INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–1510/2020/23–4–777–785
DOI: 10.17957/IJAB/15.1352
http://www.fspublishers.org

Full Length Article

Effect of Chilling Temperature on Chlorophyll Florescence, Leaf Anatomical Structure, and Physiological and Biochemical Characteristics of Two *Camellia oleifera* Cultivars

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Received 30 September 2019; Accepted 06 December 2019; Published 13 February 2020

Abstract

Camellia oleifera Abel. is an economically important tree that produces edible oils and flowers and fruits in late autumn and early winter. However, low temperatures lead to low yields because of the absence of normal pollination and fertilization. In this study, a pot experiment was conducted to understand the effects of temperature (6°C, 25°C, and control) on florescence, photosynthesis, physiological response, and anatomical structure in two *Camellia* cultivars (Hua Shuo and Hua Xin) for 25 days. Low-temperature stress (6°C) decreased net photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, and maximum quantum yield of PSII photochemistry of the two cultivars. Initial fluorescence, leaf thickness, and soluble sugar and malondialdehyde contents increased in the low-temperature treatment compared to the normal temperature (25°C). Low-temperature stress (6°C) destroyed chloroplast morphology and structure. In addition, it inhibited the normal opening of Hua Xin and normal temperature promoted the growth of young fruits. However, the flowers of Hua Shuo bloomed, the stamens and pistils withered but did not fall off, and for the first time it was discovered that a significant amount of mucus appeared at the base of the flower under low temperature. The mechanism of secretion of the mucus is unknown and merits further investigation. In summary, these results suggest that *C. oleifera* has a high fruit setting rate under normal temperature. Low temperature, even 6°C, significantly reduced photosynthetic efficiency and the fruit setting rate. In addition, Hua Shuo had better cold tolerance than Hua Xin, but young fruit of Hua Xin grew faster than Hua Shuo at the normal temperature.

Key words: Cold resistance; Chloroplast ultrastructure; Chlorophyll fluorescence; *C. oleifera*

Introduction

Tea-oil tree (*Camellia oleifera* Abel; family Theaceae) is an evergreen shrub and oil plant that has been cultivated for more than 2,000 years in China (Tan *et al*. 2011b). *C. oleifera* is one of the four major oil plants in the world (Li *et al*. 2016); the others are the olive tree (*Olea europaea*), oil palm (*Elaeis guineensis*), and coconut palm (*Cocos nucifera*). The tea oil extracted from *C. oleifera* seeds is edible oil called "eastern olive oil," because of its high nutritional value and health care functions (Qu *et al*. 2019). This oil has a similar chemical composition as that of olive oil, as both contain high amounts of unsaturated fatty acids (Gao *et al*. 2015; Yang *et al*. 2016). Tea oil is not only edible but also a traditional Chinese medicine and superior nutritional dietary supplement that benefits human digestive system, reduces blood cholesterol and prevents hypertension and hardening of the arteries (Feás *et al*. 2013; Zeng *et al*. 2015). It is also an important raw material for the pharmaceutical and chemical industries. For example, tea meal can be used to extract saponin and produce feed, and the tea shell can be used to produce potassium carbonate or cultivate edible and medicinal fungi (Zhang and Liu 2007; Hu *et al*. 2012; Zhu *et al*. 2018). In recent years *C. oleifera* has been widely planted in the red soil at hilly regions of southern China due to the rapid development of the *C. oleifera* industry.

C. oleifera is a self-incompatible plant at the beginning of flowering in early November and usually blooms in large numbers in mid-late November (Fig. 1c). In addition, most *C. oleifera* is polyploid with many cultivars that display significant differences in cold resistance (Deng *et al*. 2018; Shi *et al*. 2019). Cultivars of *C. oleifera* with big fruits, high yields, good stability, and strong resistance have been

To cite this paper: Wu L, J Li, Y Gu, F Zhang, L Gu, X Tan, M Shi (2020). Effect of chilling temperature on chlorophyll florescence, leaf anatomical structure, and physiological and biochemical characteristics of two *Camellia oleifera* cultivars. *Intl J Agric Biol* 23:777–785

planted on a large scale by farmers. Hua Shuo and Hua Xin are new high-yielding *C. oleifera* cultivars bred from common *C. oleifera* in 2009. Hua Shuo (Fig. 1a) has large fruits, high yields, strong resistance, and late maturity (Tan *et al*. 2011a). Hua Xin (Fig. 1b) has high and stable yields, strong resistance, and precocity (Tan *et al*. 2012). The cold resistance of the two cultivars is unclear, particularly when the plants are flowering in large numbers.

