Effects of cold treatment on some physiological characteristics of *Dendrocalamus latiflorus* seedlings

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Introduction

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Dendrocalamus latiflorus Munro is an important large sympodial bamboo species for shoot and timber, characterized by its high quality and fast-growth.
There are more than 100,000 hectares in southern China, distributed mainly in Fujian, Taiwan, Guangdong, Guangxi, Hainan, Guizhou, Sichuan, Chongqing and Yunnan provinces.

D. latiflorus is easily to be injured by chilliness with extreme low temperature below -2℃ in winter. As global climate change, extreme low temperature occurred in recent years in south China and caused extensive damage to bamboo, especially to sympodial bamboo species.

So, it is important to explore the physiological and biochemical mechanisms of cold-tolerance formation in sympodial bamboos, identify the physiological characteristics and parts that are most closely related to cold-tolerance.

Currently cold-tolerance research of bamboo is only restricted to direct field sampling and measurement, while few reports have studied the mechanism of cold-tolerance formation in bamboos under controlled low temperature.

Cold-resistant sympodial bamboo species

The membrane-fluidity theory is based on the fact that membranes from cold-resistant plants are often richer in unsaturated fatty acids than those from cold-sensitive plants.

Cold-sensitive sympodial bamboo species

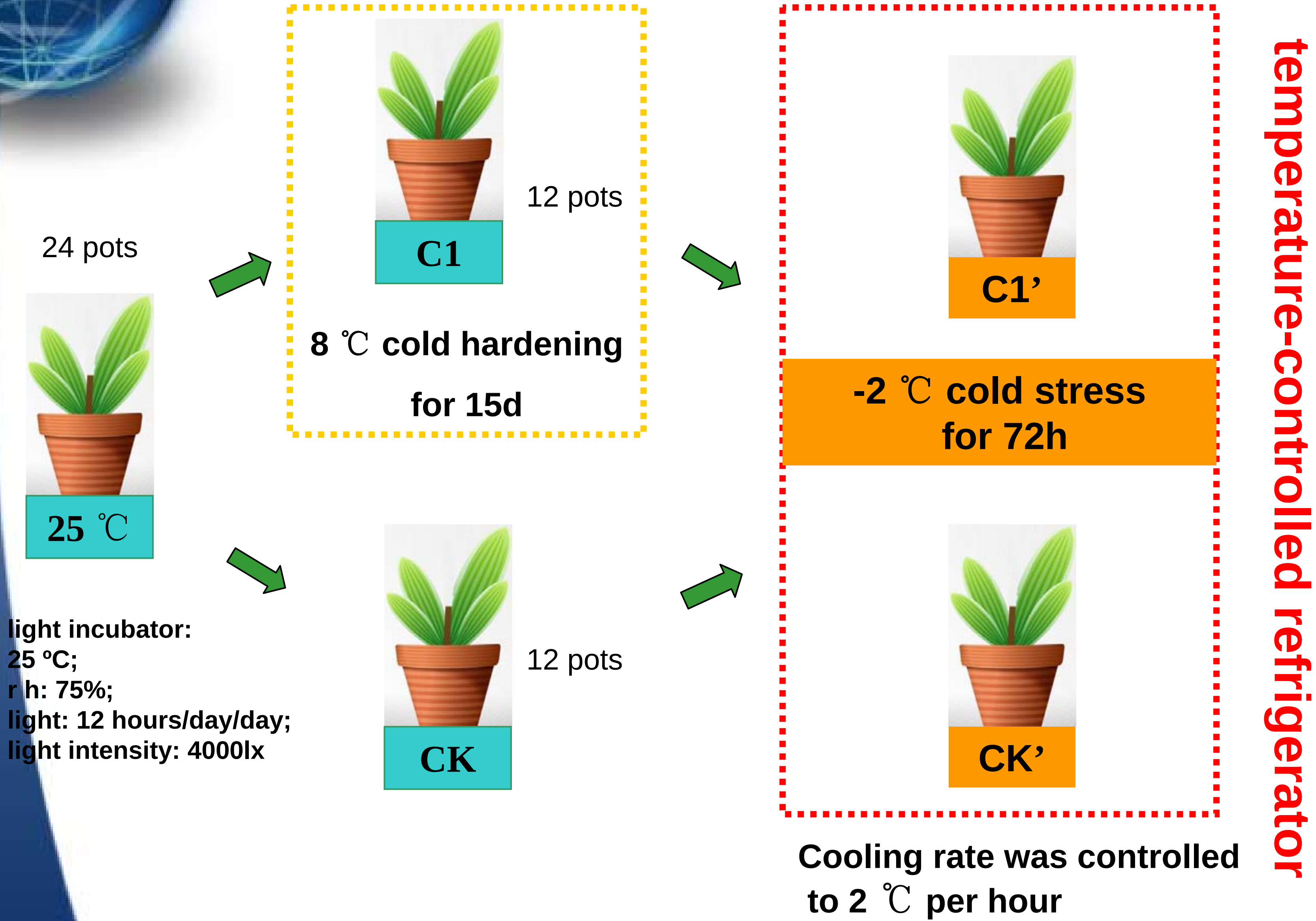
Materials and methods

In this experiment, we used 0ne-year old *D. latiflorus* seedlings from tissue culture as materials and tested the changes of physiological characteristics in leaves and roots at low-temperature through indoor low-temperature induced treatment methods.

The results provide a basis for the future selection of cold-tolerance bamboo species.

One-year old *D. latiflorus* monoclonal tissue culture seedlings were provided by the Seedling Center of Yong’an Forestry Ltd. Co, China.

Experiment processing



CK
C1
CK'
C1'

leaf

root

root samples were washed with the corresponding temperature of pure water to remove the soil, and -2 ℃ treatment was rinsed with ice water

- electrolyte leakage
- malondialdehyde (MDA)
- soluble sugar content
- soluble protein content
- superoxide dismutase (SOD) activity
- peroxidase (POD) activity
- membrane lipid fatty acids

membrane lipid fatty acids analysis

Total lipid extraction ← Chloroform and methanol

↓

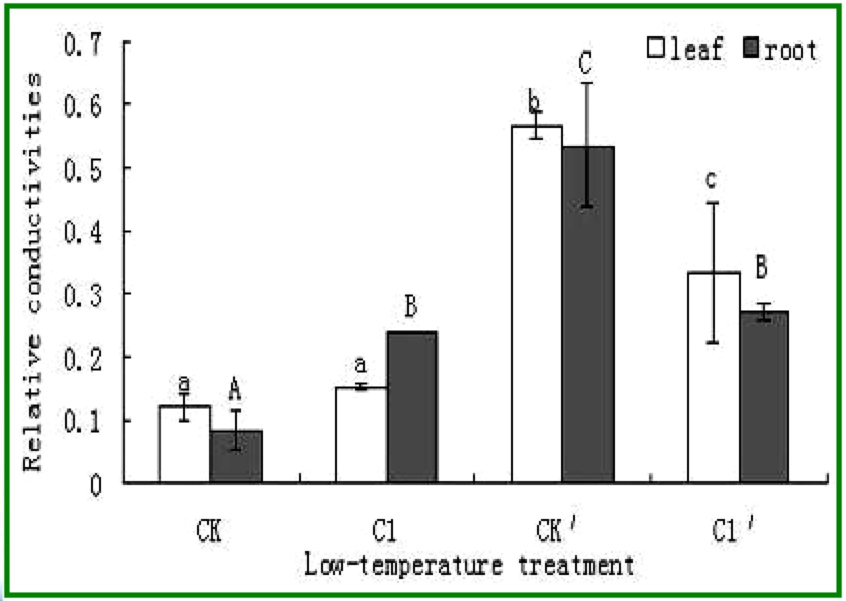
Polar lipid purification ← Methanol and petroleum ether

