

Full Length Research Paper

## Effect of sunlight shielding on leaf structure and amino acids concentration of light sensitive albino tea plant

Wang K. R.<sup>1,2</sup>, Li N. N.<sup>1</sup>, Du Y. Y.<sup>1</sup> and Liang Y. R.<sup>1\*</sup>

<sup>1</sup>Zhejiang University Tea Research Institute, Hangzhou 310029, China.

<sup>2</sup>Ningbo Extension Station of Forestry and Speciality Technology, Ningbo 315012, China.

Accepted 2 August, 2013

Light sensitive albino tea cultivar 'Jinguang' (*Camellia sinensis*) which grows albinism leaf in yellow colour, results to high level of amino acids but low levels of photosynthetic pigments including chlorophylls, neoxanthin, violaxanthin, phytoanthin and  $\beta$ -carotene when it is exposed to high sunlight illumination in the summer season. In this case, the chloroplasts showed partially lysed, with few thylakoids. The leaf albinism was reverted when the leaf was shielded from direct illumination of strong sunlight. It is considered that the blocked development of chloroplast and photosynthetic pigments in the albinism leaf inhibited the biosynthesis of leaf proteins, resulting in an accumulation of free amino acids.

**Key words:** *Camellia sinensis*, leaf albinism, light intensity, photosynthetic pigments, amino acids, chloroplast.

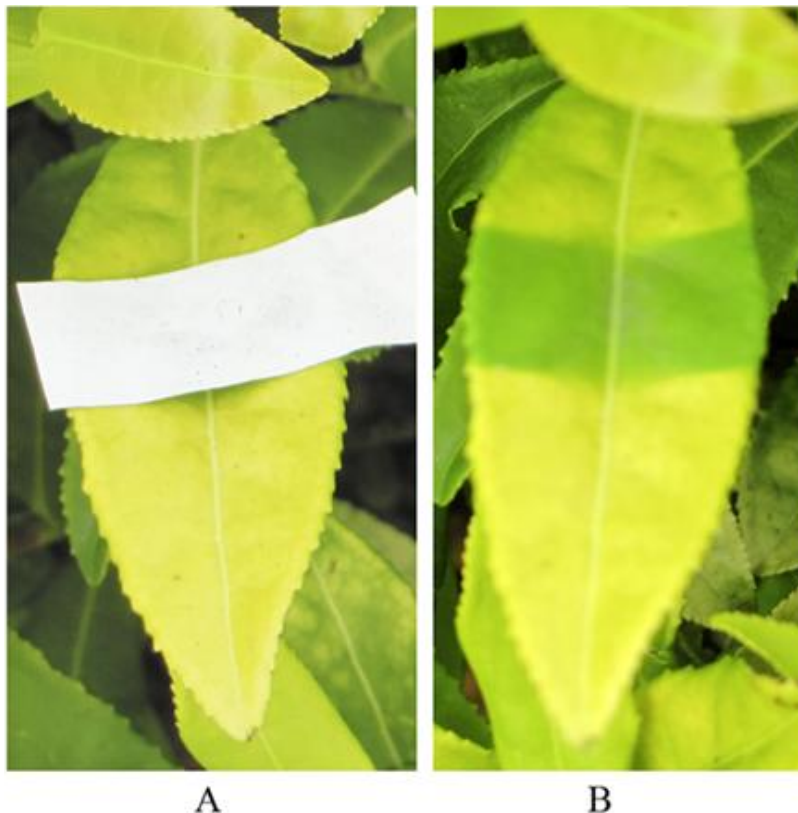
### INTRODUCTION

Amino acids are considered to be a group of quality and health benefit related components in tea (Yokogoshi et al., 1995; Liang et al., 2003; Liang et al., 2008). Albino tea cultivars are characterized by high concentration of amino acids (Du et al., 2006, 2008). There are two kinds of albino tea cultivars, that is, temperature sensitive one and light sensitive one. The temperature sensitive albino tea cultivars grow albinism shoots with high level of amino acids during early spring period when temperature is below 20°C, which can be used as good materials to processing quality green tea. However, in the summer and autumn seasons when the temperature is higher than 20°C, they grow green shoots resulting to decreased amino acids (Du et al., 2009). Normally, leaf materials from normal tea cultivars have low concentrations of amino acids and high concentration of polyphenols during summer and autumn seasons, resulting in low quality of green tea owing to their bitter taste. However, the light sensitive albino tea cultivars grow albinism shoots during

the low temperature spring and the strong sunshine summer and autumn. These albinism shoots have high level of amino acids, which are suitable for processing quality green tea even in the summer and autumn seasons.