The primary problem during *C. oleifera* production is low fruit setting rate caused by low temperature and rainy weather in southern China, resulting in a lower yield and reduction in the distribution area (Peng and Chen 2008; Wang *et al*. 2017). Chen (2018) showed that sufficient sunshine and suitable temperatures improve the seed setting rate of *C. oleifera*. In addition, *C. oleifera* plants flowering in the winter will encounter freeze injury, and low insects activities, resulting in abnormal pollination and fertilization of *C. oleifera*, which seriously affect the development of *C. oleifera* industry in China (Fig. 1). The lowest temperature for suitable growth of the two *C. oleifera* cultivars is unknown, particularly in southern China, where rainy and low temperature days are frequent. Little information is available about the effects of prolonged low temperature on the growth of the two *C. oleifera* cultivars during the flowering phase.

We investigated the differences in physiological indices between the two cultivars during the flowering period. We compared cold resistance by exploring the long-term low-temperature stress on physiological and biochemical processes of the two *C. oleifera* cultivars. For this purpose, we measured chlorophyll content, photosynthesis, chlorophyll fluorescence, and observed the leaf anatomical structure and chloroplast ultrastructure of the two *C. oleifera* cultivars.

Materials and Methods

Plant materials and treatments

The experimental materials for this study were obtained from 4-year-old *C. oleifera* potted plants cultured by grafting a shoot each of *C. oleifera* Hua Shuo and Hua Xin onto germinated hypocotyls of seeds on the same tree as rootstock. On February 18, 2017, 120 two year-old C. *oleifera* young plants were selected and transplanted into plastic containers (22 × 22 × 20 cm) filled with a 2:1:1 mixture of peat soil, loess, and perlite. The plants were grown under natural conditions with the same water and fertilizer management at the Life Science Building of Central South University of Forestry and Technology, Changsha, China *(28°10' N; 113°23' E)*.

On November 3, 2018, 54 plants of each cultivar with similar growth rates were divided randomly into three groups. Each group consisted of 18 plants. Four year-old *C. oleifera* potted plants were placed in three different temperature for the experiments: (1) The *C. oleifera* potted plants were placed in field conditions (CK); (2) low temperature of 6°C in an artificial climate chamber (6°C); (3) normal temperature of 25°C in an artificial climate chamber (25°C). Other parameters in each room of the artificial climate chamber were the same with 70% relative humidity, a 12 h photoperiod at a photosynthetic photon flux density of 200 μmol·m^{-2}·s^{-1}, and an average CO_2 concentration of 450 μmol·mol^{-1}.

After 25 days, florescence, chlorophyll content, photosynthesis, physiological response and anatomical structure were measured in different treatments. Immediately after measuring gas exchange, the leaves were cut, weighed, wrapped in tin foil, frozen in liquid nitrogen, and stored at –80°C until the physiological response measurements were taken. Plants in all treatments were watered (500 mL/plant) twice and fertilized once per week with 500 mL Hoagland solution during the experimental period (Li *et al*. 2017).

Chlorophyll content analyses

Six plants of each of the two cultivars in each treatment were used for the test. Chlorophyll content was measured with 10 mL an acetone-ethanol solution (1:1, v/v) (Zhang 1986). The samples were soaked in the solution for 24 h at 4°C in the dark. The absorbance values at 663 nm (OD_{663}) and 645 nm (OD_{645}) of the solution were measured with a spectrophotometer (UV-1100 MAPADA, Shanghai, China). Chlorophyll content was calculated with the following equations:

$$\text{Chl a (mg/dm}^2) = 12.72 \times OD_{663} - 2.59 \times OD_{645}$$
$$\text{Chl b (mg/dm}^2) = 22.88 \times OD_{645} - 4.68 \times OD_{663}$$
$$\text{Chl (a + b) (mg/dm}^2) = \text{Chl a + Chl b}$$

Photosynthetic characteristic measurements

The photosynthetic characteristics were measured using an Li-6400xt instrument (LI-COR Biosciences, Lincoln, NE, USA). Six plants of each of the two cultivars from the treatments and control were used for the measurements. The photosynthetic parameters were measured between 9:00 am and 11:00 am with red–blue light of 1,000 μmol·m^{-2}·s^{-1} and a CO_2 concentration of 400 μmol·mol^{-1}.

Chlorophyll fluorescence analyses

The chlorophyll fluorescence parameters were measured using the Li-6400xt device. Six plants of each of the two cultivars from the treatments and control were used for the measurements. After a 2 h dark adaptation from 20:00–22:00, the plants were given a saturation pulse for 0.8 s at a light intensity of 7,200 μmol·m^{-2}·s^{-1} in the dark. Then the Li-6400xt collected the initial fluorescence (F_o) and maximal photochemical efficiency (F_v/F_m) data. The actual photochemical quantum efficiency (Φ_{PSII}) and electron transport rate (ETR) were determined after activation with light.