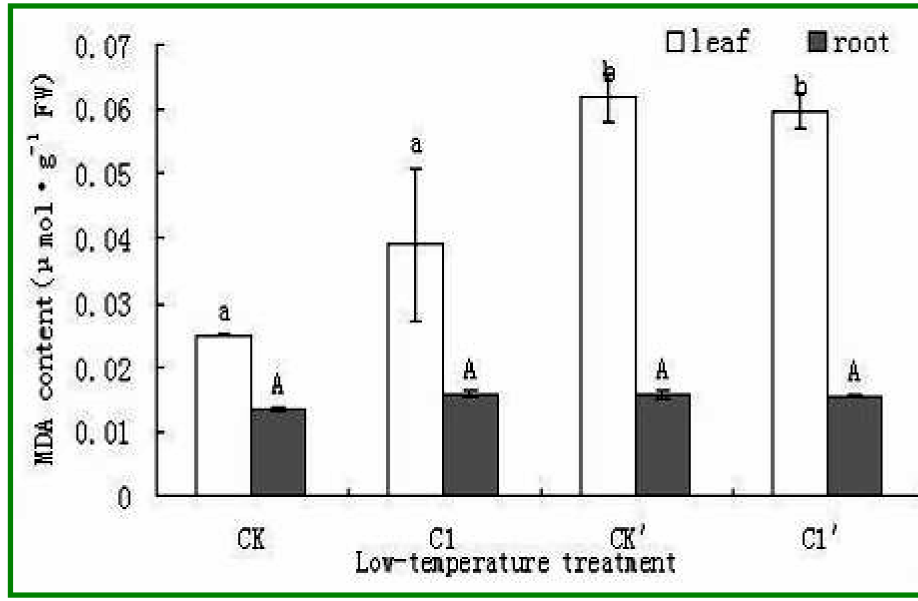
↓

Fatty Acid Quantification ← Gas chromatography analysis

Results

1. Effects of low temperature on membrane permeability and lipid peroxidation of *D. latiflorus* seedlings

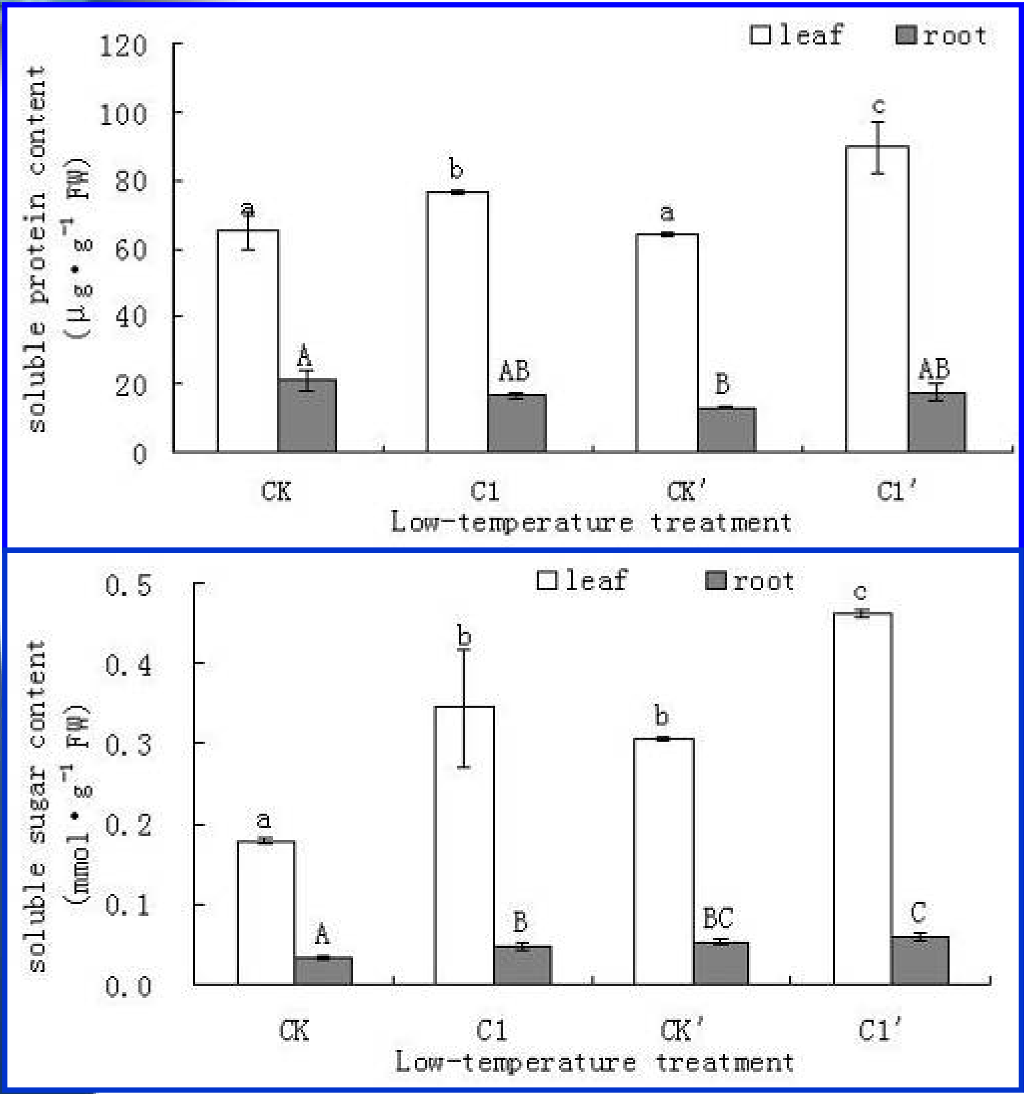




After -2 ºC cold treatment, the membrane permeability of control (CK') leaves and roots had significantly increased. In pretreatment group (C1'), the membrane permeability in both leaves and roots was significantly lower than control (CK').

