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EFFECTS OF LOW TEMPERATURE STRESS ON THE PHYSIOLOGICAL CHARACTERISTICS DURING GREENING SEEDLING PERIOD OF THREE TREE SPECIES

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Key words: Greening seedling, Low temperature stress, Physiological index

Abstract

Some physiological indexes of seedlings from tree species: *Carallia brachiata* (Lour.) Merr., *Ficus microcarpa* Linn. and *Phoebe zhennan* S. Lee were measured under low temperature stress (6ºC) for 2, 4 and 6 days (d) elaborate at the beginning and 2 d after release by chilling, respectively. The results show that with increasing stress time, chlorophyll content in *C. brachiata* and *F. microcarpa* increased 4 d after low temperature treatment and decreased from 4 to 6 d, whereas in *P. zhennan* decreased 2 d after low temperature treatment and then increased. Soluble sugar content in *C. brachiata* increased 2 d after low temperature treatment and decreased from 2 to 4 d, and then increased again, in *F. microcarpa* decreased 2 d after low temperature treatment, remain in stable condition from 2 to 4 d and then increased, whereas in *P. zhennan* tended to increase**.** Soluble protein content in C. *brachiata* and *P. zhennan* increased 6 d after low temperature treatment, whereas in *F. microcarpa* remained in stable condition. Content of proline increased in *C. brachiata* and *F. microcarpa* 2 d after low temperature treatment and in *P. zhennan* 4 d after low temperature treatment and then tended to decrease. The activity of SOD remained in stable condition 4 d after low temperature treatment and then significantly decreased in C. *brachiata*, whereas it fluctuated in *F. microcarpa* and *P. zhennan*. MDA content increased in *C. brachiata* and *P. zhennan* 2 and 4 d after low temperature treatment, respectively, and then decreased in the both species from 2 to 4 d and 4 to 6 d after low temperature treatment, respectively, while in *F. microcarpa* it gradually increased. Two days after low-temperature release, contents of chlorophyll, soluble sugar, soluble protein and proline in seedlings of all three species were higher or similar to control levels. The SOD activity in *C. brachiata* was lower than the control, and in other species was similar to the control. The MDA content in *C. brachiata* was lower than the control; in *P. zhennan* was higher, whereas in *F. microcarpa* it was similar to the control. The evaluation of cold-resistance ability of seedlings of three analysed tree species, done by using principal component analysis, shows decreasing in order of *C. brachiata* > *P. zhenna n* > *F. microcarpa*.

Introduction

Temperature is one of the important environmental factors affecting plant growth and development; low temperature limits plant growth and distribution and causes natural disasters in agroforestry production (Xu *et al*. 2007). Low temperature has an influence on the physiological and biochemical, photosynthetic and fluorescence characteristics of plants. The physiological and biochemical indexes such as contents of chlorophyll, soluble sugar, soluble protein, proline, malondialdehyde (MDA) and activity of superoxide dismutase (SOD) changes during low temperature stress (Shao *et al*. 2013), so these indexes can be used to evaluate cold resistance ability of plants. Under the effects of the global climate change and more frequent occurrence of extreme weather conditions worldwide, fully understanding the process of plant response to temperature changes, especially plant adaptability and reaction mechanism to low temperature is one important way of understanding relationship between plants and environment (Su 2007). *Carallia brachiata* (Lour.) Merr., *Ficus microcarpa* Linn. f. and *Phoebe zhennan* S. Lee belong to

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the families Rhizophoraceae, Moraceae and Lauraceae, respectively, and the three species are important timber and greening tree species in South China, however, their physiological characteristics under low temperature stress have remained unclear. Studying the physiological characteristics of these tree species in low temperature environment can provide reference for future selection of cold-tolerant greening tree species.