Anatomical leaf feature analyses

Six plants of each of the two cultivars from the treatment and control were used for the measurements. The leaf anatomical structure was studied in paraffin sections using an optical microscope. Mature leaf samples from the plants were cut into 5 × 4 mm pieces which were then soaked in FAA fixative solution containing 70% ethanol, glacial acetic acid, and formaldehyde (95:5:5, v/v/v) for 24 h. The samples were dehydrated in a graded ethanol series (70, 80, 90, 95, and 100%), embedded in paraffin, microtome sliced (Leica RM2235, Germany), and stained using a Safranin-O and acid fast green staining procedure (Zeng *et al.* 2008). Using the Leica DMi8 inverted microscope (Leica Inc. Jena, Germany) to observe the images, the structure of the palisade and spongy tissues was analyzed with application software (version 4.12.0).

Determination of malondialdehyde (MDA) and soluble sugar contents

Fresh leaves (0.3 g) were collected at a similar position to determine MDA and soluble sugar contents. Six plants of each of the two cultivars from the treatments and control were used to provide the leaf tissues. All samples were wrapped in sterilized tin foil (Solarbio) and stored at –80°C for later analyses. MDA content was measured as described by He *et al*. (2015), and soluble sugar content was determined as described by Wang *et al*. (2015) and Irigoyen *et al*. (1992). Each determination included three biological and technical replicates.

Chloroplast ultrastructural observations

The middle portion of each leaf was cut into 1 mm^2 strips and fixed in 2.5% glutaraldehyde solution (prepared with 0.1 mol L^{-1} sodium phosphate buffer, pH 7.3) for 24 h at 4°C. After washing three times (30 min each), the leaf samples were dehydrated in a series of graded ethanol solutions. After fixing in 1% osmium tetroxide for 2 h at room temperature, the strips were embedded in epoxy resin and placed in an ion sputtering coating machine for 20 min. The blade samples were sliced (0.5 μm) using an ultramicrotome (Leica EM UC7; Heidelberg, Germany) and mounted on copper grids. Transmission electron microscopy (HT7700; Hitachi, Tokyo, Japan) was used for the observations.

Statistical analyses

Microsoft Office Excel 2013 was used to process the data. Experiments were conducted as a completely randomized design (CRD) with eighteen replications each treatment. SPSS 19.0 software was used to analyse the variance to test for differences. Treatment means were compared using one-way analysis of variance (ANOVA) and Duncan's multiple range test with a probability of $P \leq 0.05$.

Results

Investigation of flowering and fruiting

Compared to the natural temperature (CK), both Hua Shuo and Hua Xin at 25°C flowered ahead of schedule, and the first flowering dates were advanced by 4 and 2 days, respectively. The flowering phases of Hua Shuo and Hua Xin were shortened by 20 and 11 days, respectively compared to CK at 25°C. However, the first flowering dates of Hua Shuo and Hua Xin were delayed by 4 and 5 days at 6°C, respectively compared to CK (Table 1).

Under natural conditions, the petals fell off after flowering of Hua Shuo and Hua Xin, and the young fruits were very small (Fig. 2aii, bii). The young fruits of Hua Xin were bigger than those of Hua Shuo at 25°C (Fig. 2ai, bi). Interestingly, a large number of Hua Xin buds did not flower at 6°C, and remained in their original state (Fig. 2biii), while Hua Shuo flowered for 49 days. The stamens and pistils withered but did not fall off, and a significant amount of mucus appeared at the base of the flower (Fig. 2aiii).

Chlorophyll content

The Chl a, Chl b, and total chlorophyll contents of Hua Shuo and Hua Xin were highest at 25°C, but significantly decreased in both cultivars at 6°C compared to the normal temperature of 25°C, except for Chl b of Hua Shuo (Table 2). In Hua Xin, they significantly decreased by 30.33, 36.88 and 31.75%, respectively, compared to controls (CK) ($P < 0.05$), at 6°C, but total chlorophyll content of Hua Shuo was not significantly different ($P > 0.05$) (Table 2). This indicates that low temperature decreases the chlorophyll content of *C. oleifera*.

