Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section A: Chemical Sciences

CODEN (USA): JCBPAT Research Article

Comparative Study for Cold Hardiness Physiological Indicators of Four Introduced Acer rubrum

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Received: 5 October 2015; **Revised:** 26 October 2015; **Accepted:** 05 November 2015

Abstract: We chose one-year shoots of four introduced Acer rubrum (two years old) as research subjects, selecting one-year shoots of local native tree species Acer mono (Acer elegantulum) (two years old) as control, and determined relative leakage of electrolytes (relative conductivity), soluble protein contents (10% Homogenate protein concentration), SOD activity, as well as POD activity of these shoots after different freezing treatments. Then, the Logistic equation is used to calculate lethal temperature (LT_{50}) of these Acer rubrum. The results showed that as the temperature dropped, relative conductivity of these samples presented S-shaped, increasing, soluble protein contents were firstly decreased and then increased, superoxide dismutase (SOD) activity was firstly increased and then decreased as well as peroxidase (POD) activity. There is greatly gap in cold hardiness among these introduced Acerrubrum, with the order: 2#>1#>4#>3#, but they all less than local native tree species Acer mono. 1#,2#,4# can get through the winter in Liaoning.

Key words: Acer rubrum; Physiological indicators; Cold hardiness; Relative electrical conductivity; Antioxidant enzymes

INTRODUCTION

Acer rubrum, also called red maple, is affiliated with an Acre of Aceraceae, which is one of perennial deciduous trees. The plant native to the Northeastern of the America, and it becomes one of the most popular tree species because of its advantages of upright tree gesture, fast-growing, high value of ornamental, gorgeous colours of leaves in autumn, as well as a high tolerance to cold, drought or waterlogging[1], which had widely used in parks, streets and other places of greening. Recently, introduced Acer rubrum from North American areas are cultivated in Northern China, among them, there are a large of the introduction in Shandong, Beijing, Tianjin and other places.

However, the results of introducing vary for the differences of climate condition, the effect in various places have different performance. Liu[2] observed the biological characteristics and cold resistance of red maple; he thought that the introduction of maple varieties had the long-term survival possibility of introducing region. For a long time at low temperature in severe winter in Liaoning province, temperature is the dominant factor of climatic factors impacting on geographical distribution of plants[3]. Low temperature influences the growth and metabolism, which can change physiological indices, caused damage even death of plants[4].

Exposure of plants to low temperatures has long been known to enhance their subsequent tolerance of exposure to sub-zero temperatures. That this process known as cold acclimation has been extensively studied, because analysis of the specific alterations associated with cold acclimation could reveal the molecular basis of freezing tolerance in plants[5, 6]. Physiological, structural and biochemical analysis identifies several consistent features of cold-acclimation which are thought to have an important role in cold tolerance. However temperature impinges on all aspects of plant metabolism and physiology and thus there are probably very many important changes in response to cold, some as yet unknown. Higher plants have developed an oxygen-scavenging system scavenging system which consists of some antioxidant enzymes, including superoxide dismutase (SOD) and peroxidase (POD) [7].

In the view of the above studies, the main objective to carry out this study. The introduction and spreading of Acer rubrum is limited by the cold climate in Northern China. All in all, this research has a certain theoretical and practical value for the introduction, domestication and cultivation[8, 9] by undertaking many physiological attributes.

MATERIALS AND METHODS

In early November 2013, collected one-year shoots of four introduced Acer rubrum (two years old) which were used to research the subjects, and selected one-year shoots of local native tree species Acer mono (Acer elegantulum) (two years old) as control[10]. We introduced these Acer rubrum from America or Canada in 2011 (see Table 1).

Dormant stem segments of these samples were selected that grew at the same rate, and cut them into branches of 15 cm, then flushing with distilled water three times, finally divided into five groups. We placed these samples into high-low temperature test chamber, and treat them 12 hours at setting temperature (0℃, -10℃, -20℃, -30℃and -40℃) by lowering 2.5℃ per hour. Every treatment repeated three times, and the results of treating at 4℃ were in control (called CK).