Materials and Methods

The study was conducted in College of Forestry and Landscape Architecture, South China Agricultural University, Tianhe district, Guangzhou city (113°18′E, 20°6′N). The climate belongs to subtropical monsoon climate type with characteristics of warm and rainy and with sufficient sunlight. The annual average temperature, the coldest month and the warmest month temperature are 21.8, 13.3 and 28.1ºC, respectively. Annual rainfall is 1714.4 mm, occurring mainly from April to September, while annual average relative humidity is 79%.

One-year-old seedlings of *C. brachiata*, *F. microcarpa* and *P. zhennan* were supplied by Shenzhen Techand Ecology Environment Co. Ltd. General characteristics of experimental seedlings were measured at the beginning of experiment (Table 1).

Table 1. General characteristics of the experimental seedlings (mean ± Sd).

Tree species	Parameter (cm)		
	The average diameter	The average seedling height	The average crown width
Carallia brachiata	0.48 ± 0.02	27.8 ± 2.1	22.1 ± 0. 8
Phoebe zhennan	0.39 ± 0.04	20.2 ± 1.2	16.2 ± 0.8
Ficus microcarpa	0.79 ± 0.60	44.3 ± 1.5	20.7 ± 0.9

The experimental seedlings were put into the RXZ intelligent artificial climate box for 6 d. Lighting time was from 8:00 to 17:00 with a light intensity of 120 mmol (photons)•m^{-2}•s^{-1} and a relative humidity of 80 ~ 85%. The seedlings were treated with 6 ± 0.5ºC low temperature (0 d after low temperature treatment as the control). Temperature of intelligent artificial climate box was reduced with 6ºC/h. When temperature dropped to 6ºC and then maintained temperature stability. Six seedlings of each tree species were selected after low temperature treatments for 0, 2, 4 and 6 d, taking 3 to 8 function leaves from the top seedlings, and then leaf chlorophyll, soluble sugar, soluble protein, proline, SOD and MDA contents were determined. The seedlings 6 d after low temperature treatment were moved from intelligent artificial climate box to outdoor for restoring growth for 2 d, and then the above physiological indexes were determined. Each index was determined in triplicate.

Chlorophyll, soluble sugar, soluble protein, free proline, superoxide dismutase (SOD) and malondialdehyde (MDA) were determined by acetone method, anthrone method, coomassie brilliant blue G-250 dyeing method, acidic indene three ketone, NBT photoreduction method and glucosinolates barbituric acid method, respectively (Li 2000, Chen and Wang 2005).

All statistical analyses were performed using Excel 2010 and the statistical Analysis System (SAS 8.1).

Results and Discussion

With increasing stress time, chlorophyll content in *C. brachiata* and *F. microcarpa* increased 4 d after low temperature treatment and decreased from 4 to 6 d, whereas in *P. zhennan* decreased 2 d after low temperature treatment and then increased (Fig. 1). There were no significant difference between content of chlorophyll in seedlings of three analyses tree species and the controls 2 d after low-temperature release.

The chlorophyll content is one of the important physiological indexes for temperature resistance in plants. In this study, chlorophyll content of *P. zhennan* seedlings was lower than the control for 2 d after low temperature treatment, which is similar with results reported by Zhang (1990). The reason may be continuous accumulation of MDA, which inhibited leaf photosynthetic rate, reduced the enzyme activity and strengthen chloroplast decomposition. In the late stages of low temperature stress, chlorophyll content in seedlings of three analysed species increased, indicating that seedlings had certain adaptability to low temperature.

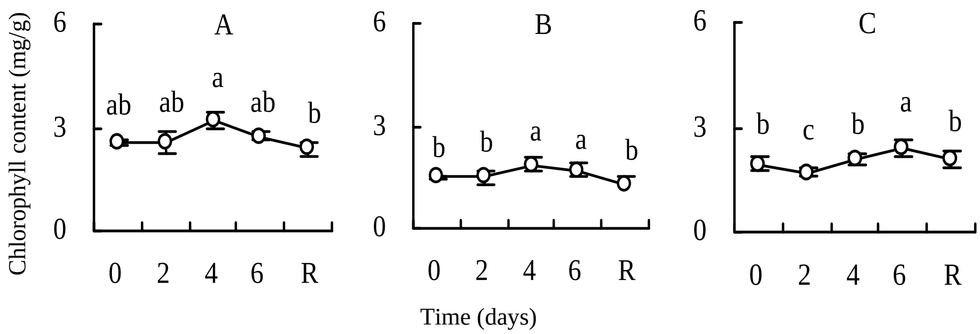

Fig. 1. Effect of low temperature stress on the chlorophyll content in seedling leaves. A, *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.* R: Seedlings restore growth for 2 d. Different letters represent significantly different values, $p < 0.05$.