Photosynthetic characteristics

Net photosynthetic rate (P_n), transpiration rate (T_r), and stomatal conductance (G_s) were significantly affected by temperature, and were shown to be the highest values at 25°C in both cultivars (Fig. 3a–c). The values for Hua Shuo decreased by 40.25, 79.43 and 75.00% ($P < 0.05$), respectively, at 6°C compared to controls, and those of Hua Xin decreased by 54.45, 74.23 and 80.95% ($P < 0.05$), respectively. However, C_i was not significantly different between the two cultivars, except under control conditions (Fig. 3d). In addition, the P_n of Hua Shuo was lower than that of Hua Xin at 25°C, but was higher than that of Hua Xin at 6°C.

Chlorophyll fluorescence

Different temperature treatments had variable effects on the chloroplast fluorescence parameters of the two cultivars. Initial fluorescence (F_o) significantly improved and maximum phototchemical efficiency (F_v/F_m) decreased for

Table 1: Florescence of two *C. oleifera* cultivars under different temperatures

Species	Treatment	Phenological phase			Florescence (days)
		First flowering stage	Flowering stage	Late flowering stage	
Hua Shuo	CK	11/8-11/14	11/15-12/5	12/6-12/16	38 d
	25ºC	11/4-11/8	11/9-11/19	11/20-11/22	18 d
	6ºC	11/12-11/15	11/16-12/20	12/21-12/31	49 d
Hua Xin	CK	11/5-11/7	11/8-11/21	11/22-12/4	31 d
	25ºC	11/3-11/4	11/5-11/11	11/12-11/23	20 d
	6ºC	11/10-11/28	/	/	/

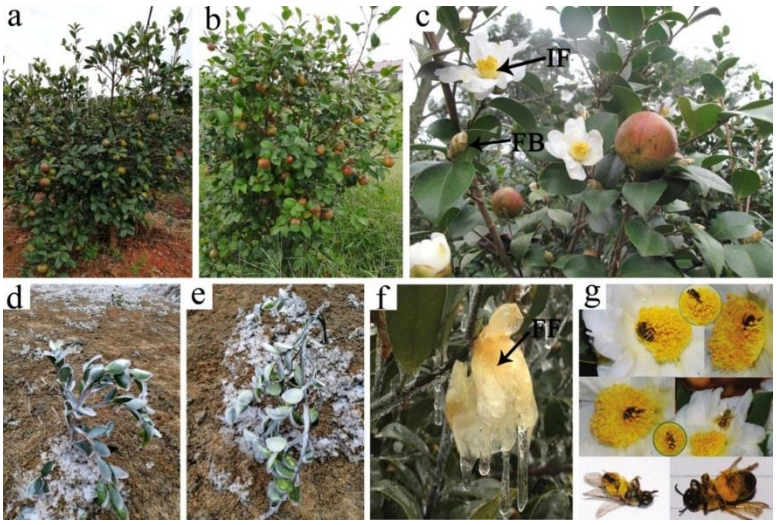

Fig. 1: The growth habit of the two *C. oleifera* cultivars
a) *Camellia* Hua Shuo with fruit; **b**) *Camellia* Hua Xin with fruit; **c**) *Camellia* Hua Xin with fruit and flowers (IF: initial flowering; FB: flower bud); **d**) Frozen *Camellia* Hua Shuo; **e**) Frozen *Camellia* Hua Xin; **f**: Frozen flower (FF: frozen flowers); **g**: Bees stop moving at low temperatures

Fig. 2: Growth of two *C. oleifera* cultivars under different temperature at 25 DAT
a1: 25°C of Hua Shuo (bar = 0.5 cm); **a2**: CK of Hua Shuo (bar = 0.5 cm); **a3**: 6°C of Hua Shuo (bar = 0.3 cm);
b1: 25°C of Hua Xin (bar = 0.5 cm); **b2**: CK of Hua Xin (bar = 1 cm); **b3**: 6°C of Hua Xin (bar = 0.5 cm)

both Hua Shuo and Hua Xin at 6°C compared to the controls (Fig. 4a, b). No significant differences were observed between 25°C and CK for either strain. The actual Φ_{PSII} and ETR values were higher at 25°C than those at 6°C ($P > 0.05$) (Fig. 4c, d). The ETR values of Hua Shuo and Hua Xin decreased by 52.15 and 36.37% ($P < 0.05$), respectively, at 6°C compared to the controls (Fig. 4d).