As the treatment temperature decreased, the level of lipid peroxidation in *D. latiflorus* leaves increased, while the lipid peroxidation level in roots was not correlated with the temperature.

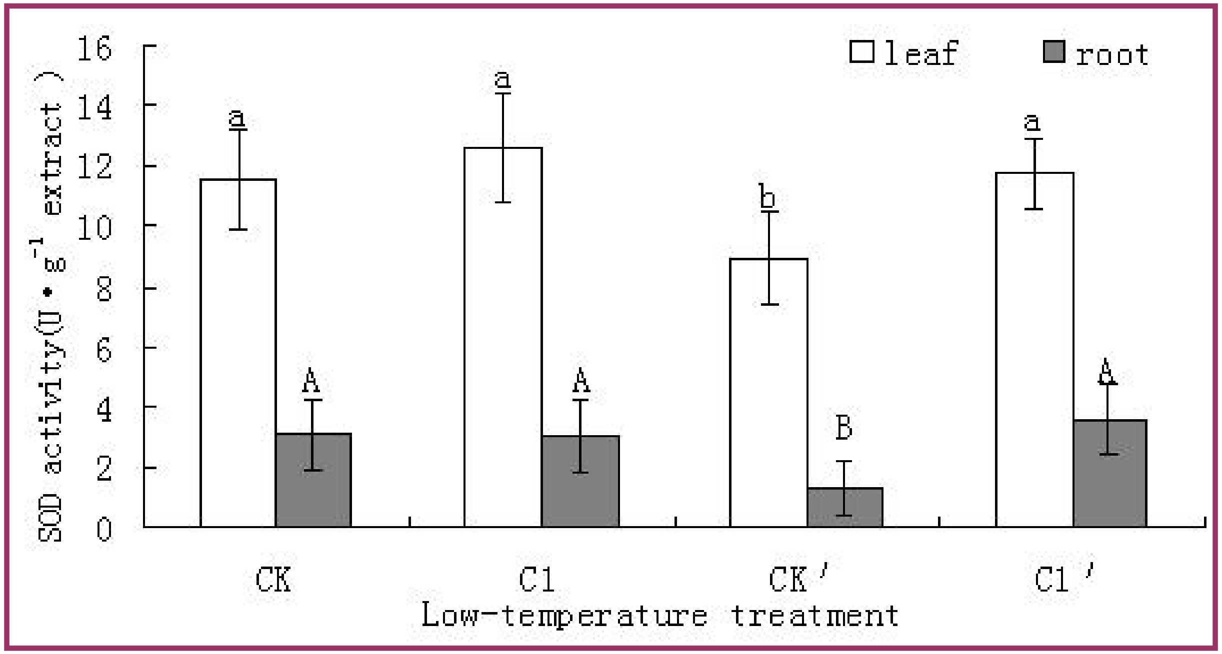
2. Effects of low temperature on soluble protein and soluble sugar content in *D. latiflorus* seedlings



After pretreatment of cold-hardening, soluble protein and soluble sugar contents in *D. latiflorus* (C1) leaves all significantly increased compared to control (CK).

