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Effects of Low Temperature on Morphology and Photosynthetic Fluorescence Characteristics of Tea Plants

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Abstract To examine how low-temperature frost affects tea production in the Dabie Mountains by using Lu'an Guapian tea plants as the study material, four groups of temperature treatments were applied in an artificial climate chamber to conduct controlled low-temperature experiments, and measurements of leaf photosynthetic and chlorophyll fluorescence parameters were taken to assess the effects of varying low temperatures on photosynthetic performance and fluorescence characteristics. The results show that all low-temperature conditions cause visible leaf damage, with young leaves exhibiting greater sensitivity than mature leaves, and colder temperatures lead to more severe injury. Low temperatures also reduce photosynthetic capacity and fluorescence indicators, and the magnitude of these effects increases as temperature decreases. These impacts ultimately hinder plant growth and negatively affect tea yield and quality.

Key words Tea plant; Lu'an Guapian; Photosynthetic parameters; Chlorophyll fluorescence parameters; Low temperature stress

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Low temperature is a crucial factor that limits the growth and distribution of tea plants[1]. Anhui Province has large terrain undulations and significant vertical climate differences, with frequent occurrences of low-temperature freezing damage[2]. Freezing damage refers to the sudden drop in the lowest temperature below 4 ℃, which can cause partial morphological damage or death of tea plants[3]. After the tea leaves and buds are frostbitten by low temperature, they generally exhibit symptoms such as thinning and browning of the edges of the tender buds, and curling of the tender buds, leading to a decrease in tea quality and economic value. The effect of low temperature on photosynthesis and fluorescence characteristics of tea plants is highly sensitive. Existing research indicates that low temperatures can inhibit the activity of protective enzymes and disrupt the normal synthesis process of chlorophyll. At the same time, it can also damage the structure of chloroplasts, causing changes in chlorophyll content and composition, thereby weakening the efficiency of photosynthesis[4]. The lower the temperature, the more significant the inhibitory effect on the fluorescence parameters and photosynthetic capacity of tea plants. In order to explore the influence of different low temperature conditions on tea plants, the 5-year-old tea plant Lu'an Gua Pian was taken as the research object. The low temperature control experiment was carried out using an artificial climate chamber to measure the photosynthetic and fluorescence parameters of tea leaves after different low temperature stress, so as to reveal the influence of different low temperature conditions on tea plant morphology, photosynthetic capacity and fluorescence characteristics, and provide a basis for the low temperature defense mechanism of tea plants.

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1 Experimental plan and indicator measurement method