Table 1: Provenance Location of introducing Acer rubrum

Number	Provenance	Provenance Location
1#	Quebec 6559#	Latitude (N) 47.43° Longitude (W) 76.95°
2#	Ontario 6562#	Latitude (N) 47.01° Longitude (W) 75.32°
3#	Ontario 6566#	Latitude (N) 45.82° Longitude (W) 77.14°
4#	Pennsylvania 6500#	Latitude (N) 47.37° Longitude (W) 71.28°
CK	Acer mono (Acer elegantulum)	Latitude (N) 42.34° Longitude (W) 125.03°

Above these for study of cold hardiness indicators as:

- Relative Leakage of Electrolytes
- Protein Contents
- SOD Activity
- POD Activity

Relative Leakage of Electrolytes: Samples were soaked for 10 hours in deionized water at room temperature, and measured the initial electrical conductivity (S1) by the DDS-IIA conductivity meter after mixing well, then measured the final electrical conductivity (S2) after boiling for 10 minutes. According to the values of electrical conductivity both percentage of electrolytes and index of injury were calculated as:

$$L\ (\%) = S1/S2 \times 100$$

$$I\ (\%) = (L0\text{-}LCK) \times 100 / (100\text{-}LCK)$$

Where:

L- Relative conductivity, namely relative Leakage of Electrolytes;

S1- The initial electrical conductivity;

S2- The final electrical conductivity;

I- Index of injury;

LCK- Relative conductivity of the control groups[11, 12].

Process and analyse data with SPSS software and Excel software, and build a fitting equation of the relative conductivity and Logistic curve equations: y=k/(1+ae-bt) [13].

y- Relative conductivity

t- Temperature

k-The maximum limit of y

a、b-Undetermined parameters

The nonlinear regression was obtained, and constraint conditions were k≤100, a≥1, b≤0. The turning point of the logistic equation was defined as lethal temperature[14] (LT50), LT50=ln a/b.

Determination of Soluble Protein Contents: Soluble protein concentration was measured by Coomassie brilliant blue[15, 16] G-250. The samples mixed with 9 times the volume Phosphate buffered saline (pH7.8) were crushed into 10%homogenate in the mortar, and then centrifuged for 10min (3500 rpm), the concentration of soluble protein was calculated from the absorbance at 595 nm. 10% Homogenate protein concentration of samples was calculated as:

C=C0×N × (OD2-ODCK) / (OD1-ODCK)

C-10% Homogenate protein concentration of samples (mgprot/ml)

C0-Standard protein concentration (0.563mgprot/ml)

ODCK-The absorbance of control group at 595 nm

OD_1-The absorbance of standard group at 595 nm

OD_2-The absorbance of samples at 595 nm

N-Diluted times of samples

SOD Activity Assay: Superoxide Dismutase (SOD) activity was measured by nitroblue tetrazolium (NBT). The photochemical reduction[17] of NBT was measured at 560 nm, and one unit of SOD activity was defined as being present in one milligram protein that caused inhibition of the photoreduction of NBT by 50%.

The SOD activity was calculated as:

S = (ODCK-OD1)/ODCK × (V/V1)/C/50%

S-SOD activity (U/mgprot)

C-10% Homogenate protein concentration of samples (mgprot/ml)

ODCK-The absorbance of control group at 560 nm

OD1-The absorbance of samples at 560 nm

V-The total volume of reaction solutions (ml)

V1- Sample volume (ml)

POD Activity Assay: Peroxidase (POD) activity was measured with guaiacol[18]. The samples mixed with 9 times the volume Phosphate buffered saline (pH7.8) were crushed into 10% homogenate in the mortar,

then centrifuged for 10min (3500 rpm), the concentration of soluble protein was calculated from the absorbance at 470 nm. One unit of POD activity was defined as being present in one milligram protein that catalysed one milligram substrate per minute at 37 ℃. The SOD activity was calculated as:

P = (ODCK-OD1) / (12×D) × (V/V1)/C/t/×1000

P-POD activity (U/mgprot)

C-10% Homogenate protein concentration of samples (mg prot/ml)

ODCK-The absorbance of control group at 470 nm

OD1-The absorbance of samples at 470 nm

V-The total volume of reaction solutions (ml)

V1- Sample volume (ml)

D-The diameters of cuvette (1.0 cm)

t-The reaction time (30 min)

RESULTS AND ANALYSIS

Relative Leakage of Electrolytes: After different freezing treatments, four introduced Acer rubrum had various degrees of injuries. As figure1 showed, relative conductivity of these processed samples presented S-shaped increasing. Relative conductivity increased slowly at 0℃~-10℃, then increased rapidly, however, finally the increasing rate slowed down at -20℃~-30℃. Relative conductivity reached 50% when the temperature was around -20℃. Index of injury was similar to the relative conductivity curve as S-shaped **(Fig.2).** Four introduced Acer rubrum had fewer injuries at 0℃, but the injuries could increase with the decreasing of temperature.

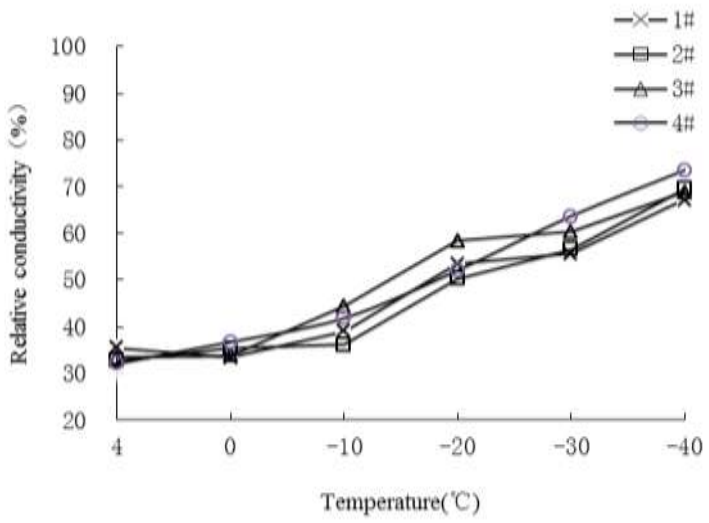

Fig.1: Relative conductivity of different treatments

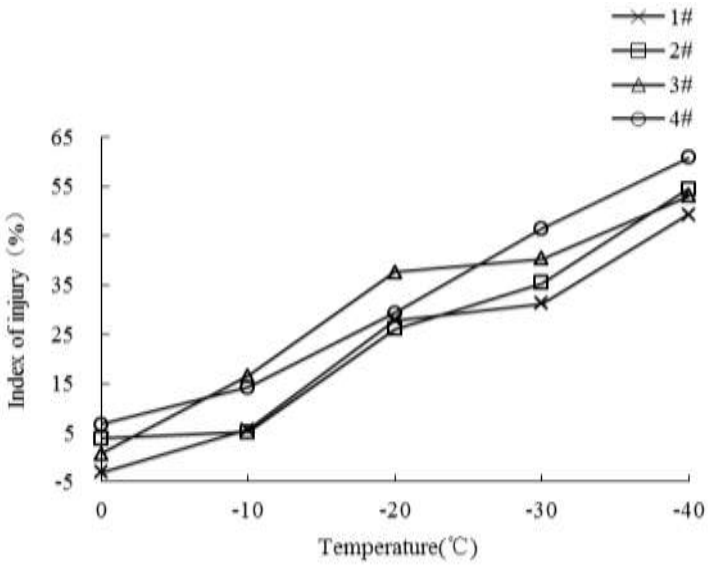

Fig.2: Index of injury of different treatments

Calculating lethal temperature (LT_{50}): According to Analysis of Variance (ANOVA), relative conductivity existed significant differences among these introduced Acer rubrum after freezing treatments. To calculate LT_{50}, we build a fitting equation of the relative conductivity and Logistic curve equations: y=k / (1+ae-bt) [19], and these equations were approved by significant testing. As **Table 2** showed, K expressed the maximum limit of relative conductivity, equaling 100 except that 3# was 73.9.