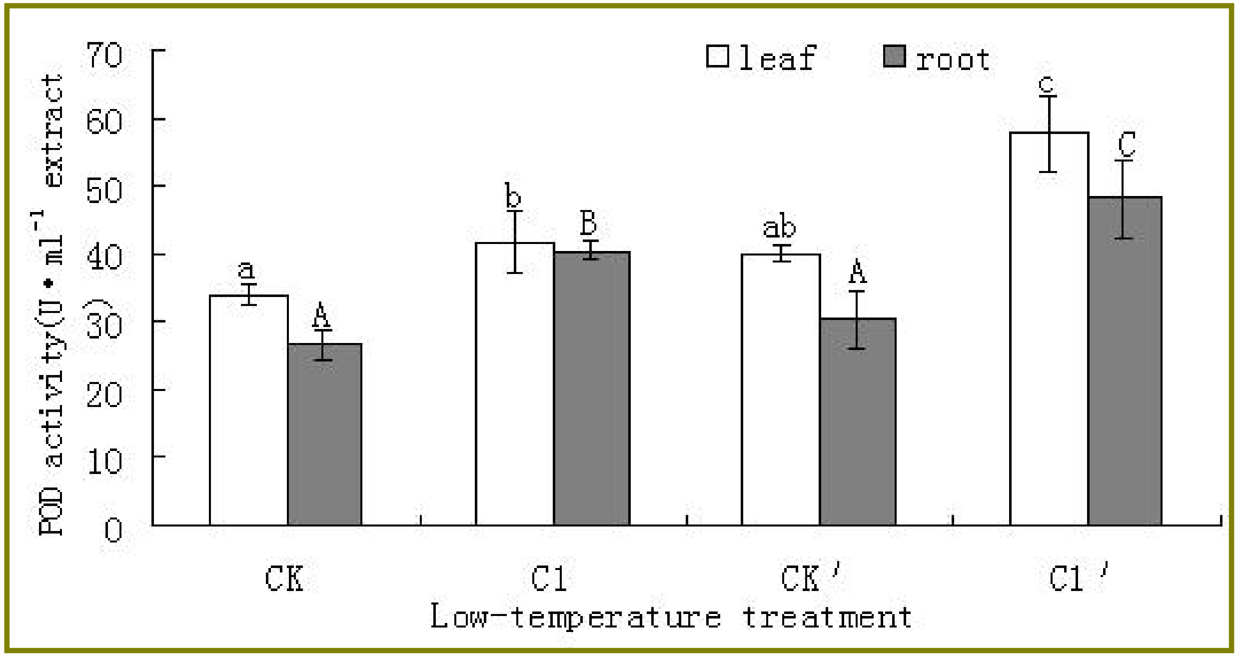
After -2ºC low temperature treatment, the soluble protein and soluble sugar contents in *D. latiflorus* (C1 ') roots had no significant differences compared with the control (CK ')

3. Effects of low temperature on superoxide dismutase (SOD) activity in *D. latiflorus* seedlings



the SOD activity of *D. latiflorus* (C1) leaves increased by 9.3% comparing to that in control (CK), After -2ºC low temperature treatment, the SOD activity of *D. latiflorus* leaves in the treatment group (C1') decreased by 6.6% compared to that before treatment, but the difference was not significant, while the SOD activity in control *D. latiflorus* leaves significantly decreased by 22.4%. The SOD activity changes in *D. latiflorus* roots showed similar trend as in leaves.

4. Effects of low temperature on peroxidase (POD) activity in *D. latiflorus* seedlings



The results show that the POD activity in *D. latiflorus* (C1) leaves and roots after pretreatment was significantly higher than that without low temperature treatment, indicating that the POD activity in *D. latiflorus* seedlings (C1) is more sensitive to low temperature conditions, and POD activity can be induced by low temperature and significantly increased

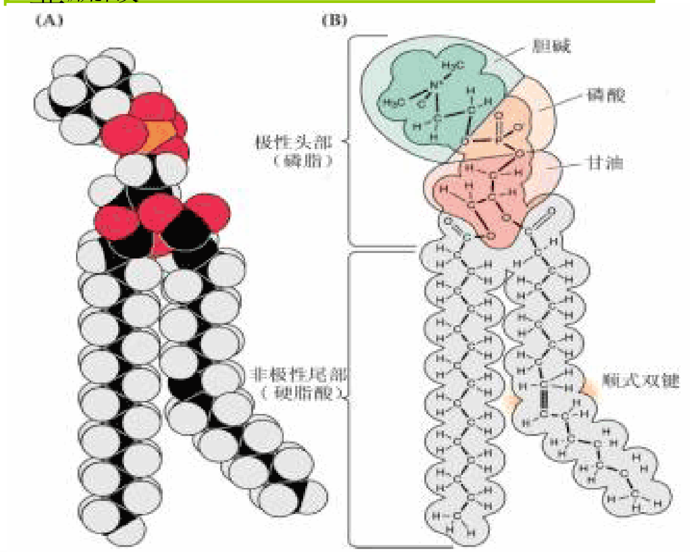
5. Effects of low temperature on relative content of membrane lipid fatty acids

Analysis of membrane lipid fatty acids in *Dendrocalamus latiflorus* leaves and roots showed that the membrane lipid fatty acid composition was similar.

