Mechanism of accumulation of taste constituents in tea leaves by light shading

Takeshi Ogawa[1,2], Shoko Watanabe[1], Eiji Kobayashi[3], Yoriyuki Nakamura[3], and Hirokazu Kobayashi[1]

1: Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan
2: Local Collective Promotion Office, Food Science Center, The Shizuoka Organization for Creation of Industries, 44-1, Oute-machi, Aoi-ku, Shizuoka, 420-0853, Japan
3: Tea Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry, 1706-11, Kurasawa, Kikugawa, Shizuoka,439-0002, Japan
Correspondence email: hirokoba@u-shizuoka-ken.ac.jp

Summary

When tea plants (*Camellia sinensis*, cv. Yabukita) are shaded from sunlight, it has been known that amino acid contents increase. We determined contents of photosynthetic pigments and transcript levels for components of protein degradation systems. It has been observed that (1) protein degradation was significant under the 100%-shaded condition; (2) the incomplete shade such as 98% enhanced the content of each photosynthetic pigment, whereas 100%-shade diminished its content; (3) phenylalanine content increased under the 100%-shade, where amounts of polyphenols decreased; and (4) 98%- or 100%-shade resulted in stimulation of expression of gene for a homolog of ubiquitin-conjugating enzyme.

Introduction

It has known that amino acids including theanine for *umami* (savoriness) are accumulated and tannines for bitterness are diminished in *gyokuro* green tea, which is manufactured from leaves of tea plants maintained under a light-shaded condition. However, its underlying mechanism has not yet been clarified. We have speculated that the free amino acids may be made by degradation of proteins. Tea plants were shaded for certain periods and in different extents, and subjected to analysis of the decay of proteins and the expression of genes for the protein degradation systems such as ubiquitin-proteasome(Hellman *et al*., 2002).

Materials and methods

Tea plant (*Camellia sinensis* cv. Yabukita) were grown in fields under shading conditions (85, 98 and 100% shading) for different periods (3 days and 1, 2 and 3 weeks), and one to three young leaves attached to stems to be harvested for tea manufacture were used for the analyses. No shaded leaves were employed as a control throughout the investigation. To determine photosynthetic pigments, the leaves and stems were ground by mortar and pestle in the presence of liquid N_2, extracted with 80% acetone, and then subjected to measurement of absorbance at 433, 645, 663 and 710 nm. The contents of photosynthetic pigments were calculated as follows (Katou *et al*., 1981; Arnon, 1949)

(1) Chrolophyll *a* (μg/ml) = 12.7 (A_{663}-A_{710})-2.59 (A_{645}-A_{710})
(2) Chrolophyll *b* (μg/ml) = 22.9 (A_{645}-A_{710})-4.67 (A_{663}-A_{710})
(3) Total carotenoids (mol) = (A_{433}-A_{710})・solvent volume (ml)・dilution rate/1.4E^5

For real-time RT-PCR, total RNA was extracted from the leaves and stems, and determination of transcript levels were done by real-time RT-PCR (LightCycler), when transcript levels were standardized by expression of an actin gene.

Results and discussion

The 100%-shading resulted in maintaining the lowest level of chlorophyll *a* throughout the treatment

period, whereas 98%-shading enhanced its content. The same tendency of change of pigment content as that of chlorophyll *a* was observed. The profile of change of carotenoid content resembled that of chlorophyll *a* or *b* under 100%- or 98%-shading condition, although that under 85%-shading was kept at higher levels, being different from that of chlorophyll *a* or *b*. Expression of gene for a homolog of ubiquitin-conjugating enzyme was enhanced gradually during the period under the 100%- or 98%-shade. Any significant change was not observed in expression of an ubiquitin homolog.

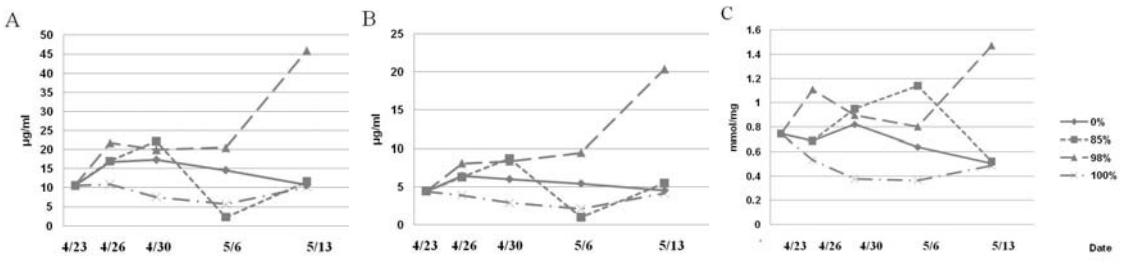

Figure 1. Time-sequential change of photosynthetic pigments under different sunlight-shading conditions. A, chlorophyll *a*; B, chlorophyll *b*; and C, total carotenoids.

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