperature, CE increased gradually with time and reached a plateau. The higher the temperature, the shorter the time to reach the plateau. When the illumination was weakened, CE decreased depending on the light intensity, reaching the minimum level at the light intensity around LCP, and in the light intensity below LCP, it increased again attaining the level of CE in the dark. The result of Fig. 1 was transferred to Fig. 2 showing CE on the vertical line and light intensity on the horizontal line. As seen here, CE showed a decreasing trend up to LCP and above that an increasing one, attaining a plateau at about 15,000 lx. As for the effect of temperature, the higher the temperature, the higher was the level of CE in all light intensities.

Furthermore, the light intensity where the bottom value of CE was obtained, shifted to the higher one with increasing temperature. These light intensities corresponded to LCP in each temperature. In this way, the value of CE changed quantitatively at
LCP in response to light intensity. Hereafter the portion of the curve below LCP will be called Phase 1 and that above LCP, Phase 2.

Fig. 3 (above) show the results of the same experiment as in Fig. 1, with a single modification that the intensity of illumination was changed to a stronger one. Fig. 3 (below) was obtained from the above figures. As shown in both the figures, the same results were obtained as in Figs. 1 and 2.

On the other hand, the result of maize, a C₄ plant, was shown in Fig. 4 where CE in Phase 1 decreased with increasing light intensity in the same way as in rice leaves. But CO₂ evolution could not be observed in Phase 2. Therefore, it can be inferred that the CO₂ evolution in Phase 2 is attributable to photorespiration. Hereupon, we measured CE of rice leaves in the low O₂ concentration, the condition which inhibits photorespiration. The result is shown in Fig. 5. Under this condition, rice leaves scarcely show any CO₂ evolution in Phase 2. From the results shown in Figs. 4 and 5, it was concluded that the CO₂ evolution in Phase 2 is attributable to photorespiration.

In order to check the possibility that the
substrate for the CO₂ evolution might become in shortage or exhausted with the process of the measurement, normal air was supplied for about 5 minutes just before the light intensity was changed, and then CE was measured at each light intensity. The result is shown in Fig. 6, which gives almost the same tendency as Fig. 2 or Fig. 3. From these it could be considered that the exhaustion of the substrates for respiration did not influence the results throughout these experiments.

Discussion

The results of this paper clearly showed that CE of rice leaves decreased in Phase 1 and increased in Phase 2 with increasing light intensity, giving a sharp break around LCP. The nearly same results were obtained in orchard grass by CARLSON et al.⁵, in Douglas-fir by BRIX⁶, in Sitka spruce by CORNIC and JARVIS⁷ and in sunflower by HAW et al.⁸. This tendency of CE is explicable of the KOK effect in C₃ plant, because the decrease of CE in Phase 1 and the increase in Phase 2 seem to be correlated to the steep increase of apparent photosynthesis in Phase 1 and the gentle increase of that in Phase 2.

Meanwhile, it can be considered that CE is determined by the total respiratory CO₂ evolution minus photosynthetic refixation of it. So, the sharp change of CE around LCP is presumably caused by that either of respiratory CO₂ evolution or of photosynthetic CO₂ refixation. The ability of refixation, however, should increase with increasing light intensity. Thus, we cannot help considering that the respiratory CO₂ evolution itself sharply changed around LCP, resulting in the sharp change of CE. CE in Phase 1 can be considered to be originated from the dark respiration by the following two reasons, viz., i) it can be observed in both C₃ and C₄ plants, and ii) it is hardly affected by O₂ concentration. On the other hand, CE in Phase 2 is presumably originated from the photorespiration by the following three reasons, viz., i) it increases with increasing light intensity, ii) it cannot be observed in C₄ plant, and iii) it is inhibited by low O₂ concentration.

Therefore, from the behavior of CE in Phase 1 and Phase 2, it seems that dark respiration is inhibited by weak light and that photorespiration starts at the light intensity around LCP. But it cannot be concluded from the results of this paper that dark respiration is actually inhibited by weak light.

Summary

The relationship between the rate of CO₂ evolution into CO₂-free air (CE) and light intensity was investigated in the excised leaves of rice, a C₃ plant and maize, a C₄ plant.

1. CE of rice leaves decreased with increasing light intensity up to LCP (Phase 1) and it increased with increasing light intensity above LCP (Phase 2), showing a sharp break around LCP.
2. CE of rice in Phase 2 was influenced by the O₂ concentration in the atmosphere and CO₂ evolution was not observed in the KO₂-free air of low O₂ concentration as 2.0%.

3. Maize leaves showed the same trend of CE in Phase 1 as rice, but showed no CO₂ evolution in Phase 2.

4. It could be considered that the CO₂ evolution in Phase 1 and the one in Phase 2 were originated from dark respiration and photorespiration, respectively.

5. Seemingly, dark respiration was inhibited by weak light below LCP and the photorespiration started at the light intensity around LCP.

6. The trend of CE in rice can be rationally explicable of the Kok effect which was previously suggested to be one of the characteristics of a C₄ plant.

References


C₃, C₄ 植物の葉からの CO₂ 放出速度に及ぼす光の影響

—Kok 効果に関連して—

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著者らは Kok 効果（光一光合成曲線が光補償点近辺で折れ曲る現象）が、C₃ 植物にはみられが C₄ 植物にはみられないこと、C₃ 植物でも低酸素濃度の空気中ではみられないことを先に発表し、Kok 効果が光呼吸に関係するものであることを示唆した。本報告では、CO₂-free air への CO₂ 放出速度（CE）を、異なる光強度の下で測定することにより光呼吸、光呼吸の速度と光の強さの関係を調べ、呼吸速度と光強度の関係という観点から Kok 効果の原因を明らかにしようとした。

1. イネの CE は光補償点（LCP）以下で光が弱くなるに従い減少し、LCP 以上の光の下では光が強くなるに従い増加する。このことと、CE と光の強さの関係は LCP を境に全く逆の傾向を示し、その結果 CE は LCP 付近で鈍い傾れ曲りを示す（第 2 図）。

2. 光の強さが LCP 以下の部分を Phase 1、以上の部分を Phase 2 と呼ぶと、C₃ 植物のイネの CE は Phase 2 において酸素濃度に強く影響され、2 %の酸素濃度下ではほぼ 0 になった（第 3 図）。

3. イネの CE は Phase 1 ではほぼイネと同じ傾向を示すが、Phase 2 ではほぼ 0 であった（第 4 図）。

4. 以上のことから Phase 1 における CO₂ 放出は暗呼吸に、Phase 2 における CO₂ 放出は光呼吸に由来するものと考えられた。

5. したがって、みかけ上暗呼吸は LCP 以下の弱い光で阻害され、光呼吸は LCP 付近の光強度から始まり、光の強さにともなって増加する。

6. CE は Phase 1 においても、Phase 2 においても温度に強く影響され、温度が高くなるにつれ CE は増加すると共に、Phase 1 から Phase 2 に切り替わる光の強さは高照度側に移行する（第 2、4 図）。

7. 本実験で得られた CE と光の強さの関係は、C₃ 植物、C₄ 植物の Kok 効果の有無と対応しており、C₃ 植物における Kok 効果は主に光呼吸に起因していることが推察された。