Soluble sugar and malondialdehyde contents

Low-temperature treatment increased soluble sugar and MDA contents. The soluble sugar content at 6°C increased by 29.03 and 7.86% ($P < 0.05$) in Hua Shuo and Hua Xin, respectively, compared to the controls. At 25°C it decreased by 24.72 and 24.07% ($P < 0.05$),

Table 2: Chlorophyll content of the two *C. oleifera* cultivars under different temperatures

Species	Treatment	Chl a content (mg/dm^{-2})	Chl b content (mg/dm^{-2})	Total Chl content (mg/dm^{-2})
Hua Shuo	CK	4.56±0.06b	1.13±0.10b	5.69±0.16b
	25℃	5.92±0.08a	1.56±0.05a	7.48±0.13a
	6℃	4.71±0.06b	1.61±0.08a	6.32±0.14b
Hua Xin	CK	5.11±0.07b	1.41±0.07a	6.52±0.14ab
	25℃	5.36±0.07a	1.34±0.11a	6.70±0.18a
	6℃	3.56±0.10c	0.89±0.12b	4.45±0.22c

Different lowercase letters within a column indicate a significant difference at $P < 0.05$ a (n = 6)

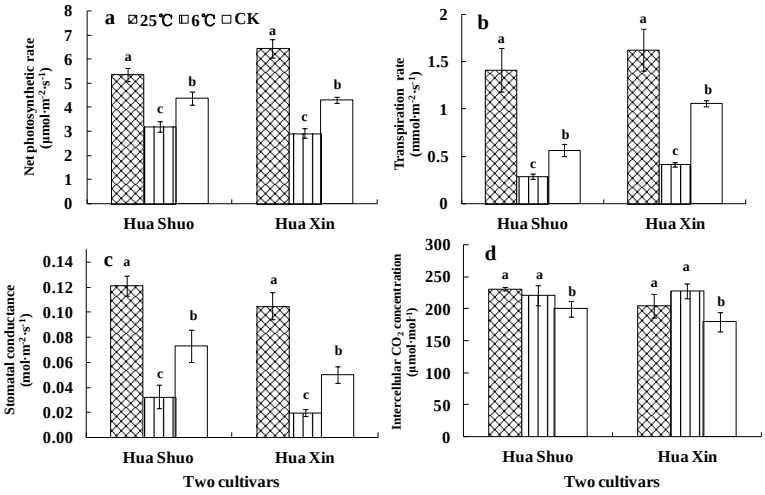

Fig. 3: Effects of different temperatures on net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) in the two *C. oleifera* cultivars. Different lowercase letters within a column indicate a significant difference at $P < 0.05$ (n = 6)

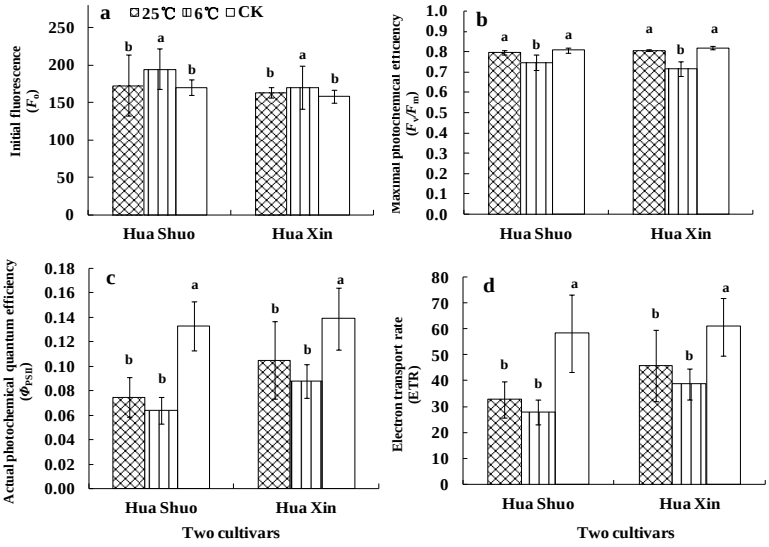

Fig. 4: Effects of different temperatures on initial fluorescence (F_o), maximum phototchemical efficiency (F_v/F_m), actual photochemical quantum efficiency (Φ_{PSII}), and electron transport rate (ETR) in the two *C. oleifera* cultivars. Different lowercase letters in the columns indicate a significant difference at $P < 0.05$ (n = 6)

respectively (Fig. 5a). MDA contents were lowest in both cultivars at 25°C and that of Hua Xin increased by 24.29% ($P < 0.05$) at 6°C compared to the controls (Fig. 5b).