With increasing stress time, soluble sugar content in *C. brachiata* increased 2 d after low temperature treatment and decreased from 2 to 4 d, and then increased again, in *F. microcarpa* decreased 2 d after low temperature treatment, remained in stable condition from 2 to 4 d and then increased, whereas in *P. zhennan* tended to increase (Fig. 2). The content of soluble sugar in *C. brachiata* was significantly higher than in the control ($p < 0.05$), whereas in other two species there were no significant difference compared to the controls 2 d after low temperature release.

With increasing stress time, soluble protein content in C. *brachiata* and *P. zhennan* increased 6 d after low temperature treatment, whereas in *F. microcarpa* remained in stable condition (Fig. 3). Soluble protein content in *F. microcarpa* was significantly higher than the control ($p < 0.05$), whereas in *F. microcarpa* and *P. zhennan* there were no significant difference compared to the controls 2 d after low temperature release.

With increasing stress time, content of proline increased in *C. brachiata* and *F. microcarpa* 2 d after low temperature treatment and in *P. zhennan* 4 d after low temperature treatment and then tended to decrease (Fig. 4). Content of proline was significantly higher compared to the control 2 d after low temperature release ($p < 0.05$).

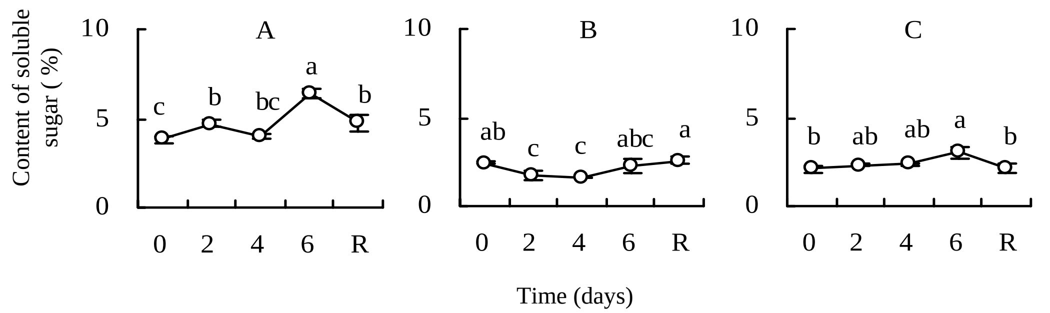

Fig. 2. Effect of low temperature stress on the content of soluble sugar in seedling leaves. A. *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.*

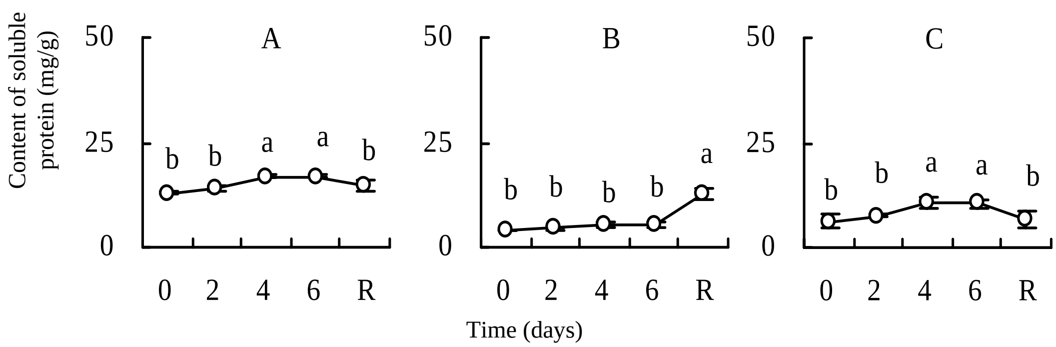

Fig. 3. Effect of low temperature stress on the content of soluble protein in seedling leaves. A, *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.*