1.1 Technical route Technical route was shown as Fig. 1.

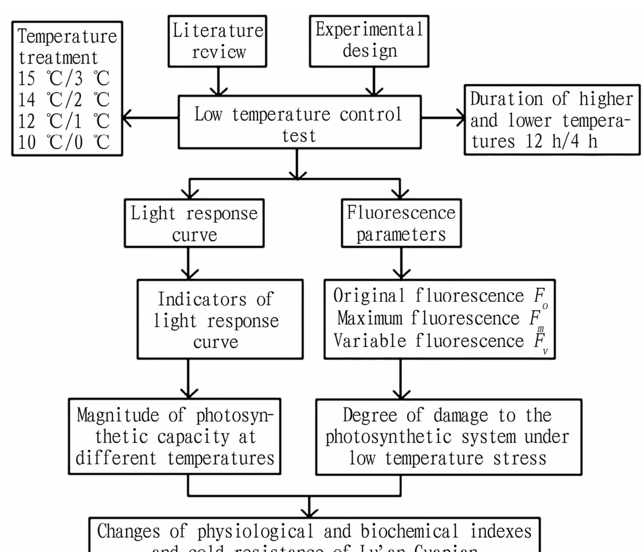

Fig. 1 Technical route

1.2 Material source and experimental plan design In this experiment, 5-year-old Lu'an Guapian tea plants were used as materials. The minimum temperature data for the Dabie Mountain tea area from February to April 2021 came from the Anhui Provincial Meteorological Disaster Prevention Technology Center, and the hourly meteorological data came from small-scale climate observation stations in tea gardens. The low-temperature control experiment was conducted from December 2021 to February 2022 at the Agricultural Experiment Station of Nanjing University of Information Science and Technology, and an artificial climate chamber (PVG-36 Conviron, volume 1 260 L; temperature −5 to 5 ℃; light exposure 0 – 1 000 μmol/(m^2·s); humidity range of 30% – 90%) was used. The soil and water fertilizer management of potted tea plants should be set consistently according to natural growth conditions. Low temperature treatment included 15 ℃/3 ℃ (T1), 14 ℃/2 ℃ (T2), 12 ℃/1 ℃ (T3), and 10 ℃/0 ℃ (T4), with lower temperatures lasting for 4 h and higher temperatures lasting for 12 h. Tender shoots were collected immediately after processing to measure photosynthetic and fluorescence indicators. The conditions of control group were 25 ℃/15 ℃, photosynthetically active radiation of 800 μmol/(m^2·s), and relative humidity of 75%. Experimental setting for different low-temperature treatments was shown as Table 1.

Table 1 Experimental setting for different low-temperature treatments

Treatment	Temperature setting//℃	Minimum temperature duration//h	Maximum temperature duration//h
CK	25/15	4	12
T1	15/3	4	12
T2	14/2	4	12
T3	12/1	4	12
T4	10/0	4	12

1.3 Determination of physiological and biochemical indicators of tea leaves

1.3.1 Measurement of photosynthetic parameters. Firstly, it was necessary to use the red blue light source (6400-02B) in the LI-6400 photosynthesis measurement system to set the photosynthetically active radiation in the leaf chamber to 12 levels, to set different PAR levels, measure the photosynthetic rate, and obtain the maximum photosynthetic rate P_{max}, light saturation point *LSP*, and light compensation point *LCP*. During the measurement of tea plants in each treatment group, the air temperature, CO_2 concentration, and relative humidity inside the photosynthesis chamber were controlled to remain constant.

1.3.2 Measurement of fluorescence parameters. The determination of fluorescence parameters included original fluorescence F_o, maximum fluorescence F_m, and variable fluorescence F_v. Functional leaves of tea plants with good growth in each treatment group were selected for the measurement of chlorophyll fluorescence parameters in experimental plant leaves. Each treatment group was tested three times, and the same tea leaves were selected. The non photochemical quenching coefficient (qN), maximum quantum yield of PSII (F_v/F_m), excitation energy capture efficiency of open PSII reaction centers under light (F_v'/F_m'), and actual photochemical quantum efficiency of PSII in the presence of reactive light (Φ_{PSII}) were calculated.

1.4 Data processing methods

The data in this paper was processed using Microsoft Excel 2003 – 2007 software.

2 Results and analysis

2.1 Impacts of different low temperature conditions on the morphology of tea leaves

Low temperature has different effects on different parts of tea plants, and the leaves are one of the most susceptible parts, especially the newly born tender buds. When the impact of low temperature is slightly mild, the tender leaves at the top could be the first to be affected. At the beginning of being frozen, the tender leaves could change color, and then gradually spread from the leaf tip and edge to the middle, eventually endangering the entire leaf. As the low temperature worsens, adult leaves could also be affected. The leaves begin to lose their luster, and then develop into leaf curl. Finally, the leaves become scorched and could fall off upon touch.

Before being subjected to low-temperature treatment, tea leaves maintained their normal shape, were generally more stretched, and had a healthier color (Fig. 2a). After low-temperature treatment, the morphology of tea leaves underwent significant changes. Tea leaves treated with low temperature lost their luster and curled from new upper leaves to old lower leaves (Fig. 2b). After one day of recovery, the leaves became burnt and withered, and both adult and new leaves were relatively fragile. The greater the morphological changes in tea leaves treated at lower temperatures, the more severe the damage to the functional structure of the leaves.

Fig. 2 Comparison of tea leaves before (a) and after (b) T4 temperature treatment

2.2 Impacts of different low temperature conditions on the photosynthetic characteristics of tea leaves

Compared with CK, low-temperature treatment showed a consistent overall slight increase in the light compensation point *LCP* of tea leaves in each group (Table 2), while the light saturation point *LSP* of tea leaves in each group significantly decreased. At the same time, the maximum photosynthetic rate P_{max} showed an overall decrease trend, which was consistent with the research conclusions of Chen Fang *et al.* [5] but slightly different. Low temperature weakened the ability of tea leaves to utilize strong light within a certain range, increased the respiration of tea leaves in each group, reduced the accumulation of dry matter in tea leaves, and thus inhibited the conversion process of photosystem light energy in tea leaves.