Chloroplast ultrastructure

The chloroplast ultrastructure of both *cultivars* changed at different temperatures (Fig. 6). At 25°C, both

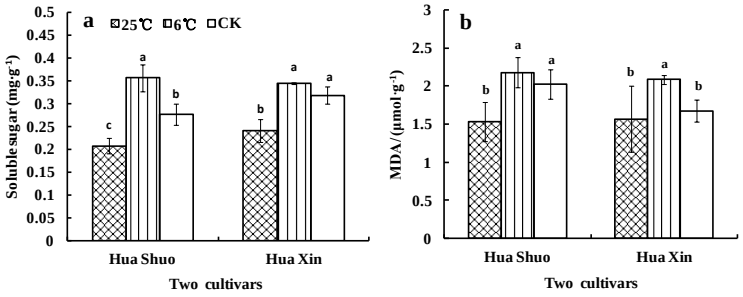

Fig. 5: Effects of different temperatures on soluble sugar and MDA contents of the two *C. oleifera* cultivars. Different lowercase letters within a column indicate a significant difference at $P < 0.05$ (n = 6)

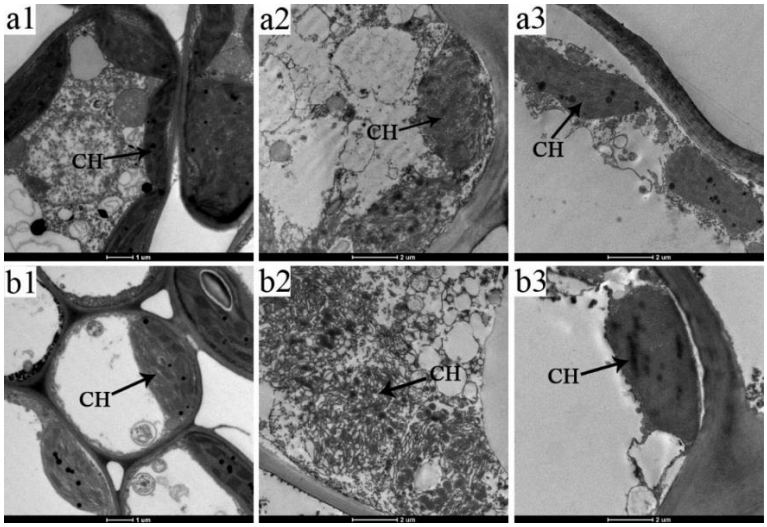

Fig. 6: Effects of different temperatures on mesophyll cell ultrastructure of the two *C. oleifera* cultivars
a: Hua Shuo; **b**: Hua Xin; **1**: 25°C; **2**: 6°C; **3**: control (CK)

cultivars had intact cell morphology, clear cell walls, and normal organelle structures (e.g., chloroplasts). Clearly, all chloroplasts were in the shape of a convex lens and distributed close to the cell edge (Fig. 6ai, bi). In addition, the grana and stroma thylakoid structures were clear, and the cytoplasm contained a small number of randomly distributed starch granules (Fig. 6ai, bi). However, at 6°C, the chloroplast reticulate structures of the photosynthetic lamellae were damaged in both cultivars, although that of Hua Xin had completely disintegrated and the thylakoid membrane was loose (Fig. 6bii), while the grana lamellae of Hua Shuo were loosely arranged and slightly swollen and dilated (Fig. 6bi). The chloroplasts of Hua Shuo was intact in the field environment, while the chloroplasts of Hua Xin were elongated into strips, deviated from the cell membranes, and loosely arranged (Fig. 6aiii, biii).

Leaf anatomical features

C. oleifera leaves are composed of the upper epidermis, lower epidermis, and mesophyll. The epidermis consisted of irregular oblong monolayers of varying sized cells, and the mesophyll consisted of a layer of palisade tissue cells and multiple layers of spongy tissue cells. The mesophyll cells were closely arranged in palisade tissue. There were more chloroplasts in the cells. The thicknesses of palisade tissue were significantly increased at 6°C in both cultivars than at 25°C. The sponge thickness of Hua Xin was the greatest among all samples. Leaf thickness significantly increased in both cultivars at 6°C compared to the controls and the leaf thicknesses of Hua Shuo and Hua Xin significantly increased by 19.49 and 13.72%, respectively (Table 3). At 6°C, the palisade tissue cells of both cultivars were elongated and the leaves were significantly thicker. The outer cuticle of the upper epidermis cells of Hua Shuo leaves was obviously thicker, while the spongy tissue cells was loosely arranged and the intercellular space was enlarged (Fig. 7). The results indicated that low temperature (6°C) increased leaf thickness of *C. oleifera*, which would help protect the plants from the impact of chilling stress.