Table 2: Results of LT_{50}

Number	Logistic equation	LT50/℃	R2	Order
1#	Y=100/(1+2.0293e0.0346t)	-20.4	0.94334	2
2#	Y=100/(1+2.2445e0.0372t)	-21.7	0.86869	1
3#	Y=73. 9/(1+1.1643e0.0634t)	-2.4	0.94833	4
4#	Y=100/(1+1.9972e0.0404t)	-17.1	0.92169	3

Besides, was between 1.2 and 2.2, b was between -0.02 and -0.6, and R2 was from 0.85 to 0.95. LT_{50} was the critical temperature of the plant when cell membrane was damaged. If the freezing temperature was below LT_{50}, relative leakage of electrolytes could increase because cell membrane was damaged seriously. Conversely, if the freezing temperature was above LT_{50}, relative leakage of electrolytes could decrease because cell membrane was damaged slightly. Cold tolerance of four introduced Acer rubrum in order from strong to weak were 2#, 1#, 4#, 3#.

Relations between cold resistance and longitude-latitude: As Table 3 showed cold resistance of four introduced Acer rubrum increased with the Acer rubrum longitude-latitude increased but not linear

positive correlation. 1#, 2#, 4# where the northerly latitude (47.43°N, 47.01°N,47.37°N), and their cold resistance was stronger, and 3# was the lowest latitude (45.82°N), but, its cold resistance was the weakest, and its LT_{50} was -2.4 ℃, that could be for adapting to the environment in the long run. However, the results may be related to different genotype among provenances, as well as other climatic factors.

Table 3: Relationship between cold resistance and longitude-latitude of four introduced Acer rubrum

Number	Latitude (N)	Longitude (W)	LT50(℃)	Order
1#	47.43	76.95	-20.4	2
2#	47.01	75.32	-21.7	1
3#	45.82	77.14	-2.4	4
4#	47.37	71.28	-17.1	3

Soluble protein contents: As the temperature dropped, soluble protein contents of four introduced Acer rubrum were firstly decreased and then increased **(Fig.3).** 1#、2#、3#、4# all reached the peak at -20℃, and 10% homogenate protein concentration was 0.0531 mg prot/ml, 0.0744 mg prot/ml, 0.0568 mg prot/ml, 0.0850 mg prot/ml. CK also reached the peak at -20℃ with 0.0991 mgprot/ml. In term of rangeability, the rangeability of 1#, 2#, 3#, 4# and CK was similar, the rangeability of them were rather great at the early stage of dropping temperature, then 10% homogenate protein concentration was rising slowly after reaching the peak at 0℃.

