Inhibition of Photosynthetic Light Reactions in Overwintering Tea Leaves during Winter Cold*

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Photosynthesis is suppressed by low temperatures in winter and restored in spring. In evergreen conifers^{6,21)}, for example, net photosynthesis and CO₂ absorption were either not found at all or seen only limitedly in winter, but both functions recovered in spring. SAKAI et al.²⁴⁾ showed that winter cold decreased the net photosynthesis of tea by 40 to 60% of the maximum rate, but partial recovery had taken place by early spring.

In tea, the growth of new shoots of the first flush is partly dependent on the photosynthesis of overwintered leaves. Almost all of these shoots are harvested as the first crop and the following shoot growth also depends on the previous photosynthesis. An understanding of the basis for the seasonal inhibition and later recovery of the process is therefore of interest.

Cold-induced inhibition of photosynthesis is thought to be the result of the blocking of feedback by starch^{7,22)}, stomatal closure^{8,11,13)} or impairment of the photosynthetic apparatus⁹⁾. The latter is influenced by photosynthetic light reactions^{12,19,20)}, photosynthetic dark reactions^{18,25)} or both¹⁴⁾. This paper suggests that the reduction of photosynthetic light reactions is the most probable cause of photosynthesis depression.

Materials and Methods

Mature tea plants (*Camellia sinensis* (L) O. Kuntze) over 15 years old were used. They were planted every 40 cm in 10 to 15m-long hedges forming an arc-shaped bush. In early October, they were skiffed (lightly pruned) to even the canopy surface. The mature

leaves, which had developed in mid-September and were at the surface after skiffing, were sampled to measure photosynthesis and cellular content.

Leaf photosynthesis was determined by measuring incorporation rate of ¹⁴C in the leaf discs¹⁾ or by the evolution rate of O₂ from leaf slices²⁾. In both methods, temperature, CO₂ concentration and light intensity were maintained at 25°C, the saturation level and 80 klx, respectively.

Hill activity was measured by ferricyanide photoreduction in broken chloroplasts (type c)³⁾. The suspension of chloroplasts (100 μl) was pipetted into an oxygen electrode vessel (Rank Brothers, England) containing 1.2 ml of reaction medium composed of 0.5 M tricine (pH 8.1), 0.03 M K₃Fe(CN)₆ and 0.2 M methylamine. After pre-incubation for 3 min, Hill reaction was started by illumination. The suspension temperature was kept at 25°C and light saturated (80 klx) oxygen evolution was measured.

Chlorophyll was measured by the method of MACKINNEY¹⁶⁾. Soluble protein, extracted with 50 mM of phosphate buffer (pH 7.0), was determined according to ITZHAKI and GILL¹⁰⁾. From a part of the soluble protein extract, fraction-1 protein was separated by electrophoresis with SDS polyacrylamide gel⁴⁾ and the content determined according to BLENKINSOP and DALE⁵⁾.

A crude extract of ribulose-1, 5-bisphosphate carboxylase (RuBPCase) was prepared using the procedure of LORIMER et al.¹⁵⁾. Activity of RuBPCase was measured by the ¹⁴C incorporation rates into acid stable substances at 25°C.

Results

The photosynthetic rate gradually in-

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Fig. 1. Changes in temperature, photosynthetic rate and chlorophyll content.

(a) Temperature (b) Photosynthetic rate (c) Chlorophyll content.

Temperatures were measured at the National Research Institute of Tea, Kanaya, Shizuoka and expressed as the average value of every five days. —; maximum, ...; mean, —minimum.

Photosynthetic rates were measured by the incorporation rate of ¹⁴C with leaf discs. Photosynthetic rates and chlorophyll contents were measured using three cultivars.

○; Hatsumomiji, △; Z1, □; NN27.

creased in October and early and mid-November, but decreased in late November when the maximum and minimum temperatures were 20°C and 10°C, respectively (Fig. 1a, 1b). It was lowest from January to the first of February, when the minimum temperature was below 0°C. The magnitude of decrease was larger in Hatsumomiji than in NN27 and Z1; in February, the rate gradually increased in Hatsumomiji but was unchanged in NN27 and Z1. In all three cultivars in March it was 40–60% of the maximum rate. These seasonal changes were similar to those shown by the results of SAKAI

Fig. 2. Changes in photosynthetic rate, soluble protein and fraction-1 protein contents.

Photosynthetic rates were measured by the evolution rate of O₂ with leaf slices. Material; cv. Hatsumomiji.

(a) ○; Soluble protein content, △; Fraction-1 protein content.

(b) Photosynthetic rate.

et al²⁴⁾.