Table 2 Photosynthetic parameters of tea leaves from Lu'an Guapian under different low temperature treatments μmol/(m^2 · s)

Temperature // ℃	*LCP*	*LSP*	P_{max}
CK (normal temperature, 25)	31.89	842.27	3.19
15/3	107.56	501.32	2.10
14/2	124.38	482.07	1.22
12/1	128.55	469.57	1.19
10/0	131.60	458.78	0.80

By comparison, it can be seen that the light compensation point *LCP* at normal temperature was the lowest, at 31.89 μmol/(m^2 · s), while the light saturation point *LSP* was the highest, at 842.27 μmol/(m^2 · s), and the maximum photosynthetic rate was the highest, at 3.19 μmol/(m^2 · s). The light compensation point under other low-temperature conditions continued to increase, while the light saturation point and the maximum photosynthetic rate continued to decrease. It can be inferred that as the temperature decreased, the light compensation point *LCP* could be higher, the light saturation point *LSP* could be lower, and the maximum photosynthetic rate could also be lower, which had a greater impact on the photosynthesis of tea plants. The growth of young tea leaves mainly relies on their own photosynthesis. The stronger the photosynthesis, the more dry matter they accumulate. Low temperatures can reduce the ability of photosynthesis, which in turn affects the yield and quality of tea.

2.3 Impacts of different low temperature conditions on fluorescence parameters of tea leaves The apparent quantum efficiency *AQY* reflects the photosynthetic ability of tea plants under low light conditions. The maximum photochemical yield F_v/F_m refers to the fluorescence yield of PSII reaction centers when they are completely closed, usually measured after 20 min of dark adaptation in leaves. Therefore, F_v/F_m is the efficiency of PSII reaction centers capturing excitation energy, and is also considered the maximum photochemical efficiency. The non photochemical quenching coefficient *qN* refers to the ability of tea plants to dissipate excess light energy into heat energy, reflecting their ability to protect themselves from light.

Table 3 Fluorescence parameters of tea leaves from Lu'an Guapian under different low temperature treatments

Temperature // ℃	*AQY*	F_v/F_m	*qN*
CK (normal temperature, 25)	0.008 5	0.80	0.58
15/3	0.007 0	0.67	0.56
14/2	0.005 9	0.63	0.55
12/1	0.005 0	0.54	0.51
10/0	0.004 1	0.42	0.45

The fluorescence parameters of tea leaves from Lu'an Guapian under different low temperature treatments were shown as Table 3. Compared with CK, low-temperature treatment reduced the apparent quantum efficiency *AQY*, maximum photochemical yield F_v/F_m, and non photochemical quenching coefficient *qN*. Moreover, these indicators showed a continuous downward trend as low-temperature stress intensified.

In summary, as the temperature decreased, *AQY*, F_v/F_m, and *qN* all continued to decline. The effect of low temperature on the fluorescence characteristics of tea plants continued to increase, with frost damage becoming more pronounced. When the temperature dropped to 0 ℃, these indicators reached their lowest point. Under T4 treatment, tea leaves showed significant frost damage, with the leaf edges turning black from bottom to top and the top young leaves being the most severely damaged. Even after returning to a normal environment, growth could not be restored. Old leaves shrank or even died, and the blackened parts of young leaves were irreversible, making it impossible to continue picking and making tea. Although it can be restored by removing frozen leaves and waiting for new shoots to sprout, new shoots need time to grow, and early freezing can directly damage the tender shoots, ultimately leading to a decrease in tea yield[6].

2.4 Impacts of different low temperature conditions on the rapid fluorescence induction kinetics curve of tea leaves Rapid fluorescence induction kinetics (OJIP) refers to the changes in chlorophyll fluorescence produced by plants under sudden light after dark adaptation. Its curve consists of four sites, O, J, I, and P, which can reflect the initial photochemical reaction and structural functional state of photosystem II (PSII). Among them, point O represents the lowest fluorescence value, and point P represents the highest peak value. The OJIP curve characteristics can reveal the electron transport status of PSII reaction centers, thereby reflecting the photosynthetic efficiency and potential of leaves.