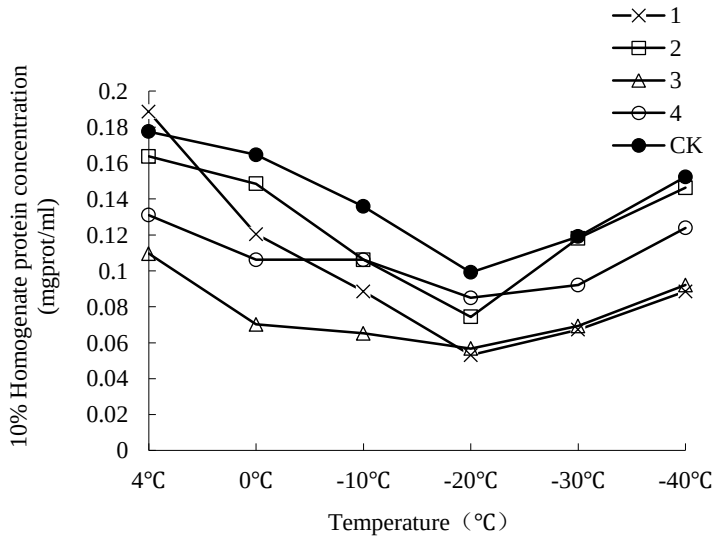

Fig.3: 10% Homogenate protein concentration of different treatments

SOD activity: As the temperature dropped, SOD activity of four introduced Acer rubrum were firstly increased and then decreased **(Fig.4)**, but still existed differences. 1# enzymatic activity maintained increasing at the early stage of dropping temperatures by 25.32%, until reaching the peak of 503.7722 U/mgprot at -10 ℃. The growth enzymatic activity of 2# was 18.18% at the early stage, reaching the peak of 485.1139 U/mgprot at 0 ℃. 3# enzymatic activity increased by 14.32% at the early stage, and then reached the summit at 0℃ by 473.2405 U/mgprot. 4# enzymatic activity increased by 19.14% firstly, and then reached the peak of 483.4177 U/mgprot at -10 ℃. CK enzymatic activity increased by 23.26% at the first stage, then reached the summit at -10 ℃ by 499.6709 U/mgprot.

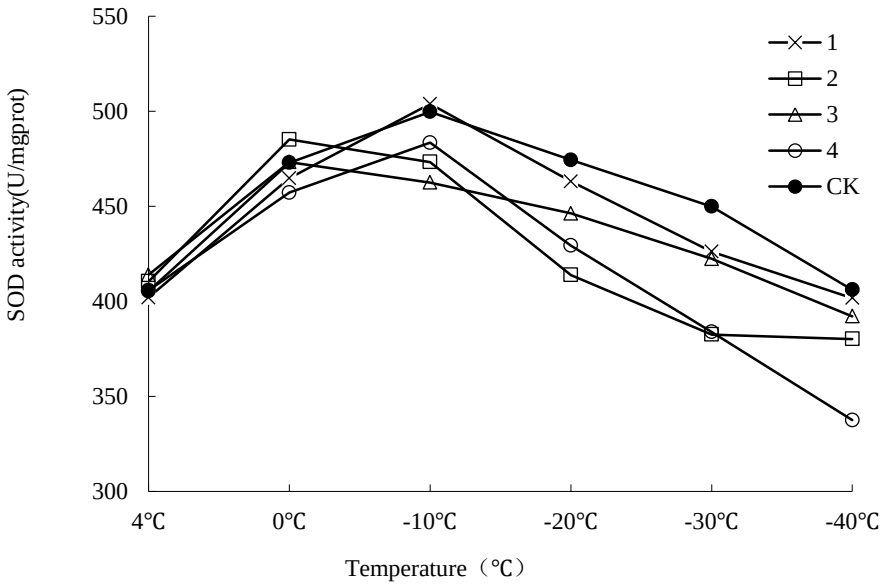

Fig.4: SOD activity of different treatments

POD activity: As the temperature dropped, POD activity of four introduced Acer rubrum were firstly increased and then decreased **(Fig.5)**. Nevertheless, POD activity varied much greatly than SOD activity despite similar to the variation of SOD activity. 1# enzymatic activity maintained increasing at the early stage of dropping temperatures by 50.28%, until reaching the peak of 532 U/mgprot at -20℃. The growth enzymatic activity of 2# was smaller than 1#, and the amplification was 15.58%, and then reached the peak of 512 U/mgprot at 0 ℃. The growth enzymatic activity of 3# was relatively large, which was 96.93%. 3# peaked at 449 U/mgprot at -10℃. 4# increased sharply at the earliest stage by 108.00%, and reached maximum 470 U/mgprot at -10 ℃. The increase of CK was 36.51% at the early stage; its summit was 587 U/mgprot at -10 ℃.