Chlorophyll decreased in mid-January when the minimum temperature was below 0°C (Fig. 1c). Sensitivity to low temperatures differed between photosynthesis and chlorophyll, for although the photosynthetic rate declined in late November and December the chlorophyll content did not. In some cases, dark green leaves changed to yellowish during winter, but leaf colour alone cannot be considered a good indicator of cold injury to photosynthesis.

The time course of the decline of photosynthetic rate in Yabukita was similar to that in Hatsumomiji, and therefore these two cultivars were used to analyse the mechanism of the depression process.

Cellular substances relating to photosynthetic dark reactions were firstly examined. As shown in Fig. 2, soluble protein decreased until late December when it began to increase sharply and thereafter maintained a constant level. Fraction-1 protein also decreased until late December when it began to increase sharply. After January, it again gradually decreased and did not recover even in early spring. RuBPCase activity

Table 1. Seasonal changes in RuBPCase activity and photosynthetic rate.

Sampling date	RuBPCase activity μmoles		Photosynthetic rate $\mu\text{moles O}_2$ per $\text{dm}^2 \cdot \text{hr}$
	per mg Sol. prot. $\cdot \text{hr}$	per mg F-1 prot. $\cdot \text{hr}$	
Oct 28	16.85 ± 3.03 (100.0)	34.50 ± 6.57 (100.0)	200.4 ± 16.7 (100.0)
Feb 1	12.31 ± 1.07 (73.1)	37.44 ± 3.51 (108.5)	84.6 ± 8.7 (42.2)
Feb 28	14.15 ± 1.06 (84.0)	35.90 ± 2.68 (104.1)	48.0 ± 7.5 (24.4)
Mar 29	15.78 ± 2.10 (93.6)	30.49 ± 3.49 (88.4)	98.7 ± 7.3 (49.3)

RuBPCase activities were measured based on soluble protein and fraction-1 protein levels. Values in parentheses are percentages. Material; cv. Yabukita.

Fig. 3. Changes in the relative values of photosynthetic rate and the initial slope light-photosynthesis curve.

○; Initial slope, △; Photosynthetic rate.

The value at the start of measurement = 100 percent.

Photosynthetic rates were measured by the evolution rate of O_2 with leaf slices. Material; cv. Yabukita.

based on soluble protein and fraction-1 protein reduced only slightly or did not change during winter (Table 1). Changes in these proteins and RuBPCase activity did not correspond with those in photosynthesis. It thus does not seem that changes in cellular content which play a role in photosynthetic dark reactions are related to the winter suppression of photosynthesis.

To examine the contribution of photosynthetic light reactions to cold injury, initial slopes of light-photosynthesis curves and light saturated Hill activity were evaluated. The relative values of the curves and the photosynthetic rates expressed as percentages of those at the start of measurement

Fig. 4. Changes in photosynthetic rate and light saturated Hill activity. Photosynthetic rates were measured by the evolution rates of O_2 with leaf slices. Material; cv. Hatsumomiji.

(a) Light saturated Hill activity.

(b) Photosynthetic rate.

are shown in Fig. 3. The initial slope declined until February and then increased, its time course being similar to the changes in photosynthetic rate. Light saturated Hill activity (Fig. 4) also decreased, corresponding with the depression of photosynthetic rate.

The results of Figs. 3 and 4 indicate that photosynthetic light reactions were damaged by low temperatures.

Discussion

The photosynthetic rate of tea leaves changes seasonally, showing the highest value in autumn; in this study the rate increas-

ed in October and November (Fig. 1b). Changes might be correlated to those in soluble protein content (data not shown).

In accordance with the decrease of temperature, the photosynthetic rate declined and was lowest when the minimum temperature was 0°C (Fig. 1a, 1b). Although the time course of this decline among varieties was similar, the amount of decrease differed and was larger in Hatsumomiji than in NN27 and Z1. While the latter two are Japanese varieties, Hatsumomiji is a hybrid of the Assam which is generally sensitive to low temperature.

The depression of photosynthesis by low temperatures is thought to be caused by feedback inhibition by starch,^{7,22)} stomatal closure^{8,11,13)} or impairment of photosynthetic apparatus⁹⁾.

Although feedback inhibition of carbon exchange was observed in alfalfa²²⁾ and pangola⁷⁾, SENSER et al²⁶⁾ showed that in spruce chloroplast starch content of plastids decreased in winter. In overwintering tea leaves also, photosynthetic rates did not correlate with the content of carbohydrate²⁴⁾. Thus the accumulation of starch might not contribute to the depression of photosynthesis in overwintering tea leaves.