In the temperature sensitive albino tea cultivar, the expressions of genes encoding chlorophyll a/b-binding protein (Lhcb), rubisco activase (RCA), D1 protein in the photosystem II core (psbA), terminal oxidase (TOX), and violaxanthin de-epoxidase (VDE) were suppressed at low temperature such as 15°C, resulting in low levels of chlorophylls and carotenoids and high level of amino acids, but the gene expression levels and pigment accumulation were reversible as the temperature increased to 20°C or above (Du et al., 2009). Specific random amplified polymorphic DNA (RAPD) markers for identifying temperature sensitive albino tea cultivars were developed (Wang et al., 2010). There has no report on the albinism mechanism of light sensitive albino tea

\*Corresponding author. E-mail: yrliang@zju.edu.cn.



**Figure 1.** Effect of sunlight shielding on albinism of tea leaf. A, Sunlight shielding treatment with white paper. B, Change in leaf colour after sunlight shielding.

cultivars. Effects of sunlight shielding on leaf structure and amino acids concentration of light sensitive albino tea cultivar 'Jinguang' were investigated in the present paper.

## MATERIALS AND METHODS

### Plant materials

The light sensitive albino tea cultivar *Camellia sinensis* cv. 'Jinguang' grown on Deshijia Tea Farm (Yuyao City, Zhejiang Province, China) was used in the experiment. A white sticky paper (2 × 4 cm) was stuck on the middle part of the second leaf from the apex bud of a shoot to make the leaf be partially shielded from sunlight in early July 2009 (Figure 1A). Ten (10) days later, the sunlight-shielded leaf portion reverted green colour and the other parts remained albinism (Figure 1B). The green and albinism parts of the leaf were excised separately for use.

### Transmission electron microscopy (TEM)

The leaf was cut into pieces (2 × 2 mm) and fixed in glutaraldehyde solution (2.5%, v/v) overnight. The fixed samples were washed using 0.1 M phosphate buffer (pH 7.0) for three times and then re-fixed in 1% (w/v) OsO<sub>4</sub> for 2 h and washed as before. The samples were dehydrated in gradient ethanol (50, 70, 80, 90, 95 and 100%, v/v), for 15 min each step. The dehydrated samples were embed-

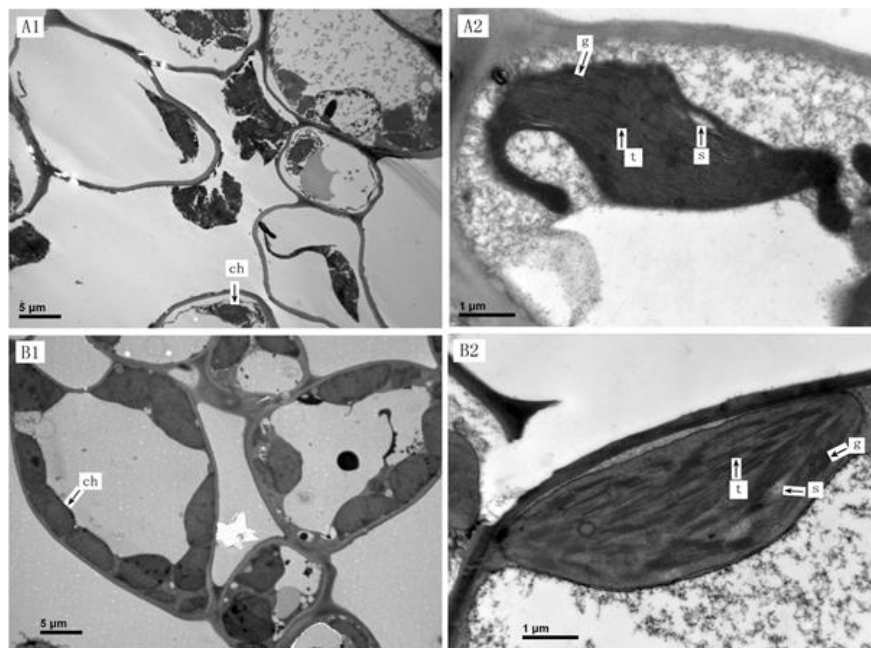
ded in 50% (v/v) epoxy resin which was dissolved in acetone for 1 h, 67% (v/v) epoxy resin for 3 h and finally in pure epoxy resin overnight. The embedded samples were sectioned and stained with uranyl acetate and lead citrate for 15 min, and examined under JEM-1200EX transmission electron microscope (JEOL Ltd., Tokyo Japan) as method described by Du et al.(2008).