Discussion

Temperature plays an important role in the flowering and fruiting of plants. Some environmental signals, particularly

Table 3: The anatomical features of leaves of the two *C. oleifera* cultivars under different temperatures

Species	Treatment	Palisade tissue thickness/μm	Sponge tissue thickness/μm	Leaf thickness/μm
Hua Shuo	25ºC	151.38 ± 7.82 b	254.48 ± 8.14 a	471.12 ± 6.72 b
	6ºC	224.67 ± 10.96 a	267.11 ± 10.97 a	544.47 ± 10.00 a
	CK	148.08 ± 9.51 b	261.19 ± 7.77 a	455.66 ± 4.09 c
Hua Xin	25ºC	155.90 ± 9.83 b	234.56 ± 7.50 b	454.97 ± 16.26 b
	6ºC	209.23 ± 18.28 a	228.99 ± 8.05 b	500.04 ± 19.45 a
	CK	107.75 ± 6.43 c	276.34 ± 7.58 a	439.72 ± 5.51 b

Different lowercase letters within a column indicate a significant difference at $P < 0.05$ according to Duncan's tests (n = 9)

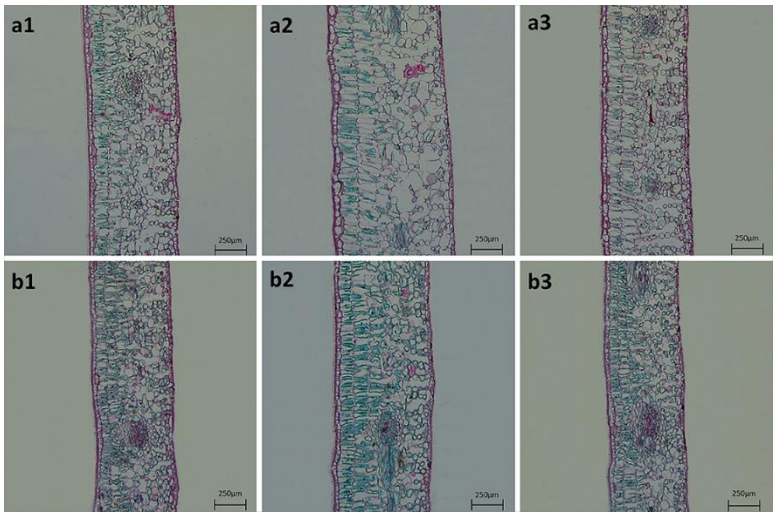

Fig. 7: Effects of different temperatures on leaf anatomical structure of the two *C. oleifera* cultivars (40×)
a: Hua Shuo; **b**: Hua Xin; **1**: 25°C; **2**: 6°C; **3**: control (CK)

warm temperatures, promote flowering by activating FT transcription; however, exposure to high temperatures reduces the activity of floral repressors (Fernández *et al*. 2016). In this study, temperature had a significant effect on the flowering stage of two *C. oleifera* cultivars. The normal temperature of 25°C during the flowering phase promoted early flowering, whereas 6°C prolonged florescence. Similarly, Daba *et al*. (2016) reported earlier flowering under long days and higher temperatures than under short days and lower temperatures. A large number of Hua Xin flower buds did not blossom at 6°C, while Hua Shuo flowered, the stamens and pistils withered but did not fall off, and a significant amount of mucus appeared at the base of the flower. The main reason may be that Hua Shuo secretes mucus to protect the young fruit against freezing injury, which is a self-protective mechanism in plants to adapt to a new environment. The phenotypic differences between the two cultivars were due to flowering response to low temperature, and that the differences were related to the expression of cold-resistance genes (Catt and Paul 2017).

Chlorophyll captures light energy in green leaves during photosynthesis, which is a series of enzymatic reactions. Studies have shown that low or high temperature stress can change the characteristics of the chloroplast membrane, leading to destruction of the chloroplast and a decrease in enzyme activities, which hinder chlorophyll synthesis and accelerate chlorophyll decomposition (Kowitcharoen *et al*. 2015; Jespersen *et al*. 2016; Li *et al*. 2018). In this experiment, the chlorophyll contents of the two *C. oleifera* cultivars showed a significant downward trend under the low-temperature stress, indicating that formation of chloroplasts and the synthetic rate of chlorophyll were significantly decreased by the low temperature (Cai *et al*. 2019). The contents of Chl-a, Chl-b, and total chlorophyll of Hua Xin were much lower than those of Hua Shuo at 6°C. The leaves of Hua Xin gradually turned yellow, indicating that Hua Shuo was more cold-resistant than Hua Xin.