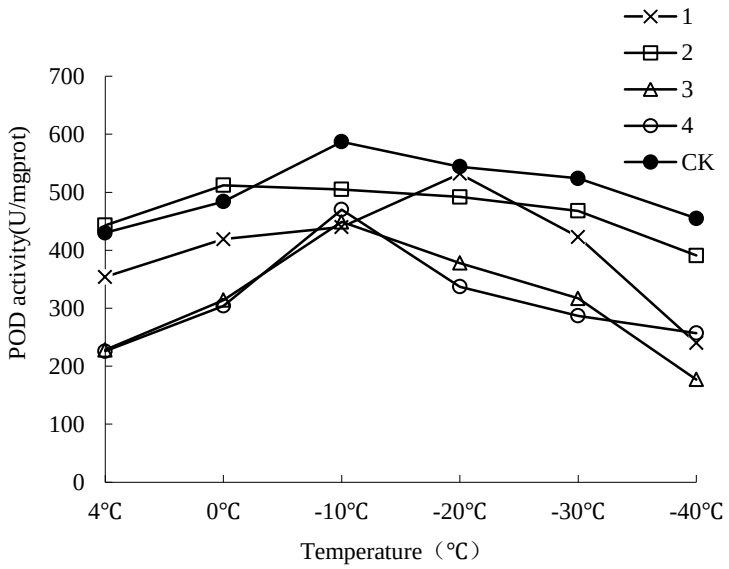

Fig.5: POD activity of different treatments

DISCUSSIONS

As one of the most popular introduced tree species, Acer rubrum is extensive cultivated in northern areas of China. At the same time, low temperature is the primary environmental factor limiting the productivity and the geographical distribution of Acer rubrum. Based on this background, we carry out the cold resistance of different introduced Acer rubrum.

Effects of low temperature stress on Plant organs, biofilm is the original parts of the plant cell which is acted on by low temperature damage[20], low temperature causes damage on the plant cell membrane structure, electrolyte leakage[21], as the relative conductivity reflects the damage degree of plant under low temperature stress[22]. In this experiment, relative conductivity of these processed samples presented S-shaped, increasing, and the LT_{50} were -20.4, -21.7, -2.4 and-17.1 respectively.

Soluble protein and antioxidant enzymes of plants also have a close relationship with the cold resistance. Some research showed that soluble protein contents were positively associated with cold resistance[23, 24]. Protein synthesis was prevented by low temperature, and protein contents were decreased. However, some research stated that plants could launch stress mechanisms to cope with cold, which gave rise to expressing new proteins or accumulating soluble protein contents. In addition, the plant could accumulate more soluble protein contents with stronger cold resistance. In a word, the relationships between cold resistance and soluble protein contents were complicated.

The study of antioxidant enzymes showed that different Acer rubrum all rapidly started defensive system after cold, then produced sufficient SOD to eliminate the excess reactive oxygen. Plenty of studies pointed to that activity of protective enzymes in plant were closely related to cold resistance[25]. As an important member of protective enzymes, SOD had been widely studied. SOD could scavenge 02- for enhancing the adversity and maintaining normal function[26, 27]. During the cold stresses, POD activity was similar to the variation of SOD activity, but the extent of variation was unlike. POD activity varied much

greatly than SOD activity, which may be because different adaptation mechanisms handle cold[28, 29]. This supposition needed to study further.

In this experiment, there was great difference in cold hardiness among these introduced Acer rubrum, during the analysis of Relative conductivity, soluble protein, SOD and POD, cold tolerance of four introduced Acer rubrum in order from strong to weak were 2#, 1#, 4#, 3#, but they all less than local native tree species Acer mono. 1#,2#,4# could get through the winter in Liaoning province. To strengthen the cold resistance of introduction Acer rubrum, we should harden off seedling at 4 ℃ or 0 ℃ for several hours, that is more efficient than taking steps to avoid-freezing[30]. We should take into full account the differences in longitude-latitude between introduction areas and previous areas.

ACKNOWLEDGEMENTS

We express our gratitude to the anonymous reviewers for helpful comments to improve the manuscript. This work was supported by the "948" Project from State Forestry Administration, P. R. China--Introduction of Resistance Excellent Red Maple Provenances and Rapid Propagation Technology (2011-4-25) and Youth Program of National Natural Science Foundation of China (31171950).

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