### High power liquid chromatography (HPLC) of leaf pigments

Leaf pigments including chlorophylls *a* and *b*, neoxanthin, violaxanthin, phytoanthin, β-carotene, were determined by HPLC. The leaf sample (0.2 g) was extracted in 10 mL acetone, centrifuged at 13,000 rpm at 4°C for 15 min. The supernatant (10 μL) was then injected into HPLC. HPLC was carried out on LC-2010A HPLC system (Shimadzu Corp., Kyoto, Japan) using a CLC-ODS column (6.0 × 150 mm) at 30°C and flow rate 1 ml min<sup>-1</sup> as method described by Du et al. (2009). Mobile phase A was a mixture of acetonitrile/ acetic acid/ water (6/1/193, v/v/v) and mobile phase B was a mixture of acetonitrile/ methanol/ chloroform (150/40/10, v/v/v). The eluate was monitored at 450 nm using a Shimadzu SPD ultraviolet detector (Shimadzu Corp., Kyoto, Japan).

### HPLC of amino acids

Leaf sample (0.5 g) was grounded in a mortar with 10 ml 70% (v/v) ethanol and centrifuged at 13 000 rpm at 4°C for 15 min. 100 μL of the supernatant was mixed with 100 μL NaHCO<sub>3</sub> (0.5 M, pH 9.0) and 100 μL 1% (w/v) fluorodinitrobenzene (FDBN) in a 1.5-mL



**Figure 2.** Ultrastructure of tea leaf. A, Leaf under natural strong sunlight. B, Sunlight shielded leaf; ch, chloroplast; g, granum; s, stroma; t, thylakoid.

centrifuge tube, heated in water bath at 60°C for 1 h. The solution was then mixed with 400  $\mu\text{L}$   $\text{KH}_2\text{PO}_4$  (0.01 M, pH 7.0) before injected into HPLC. HPLC of amino acids was carried out on a LC-2010A HPLC system (Shimadzu Corp., Kyoto, Japan) as method described by Peris-Vicente *et al.* (2006).

## RESULTS AND DISCUSSIONS

### Effect of sunlight shielding on leaf albinism and ultra-structure

In the summer season, the albinism leaf of light sensitive albino tea cultivar was yellow in colour instead of green colour of normal tea plants. However, as the part of the leaf which was shielded from direct sunlight using a piece of white paper for 10 days, it resumed green colour (Figure 1). It shows that the albinism of the leaf depends on the intensity of sunlight and the albinism is convertible when the sunlight intensity is decreased.

The chloroplast is the organelle where photosynthesis occurs in photosynthetic eukaryotes. Differences in ultra-structures of natural sunlight and light shielding treatments were shown in Figure 2. There were many chloroplasts located close to the walls of the mesophyll cells in the light shielded leaf (Figure 2, B1), but few normal chloroplasts were observed in mesophyll cells of the leaf under natural strong sunlight condition (Figure 2, A1). Chloroplasts in the light shielded leaf had normal granum, thylakoid and stroma (Figure 2, B2). The chloroplasts in leaf under natural strong sunlight condition were partially lysed, in which thylakoid was few (Figure 2, A2). Chloro-

phylls are photosynthetic pigments which use sunlight to create sugars and thylakoid sacs are sites where chlorophyll molecules are located. Aberrant development of chloroplasts was paralleled by impairment of chlorophyll biosynthesis (Hodgins and van Huystee, 1986), resulting in deficiency in pigments (Fambrini *et al.*, 2004). This study shows that the disfunction of chloroplasts is consistent to the albinism of the tea leaf. The arrested development of thylakoids might block chlorophyll accumulation in leaf. It explains why the albinism phenomenon of cultivar 'Jinguang' occurs under high sunlight illumination in the summer. It is confirmed that photosynthetic performance of a plant can be severely inhibited following exposure to light intensities in excess of those required to saturate photosynthesis (Osmond, 1981).

Despite the essential requirement of light for the functional activity, an excessive light inhibits chloroplast electron transport, resulting in photodamages (Powles, 1984). The albinism of the light sensitive albino tea cultivar might be related to its photodamages under strong sunlight conditions in the summer.