Photosynthesis requires a balance between the light energy absorbed by the light harvesting system and the energy consumed by the plant; therefore, it is very sensitive to any change in the environmental conditions. Low temperature exacerbates the imbalance between the energy source and the metabolic sink, causing photosynthesis to significantly change (Ensminger *et al*. 2006). The main factors causing a decline in the photosynthetic rate of plant leaves can be classified into stomatal and non-stomatal limitations caused by the decrease in photosynthetic activity in mesophyll cells under external environmental stress (Gu *et al*. 2019). Farquhar and Sharkey (1982) showed that a decrease in P_n is mainly caused by stomatal constraints when both g_s and C_i decrease, while a decrease in g_s is accompanied by an increase in C_i, and a decrease in P_n is mainly caused by non-stomatal factors. In this study, the

increase in C_i lead us to hypothesize that the drop in P_n was mainly due to non-stomatal factors, such as damage to chloroplasts or reduced photosynthetic enzyme activities. However, the simultaneous decline of P_n, g_s, and C_i under natural conditions in the two *C. oleifera* cultivars clearly indicated that stomatal closure was the main factor responsible for the reduced photosynthetic assimilation rate. This result was consistent with the chloroplast ultrastructural observations. Therefore, low temperature had a serious effect on photosynthetic physiology and carbon assimilation in *C. oleifera*. In conclusion, under natural conditions, *C. oleifera* first reduced the number of CO_2 photosynthetic reaction sites entering mesophyll cells by closing a portion of the stomata or adjusting the stomatal opening, and then photoinhibition occurred to protect the photosynthetic organs from low temperature damage (Lu *et al*. 2015; Xu *et al.* 2019).

Analysis of chlorophyll fluorescence parameters is helpful to elucidate the location and extent of photosynthetic apparatus injured by stress (Kooten and Snel 1990). Chlorophyll fluorescence parameters play a unique role in the study of light absorption and transmission (Wei *et al*. 2011; Geng *et al*. 2014). The scientific community has reached a consensus that the F_v/F_m of most plants is between 0.8 and 0.85 under healthy physiological conditions. An F_v/F_m value < 0.75 indicates that the plants are under stress (Perks *et al*. 2004). PSII electron transfer is carried out after the photochemical reaction, which leads to splitting (oxidation) of water molecules. Therefore, ETR is valuable for many types of plant stress investigations. Our results show that the low temperature (6°C) significantly decreased F_v/F_m, Φ_{PSII}, and ETR in the two *C. oleifera* cultivars, indicating that PSII photochemical efficiency of leaves decreased under low-temperature stress, which could be the result of photochemical damage in the PSII reaction center or photoprotection (Demmig and Björkman 1987; Hao *et al*. 2019). In this study, F_0 increased while total chlorophyll content decreased under the low temperature. This is a clear indication that the number of inactive PSII reactive centers decreased PSII activity due to stress (Li *et al*. 2017).

Soluble sugars are an osmotic regulator used for plant cold resistance. Studies have shown that soluble sugar content is positively correlated with cold resistance in plants (Yoon *et al*. 2017; Hu *et al*. 2018). MDA is the final product of membrane lipid peroxidation. MDA binds and cross-links with proteins and enzymes on the cell membrane to inactivate the structure and function of the biofilm, thereby destroying the structure and function of the biofilm (He *et al*. 2015). In our experiments, the soluble sugars of the two *C. oleifera* cultivars significantly increased under the low-temperature stress, and the results were similar to previous studies showing the soluble sugar content is positively correlated with cold resistance in plants.

We observed the morphological structure of the chloroplasts in the two *C. oleifera* cultivars. They suffered serious injury under low-temperature stress, deformed by expansion of the inner cyst lamella (Fig. 6). This hindered metabolism and decreased photosynthetic efficiency, thus affecting the normal growth of both cultivars. Paraffin sections revealed that the low-temperature stress increased leaf thickness in both cultivars, suggesting that the plants changed their growth and morphology in response to the stress. This may be an adaptive mechanism for coping with low temperatures (Hu *et al*. 2016). The specific reasons need to be further investigated.

Conclusion

C. oleifera requires a particular temperature for flowering as low temperatures reduced chlorophyll content, photosynthetic efficiency, fruit set rate, and yield. A temperature of 6°C reduced net photosynthesis by 40 and 54% in Hua Shuo and Hua Xin, respectively, compared to the normal temperature. Hua Shuo was better adapted to low temperatures than Hua Xin as reflected by flowering phase and photosynthetic parameters. Thus, low temperatures should be avoided to ensure proper flowering and yield in *C. oleifera*.

Acknowledgments

This study was supported by the Major Projects of Science and Technology Project of Hunan Province [grant number 2018NK1030], and the Hunan Postgraduate Science and Technology Innovation Project [grant number CX2018B438] and the Central South University of Forestry and Technology Postgraduate Science and Technology Innovation Fund Project [grant number 20181004].

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