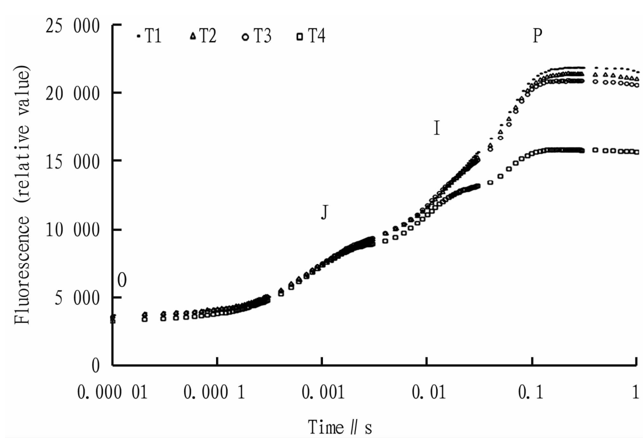

Fig. 3 OJIP curves of tea leaves treated with different low temperature conditions

Fig. 3 showed that each treatment group exhibited typical four-stage characteristics of O, J, I, and P, indicating that the leaves still had basic photosynthetic activity. As the low temperature intensified, the differences in curves at the J, I, and P stages gradually widened, and the fluorescence intensity showed T1 > T2 > T3 > T4. The results indicate that low temperature stress weakens PSII electron transport, reduces photosynthetic efficiency and potential vitality, and the degree of tissue damage increases with decreasing temperature.

2.5 Impacts of different low temperature conditions on the relative variable fluorescence of tea leaves The relatively variable fluorescence $V_t = (F_t - F_o)/(F_m - F_o)$ is obtained by standardizing the OJIP curve, and the increase amplitude can be fixed at 1, which facilitates the comparison of the reduction rate of the end electron acceptor pool at different low temperatures and the differences in the changes of each curve. ΔV_t reflects the changes in relatively variable fluorescence, and the lower the value, the stronger the PSII energy connectivity, the more fully utilized the excitation energy, and the more stable the system. The relatively variable fluorescence difference can be used to analyze the changes in the oxygen releasing complex, PSII complex unit and functional antenna size in the tea leaves from Lu'an Guapian, and is an important indicator to evaluate the energy connectivity of PSII[7].

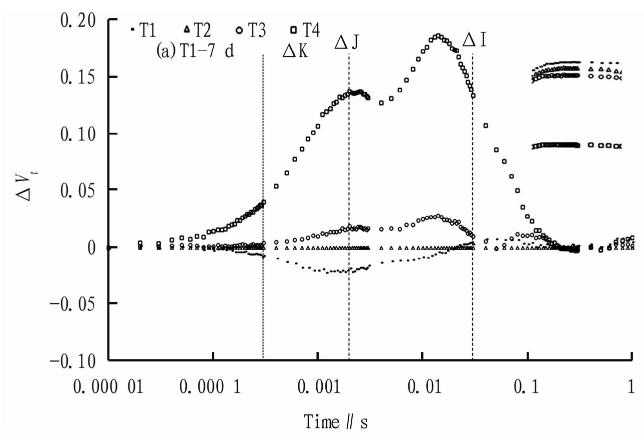

Fig.4 Impacts of different low temperature treatments on ΔV_t of tea leaves from Lu'an Guapian

Fig. 4 showed the impacts of different low temperatures on ΔV_t of tea leaves from Lu'an Guapian. Under T1 (mild stress) treatment, both ΔK and ΔJ were negative, indicating strong PSII energy connectivity and good stress resistance[8]; ΔV_t of T2 was close to 0. T3 and T4 (severe stress) both showed positive values at ΔK and ΔJ, indicating that the activity of oxygen releasing complexes was inhibited, and energy conversion and transfer were limited[9]. Among them, the ΔV_t positive value of T4 was larger and changed more significantly, indicating that PSII was the most severely damaged and had the lowest stability.

3 Conclusions

Low temperature conditions could cause irreversible damage to the morphology of tea leaves from Lu'an Guapian, and new leaves are more vulnerable to damage. The lower the temperature, the heavier the stress. The photosynthetic capacity and fluorescence parameters also deteriorate with decreasing temperature, leading to impaired electron transport in PSII reaction centers, decreased photosynthetic efficiency and potential, and aggravated leaf damage. In this paper, the morphology, photosynthesis and chlorophyll fluorescence reaction of tea leaves from Lu'an Guapian under different low temperature conditions were clarified. Later, it should be verified and compared among more tea varieties to carry out systematic research.

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