### Effect of sunlight shielding on concentration of leaf photosynthetic pigments

Leaf photosynthetic pigments including neoxanthin, violaxanthin, phytoanthin,  $\beta$ -carotene and chlorophylls *a* and *b* were significantly increased when the leaf was shaded with white paper under strong sunlight condition

**Table 1.** Concentration of leaf photosynthetic pigments ( $\mu\text{g g}^{-1}$ , FW)<sup>a</sup>.

Pigment	Natural sunlight	Shading
Neoxanthin	4.48±0.52	10.99±1.06
Violaxanthin	18.75±3.02	24.62±3.05
Phytoxanthin	54.64±0.08	110.44±8.22
$\beta$ -Carotene	67.70±0.26	151.34±9.5
Chlorophyll <i>a</i>	166.05±8.21	669.16±4.31
Chlorophyll <i>b</i>	34.22±0.75	138.18±5.28
Total	200.27±8.96	807.34±9.59
Chlorophyll <i>a</i> / Chlorophyll <i>b</i>	4.85	4.84

<sup>a</sup>FW: Fresh weight.**Table 2.** Amino acids of albino tea cultivars during illumination treated ( $\text{mg g}^{-1}$ , FW)<sup>a</sup>.

Amino acid	Natural sunlight	Shading
Aspartic	0.714 ± 0.052	0.252 ± 0.082
Glutamate	1.059 ± 0.022	0.618 ± 0.091
Asparagine	0.017 ± 0.004	0.015 ± 0.002
Serine	0.012 ± 0.003	0.007 ± 0.002
Histidine	0.130 ± 0.023	0.067 ± 0.008
Threonine	0.048 ± 0.005	0.033 ± 0.004
Theanine	2.117 ± 0.122	1.428 ± 0.105
Alanine	0.085 ± 0.007	0.000
Lysine	0.158 ± 0.022	0.129 ± 0.051
Valine	0.000	0.002 ± 0.001
Methionine	0.069 ± 0.004	0.029 ± 0.002
Cysteine	0.087 ± 0.006	0.000
Tryptophan	0.043 ± 0.012	0.006 ± 0.002
Phenylalanine	0.164 ± 0.021	0.070 ± 0.011
Leucine	0.119 ± 0.012	0.064 ± 0.012
Tyrosine	0.085 ± 0.005	0.014 ± 0.003
Total	4.905 ± 0.476	2.733 ± 0.257

<sup>a</sup>FW, Fresh weight.

in the summer (Table 1). Compared to leaf under natural strong sunlight, level of Chlorophylls *a* and *b* in the light shielded leaf increased by four times, and those of neoxanthin, phytoanthin and  $\beta$ -carotene were doubled. Violaxanthin in the light shielded leaf increased by 31%. However, there was no significant difference in the ratio of chlorophyll *a* to chlorophyll *b*, suggesting that the light shielding had a same effect on the chlorophylls *a* and *b*. The decrease in level of chlorophylls is considered to be related to the albinism of the tea leaf.

Neoxanthin, violaxanthin, phytoanthin and  $\beta$ -carotene are components of carotenoids in tea leaf and they absorb light energy for use in photosynthesis and protect chlorophyll from photodamage. Carotenoids are usually

considered to perform two major functions in photosynthesis. They serve as accessory light harvesting pigments, extending the range of wavelengths over which the light can drive photosynthesis, and they act to protect the chlorophyllous pigments from the harmful photodestructive reaction or photodamage which occurs in the presence of oxygen under excessive sunlight condition (Cogdell, 1978).

Photodamage to photoautotrophic organisms under unfavorable environmental conditions proceeds primarily via the increased generation of reactive oxygen species (Solovchenko and Merzlyak, 2008). It is considered that these pigments work as screening pigments when illumination is above the light saturation point, during which they were partially consumed, resulting in their low levels in the albinism leaf under natural strong sunlight condition in the summer.

### Effect of sunlight shielding on concentration of amino acids

There was significant difference in amino acids concentration between natural strong sunlight and sunlight-shielded treatments (Table 2). Total concentration of amino acids in sunlight-shielded leaf was decreased by 44.3%, compared to that under natural strong sunlight. Theanine was the most abundant component of tea leaf amino acids and glutamate the next in the two treatments. Valine was not detected in the natural strong sunlight sample. Alanine and cysteine were not detected in sunlight-shielded sample (Table 2). This suggests that biosynthesis and accumulation of amino acids in the light sensitive albino tea plant leaf is affected by light intensity.