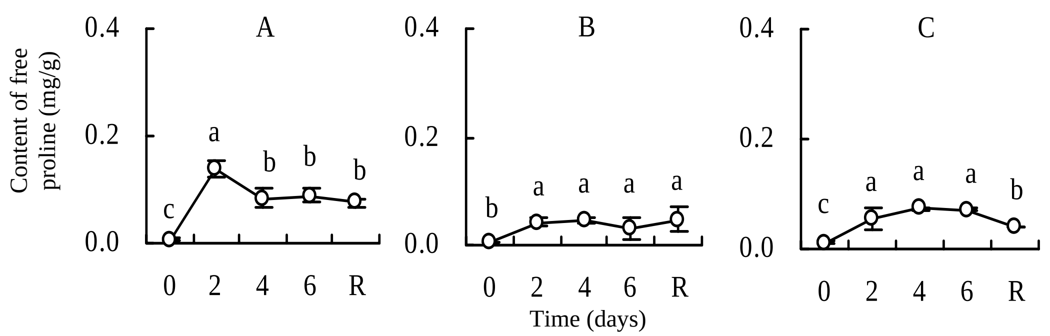

Fig. 4. Effect of low temperature stress on the content of free proline in seedling leaves. A, *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.*

The soluble sugar, protein and proline are the main osmotic regulation substances in plant cells (Xu and Dai 2011), which can increase plasma concentration and, decrease protoplasm suffering from water loss (Wang *et al*. 1996). During low temperature stress, content of soluble sugar and protein in *C. brachiata* and *P. zhennan* were significantly higher than the control, which can increase plasma concentration inside the cell, avoid cell coagulation encountering cold and increase its cold resistance (Liu *et al*. 2012). In the late stages of low temperature stress, soluble sugar and protein content in *F. microcarpa* did not show significant difference compare to the control, illustrating that its osmotic regulation ability was lower than in other seedlings. With the extension of low temperature time, proline content of seedlings in three analysed species was higher than to the control, which can reduce low temperature harm to the seedlings.

With increasing stress time, the activity of SOD remained in stable condition 4 d after low temperature treatment and then significantly decreased in *C. brachiata* ($p < 0.05$), whereas it fluctuated in *F. microcarpa* and *P. zhennan* (Fig. 5). The activity of SOD in *C. brachiata* was significantly lower than the control ($p < 0.05$), whereas in *P. zhennan* and *F. microcarpa* there were no significant difference compared to the control 2 d after low temperature release.

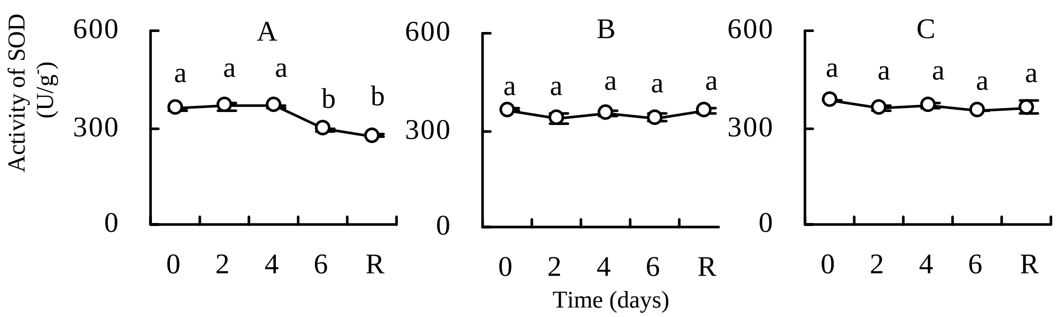

Fig. 5. Effect of low temperature stress on the activity of SOD in seedling leaves. A, *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.*