Stomatal closure was accompanied by the reduction of photosynthesis in many plants^{8,11,13)}, while PARKER²¹⁾ showed that transpiration continued in winter when CO₂ absorption ceased. MARTIN et al¹⁷⁾ also indicated that a reduction in stomatal aperture could be an indirect result of chilling. In this study, the effects of stomatal conditions on photosynthetic rates were eliminated by using a ¹⁴C and O₂ electrode method^{1,2)}. This leaves only the impairment of the photosynthetic apparatus of the mesophyll tissue as the reason for the rate inhibition. The cause of this action is still controversial, since it is influenced by photosynthetic light reactions^{12,19,20)}, photosynthetic dark reactions^{18,25)} or both¹⁴⁾.

In tea leaves, changes in soluble protein and fraction-1 protein were not correlated with changes in photosynthesis (Fig. 2). Nor did RuBPCase activity decrease in a manner similar to the photosynthetic rate (Table 1). The initial slope of light-photosynthesis

curves and light saturated Hill activity, on the other hand, did decrease in a parallel manner (Figs. 3 and 4). These results suggest that the depression of photosynthesis during winter is caused by the inhibition of photosynthetic light reactions.

In early spring, the photosynthetic rate recovered but was only 40–60% of the maximum rate. Incomplete recovery was due to the low activity of the photosynthetic light reaction system. In early spring, the initial slopes of light-photosynthesis curves and light saturated Hill activity were 80 and 40% of the maximum values, respectively (Figs. 3 and 4). These facts are important in considering the yield of the first crop of tea, because the growth of new shoots is dependent on the photosynthesis of overwintered leaves. If the leaves could be protected from cold injury or the recovery hastened, yield of the first crop might be increased.

It has been reported that light accelerates the cold injury to photosynthesis^{23,27)}. SAKAI et al²⁴⁾ and the present author's preliminary experiments found that shading did indeed lessen the impact of suppression of photosynthetic rates. A further study to increase yield by shading during winter is in progress.

Summary

Seasonal changes in autumn and winter and cause of photosynthesis depression induced by winter cold were investigated.

The photosynthetic rate gradually increased in October and early and mid-November, decreasing in the latter part of November when maximum and minimum temperatures were 20°C and 10°C, respectively. When the latter was below 0°C from January to the first of February, photosynthetic rates were lowest. The magnitude of decrease was larger in Hatsumomiji than in NN27 and Z1, although the time course of decline was similar among the cultivars. The rates then gradually increased but the recovery was partial, remaining at 40–60% of maximum rate.

Although chlorophyll content also decreased in winter, this was only observed when the minimum temperature was below 0°C, indicating that leaf colour is not a good

indicator of cold injury on photosynthesis.

Changes in soluble protein and fraction-1 protein contents did not correspond to the changes in photosynthetic rate. RuBPCase activity based on soluble protein and fraction-1 protein reduced only slightly, if at all, during winter. The initial slopes of light-photosynthesis curves and light saturated Hill activity, however, changed corresponding to the depression of photosynthetic rate.

Cold injury to photosynthesis is thus shown to be caused by the depression of photosynthetic light reactions rather than photosynthetic dark reactions in overwintering tea leaves.

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- * In Japanese with English Summary.

〔和 文 摘 要〕

冬季の低温によるチャ越冬葉の光合成明反応の阻害

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秋冬季におけるチャ越冬葉の光合成速度の季節的变化, とくに冬季の低温による光合成の低下の原因について検討した。

10月から翌春3月までの光合成速度の変化を測定したところ, 11月まで上昇し, 11月下旬に最高気温 20℃, 最低気温 10℃ となると, 光合成速度は低下を始めた。1月から2月初旬にかけて最低気温が 0℃ 以下のときに, 光合成速度は最低となった。低下時には品種間差異はみられなかったが, 低下程度ははつもみじが NN27, Z1 よりも大きかった。その後, 光合成速度は徐々に増加したが, その回復は部分的であり, 最高速度の 40—60% であった。

クロロフィル量も冬季に低下したが, その低下は最低気温が 0℃ 以下になったときにみられ, 光合成速度の低下する時期とは異っていた。このことは葉色の変化では光合成の低温阻害を確める指標とはなり得ないことを示している。

つぎに, 光合成の低温阻害機構を検討するために, はつもみじとやぶきたを用いて, 冬季における光合成明反応と暗反応の変化を測定した。

可溶性タンパク質とフラクシオン-I タンパク質量の変化は光合成速度の変化とは対応せず, またリブローズ 1, 5-ジリン酸カルボキシラーゼ活性によっても光合成速度の低下を説明できなかった。一方, 光強度—光合成曲線の初期勾配及び光飽和下のヒル反応速度は光合成速度と対応して変化していた。

以上の結果から, チャ越冬葉における光合成の低温阻害は光合成暗反応よりも明反応の抑制によることが示された。