Photosynthesis-related plastid mRNAs and plastid tRNAs were confirmed to be down-regulated in albino barley and maize leaves (Zubko and Day, 2002). Some protein synthesis is independent on chlorophyll and carotenoid development in the plastids (Fambrini et al., 2004). A light-yellow mutant of *Plantago insulari* grown in the dark became bleached on exposure to light, yet a moderate increase in nitrogen occurred during exposure to light (Michael and Spurr, 1975). The present study shows that the development of chloroplast and photosynthetic pigments including chlorophyll, neoxanthin, violaxanthin, phytoanthin and  $\beta$ -carotene was blocked in the albino leaf. Amino acids are the monomers for synthesizing proteins in plant. It is considered that the blocked development of chloroplast and photosynthetic pigments inhibited the biosynthesis of leaf proteins, resulting in accumulation of free amino acids.

### ACKNOWLEDGEMENT

This is a partial work of the project "Research on Key cultivation technologies of all year round albino tea cultivars and their industrialization" financially supported

by the Science and Technology Bureau of Ningbo City.

## REFERENCES

- Cogdell RJ (1978). Carotenoids in Photosynthesis. *Phil. Trans. R. Soc. Lond. B.* 284: 569-579.
- Du YY, Chen H, Zhong WL, Wu LY, Ye JH, Lin C, Zheng XQ, Lu JL, Liang YR (2008). Effect of temperature on accumulation of chlorophylls and leaf ultrastructure of low temperature induced albino tea plant. *Afri. J. Biotechnol.* 7:1881-1885.
- Du YY, Liang YR, Wang H, Wang KR, Lu JL, Zhang GH, Lin WP, Li M, Fang QY (2006). A study on the chemical composition of albino tea cultivars. *J. Hort. Sci. Biotechnol.* 81:809-812.
- Du YY, Shin S, Wang KR, Lu JL, Liang YR (2009). Effect of temperature on the expression of genes related to the accumulation of chlorophylls and carotenoids in albino tea. *J. Hort. Sci. Biotechnol.* 84:365-369.
- Fambrini M, Castagna A, Vecchia FD, Degl'Innocenti E, Ranieri A, Vernieri P, Pardossi A, Guidi L, Rascio N, Pugliesi C (2004). Characterization of a pigment-deficient mutant of sunflower (*Helianthus annuus* L.) with abnormal chloroplast biogenesis, reduced PS II activity and low endogenous level of abscisic acid. *Plant Sci.* 167:79-89.
- Liang YR, Lu JL, Zhang LY, Wu S and, Wu Y (2003). Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chem.* 80:283-290.
- Liang YR, Ye Q, Jin J, Liang H, Lu JL, Du YY, Dong JJ (2008). Chemical and instrumental assessment of green tea sensory preference. *Intl J. Food Prop.* 11:258-272.
- Michael SD, Spurr AR (1975). An albino mutant in *Plantago insularis* requiring thiamine pyrophosphate-role of tpp in pigment and protein synthesis. *New Phytol.* 74:221-225.
- Osmond CB (1981). Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. *Biochim. Biophys. Acta*, 639:77-98.
- Peris-Vicente J, Adelantado JV, Carbo MTD, Castro RM, Reig FB (2006). Characterization of proteinaceous glues in old paintings by separation of the o-phthalaldehyde derivatives of their amino acids by liquid chromatography with fluorescence detection. *Talanta*, 68 (5): 1648-1654.
- Powles S B (1984). Photoinhibition of Photosynthesis induced by visible light; *Annu. Rev. Plant Physiol.* 35:15-44.
- Solovchenko A, Merzlyak M (2008). Screening of visible and UV radiation as a photoprotective mechanism in plants. *Russ. J. Plant Physiol.* 55:719-737.
- Wang KR, Du YY, Shao SH, Lin C, Ye Q, Lu JL, Liang YR (2010). Development of specific RAPD markers for identifying albino tea cultivars 'Qiannianxue' and 'Xiaoxueya'. *Afri. J. Biotechnol.* 9:434-437.
- Yokogoshi H, Kato Y, Sagesaka YM, Matsuura TT, Kakuda T, Takeuchi N (1995). Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. *Biosci. Biotechnol. Biochem.* 59: 615-618.
- Zubko MK, Day A (2002). Differential regulation of genes transcribed by nucleus-encoded plastid RNA polymerase, and DNA amplification, within ribosome-deficient plastids in stable phenocopies of cereal albino mutants. *Mol. Genet. Genomics*, 267:27-37.