Antioxidant enzyme SOD is one of the enzymes for removal of the excessive reactive oxygen in plant cells, which maintains the metabolism balance of active oxygen and protects film structure and protects plant for resistance to low temperature stress. Compared to the control, SOD activity in *C. brachiata* remained at stable condition for 4 d after low temperature treatment, in *F. microcarpa* and *P. zhennan*, and it remained stable during low temperature stress, which is advantageous to slow the speed of excess free radical damage to cells.

With increasing stress time, MDA content increased in *C. brachiata* and *P. zhennan* 2 and 4 d after low temperature treatment, respectively, and then decreased in the both species from 2 to 4 d and 4 to 6 d after low temperature treatment, respectively, while in *F. microcarpa* it gradually increased (Fig. 6). MDA content in seedlings of three analysed species decreased significantly 2 d after low-temperature release ($p < 0.05$).

Low temperature damages metabolism of plant cell, increases activities of free radicals and causes damage to membrane system, leads to membrane lipid peroxidation and unsaturated fatty acid degradation, and eventually forms the MDA. The MDA content in *C. brachiata* and *P. zhennan* reached the maximum and then decreased 2 d and 4 d after low temperature treatment, respectively, suggesting that low temperature stress caused membrane damage from membrane lipid peroxidation, and afterwards alleviate membrane peroxidation substance. The MDA content in *F. microcarpa* increased with the extension of stress time, suggesting that membrane damage from membrane lipid peroxidation increased. The MDA content in seedlings of the three analased tree species significantly decreased after low temperature release, indicating that cell membrane lipid peroxidation became weaker after low temperature release.

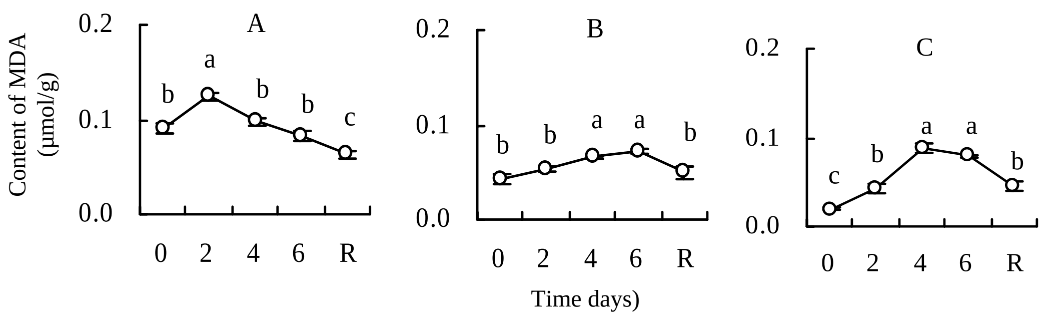

Fig. 6. Effect of low temperature stress on the content of MDA in seedling leaves. A, *Carallia brachiata*; B, *Ficus microcarpa* and C, *Phoebe zhennan.*

The physiological indexes of the seedlings were evaluated using principal component analysis. Principal component analysis is important to the study of multivariate data, which find the principal components of observed data presented by a large numbers of random variables, i.e. components of a smaller number of variables which preserve principal features of observed data. This means that the original variables can be reconstructed from the smaller ones with the least possible error. The obtained scoring values: 1.44 for *C. brachiata*;, 0.88 for *P. zhennan* and 0.54 for *F. microcarpa*.

The results of this study revealed differences in cold tolerance among three species by examining physiological indices including chlorophyll, soluble sugar, soluble protein, proline, SOD and MDA during low temperature treatment. Our results demonstrate that cold-resistance ability of the seedlings decreased in the order of *C. brachiata* > *P. zhennan* > *F. microcarpa* and this knowledge could be utilized for a better understanding of the cold tolerance characteristics of seedlings of three species.

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