Saturated fatty acid	Unsaturated fatty acid
palmitic acid (C16:0) 棕榈酸	oleic acid (C18:1) 油酸
stearic acid (C18:0) 硬脂酸	linoleic acid (C18:2) 亚油酸
arachidic acid (C20:0) 花生酸 2%	linolenic acid (C18:3) 亚麻酸

numbers of carbon atom

numbers of double bond



Effects of low temperature treatment on relative content of membrane fatty acid in leaves and roots of *Dendrocalamus latiflorus* seedlings

	Palmitic acid C16:0(%)	Stearic acid C18:0(%)	Oleic acid C18:1 (%)	Linoleic acid C18:2(%)	Linolenic acid C18:3(%)	Total unsaturated fatty acid
CK-leaf	14.33±0.39	1.90±0.13	2.63±0.70	9.15±0.36	69.98±0.56	81.76±0.97a
C1 -leaf	15.37±0.61	2.07±0.02	2.52±0.75	8.15±0.21	71.88±0.10	82.55±0.79ab
CK’ -leaf	13.84±0.34	1.90±0.11	2.22±0.12	8.44±0.18	73.04±0.95	83.7±0.97b
C1’ -leaf	14.85±0.19	2.09±0.03	2.28±0.22	6.55±0.10	74.22±0.15	83.05±0.28ab
CK-root	25.23±0.82	4.42±0.01	4.50±0.01	44.29±0.95	20.78±0.13	69.57±0.96A
C1- root	25.63±0.91	3.14±0.41	3.00±0.23	50.34±1.10	17.03±0.26	70.37±1.15A
CK’- root	28.70±0.10	3.93±0.08	4.86±0.09	44.49±0.44	16.03±0.81	65.38±0.93B
C1’- root	24.90±0.20	3.70±0.03	1.10±0.01	52.41±0.05	16.77±0.02	70.28±0.05A

The relative content of *D. latiflorus* unsaturated fatty acids in leaf and root membrane lipid of seedlings (C1) increased by 0.97% and 1.1% respectively. After -2ºC low temperature treatment, unsaturated fatty acids in *D. latiflorus* leaves showed no significant difference between C1' and CK'. However, the unsaturated fatty acid content in *D. latiflorus* (CK') roots significantly decreased.

Discussion

The physiological changes during cold-tolerance, such as the increased content of sugar and other carbohydrates as well as the accumulation of proline and soluble protein are the basis for the formation of cold-tolerance.

Malondialdehyde (MDA) is a membrane lipid peroxidation product in plants. Its content reflects the level of cellular reactive oxygen induced lipid peroxidation that causes plant cell injury. After -2ºC cold treatment, the membrane permeability and lipid peroxide levels in roots were both lower than in leaves, indicating the stronger cold-tolerance of roots than leaves.

Sudden dramatic cooling is not conducive to increase of unsaturated fatty acid content in organs. In the chilling conditions, there was a close relationship between the increase of unsaturated fatty acids and the addition of lipid peroxidation, and both of them were relative to the cold adaptation in bamboo. The unsaturated fatty acids may be an indicator of chilling resistant. The changes of fatty acid composition and protection system on membranes may be the key factor of cold adaptation, especially the latter.

Conclusion

In summary, as the treatment temperature decreased, the membrane permeability increased in *D. latiflorus* leaves, the lipid peroxidation products (MDA) augmented.

During cold acclimation leaves of *D. latiflorus* had higher soluble protein, soluble sugar contents and POD activities to avoid low temperature injuries while roots had higher SOD, POD activities and higher membrane lipid unsaturated fatty acid content to avoid membrane lipid peroxidation and membrane injuries. It is hinted that the different strategies of resisting low temperature have been adopted by them.

ACKNOWLEDGEMENT

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Thank you for Your Attention!