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EVALUATION OF COLD RESISTANCE AND SELECTION OF CHILL-PROOF MEASURE OF THREE *PHLOX* CULTIVARS

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ABSTRACT

In this study, *Phlox paniculata* cultivars 'Aida,' 'Purpurea,' and 'Rosea' were investigated in a cold resistance experiment. Relative electrical conductivity, soluble sugar content, and free proline content were selected as physiological targets, and the states of growth of the three *Phlox* cultivars under cold stress were observed after a year. Results showed that *P. paniculata* 'Purpurea' was more tolerant to cold than the two other cultivars. Earth covering, use of straw curtains, and frost-proof water were used as chill-proof measures in winter, and field conditions were analyzed for comparison. The most feasible chill-proof measure for the three *Phlox* cultivars was found to be earth covering. Overall, this study plays an important role in the introduction of *Phlox* species in Northern China.

Key words: *Phlox paniculata*; cold resistant; relative electrical conductivity; soluble sugar content; proline content; chill-proof measure.

INTRODUCTION

Phlox species are perennial flowers with great ornamental characteristics. The florescence of *Phlox* species occurs in summer. Compared with herbaceous flowers, *Phlox* species require simple management and low cost for application. As the climates limited in northern, plant variety application is scarce, thereby making *Phlox* species popular for its excellent features. Despite numerous reports on cold resistance and its application for many landscape garden flower varieties, little attention has been paid to *Phlox* species. In the present study, *P. paniculata* cultivars 'Aida,' 'Purpurea,' and 'Rosea' were investigated in a cold resistance experiment, and the most feasible chill-proof measure was selected; the results play an important role in the introduction of *Phlox* species in Northern China (Patterson *et al*., 1984; Acar and Var, 2001)

MATERIALS AND METHODS

State of growth under field conditions: *Phlox paniculata* cultivars 'Aida,' 'Purpurea,' and 'Rosea' were treated under field conditions to survive through winter. Roots that were 5 cm below the ground were collected to determine the relative electrical conductivity, soluble sugar content, and free proline content, as well as evaluate the cold resistance of these cultivars. The sampling schedule and the temperature in Harbin Province are discussed in the succeeding sections.

Table 1. The sampling schedule and the temperature in Harbin

Time	Surface temperature (℃)	Temperature (℃)	Minium tempreraturе (℃)
09-30-2013 (t1)	13.5	11.8	1.0
10-15-2013 (t2)	9.8	6.8	-4.0
11-23-2013 (t3)	1.0	-1.4	-12.0
12-26-2013 (t4)	-9.3	-11.8	-25.0
02-04-2014 (t5)	-15.1	-17.3	-30.0
03-20-2014 (t6)	9.2	6.1	-6.0
04-15-2014 (t7)	15.9	12.1	-1.0

State of growth under cold stress: Cold stress was estimated by placing the species in a refrigerator at −35 °C and −40 °C for 3, 6, and 9 h. Roots that were 5 cm below the ground were collected and considered for the analysis of relative electrical conductivity. After freezing, plant boxes were transferred to a greenhouse with temperature of 12 °C to 20 °C, and plant growth was investigated. In this way, the range of cold tolerance among the three *Phlox* cultivars was evaluated. The collected roots were also used to determine the soluble sugar and free proline contents, as well as evaluate the cold resistance of the cultivars.

Test of Phlox cultivars in the field throughout winter using chill-proof measures: Earth covering, use of straw curtain, and filling with frost-proof water were selected as chill-proof measures during winter; field conditions were also analyzed for comparison. Roots that were 5 cm below the ground were collected to measure the relative electrical conductivity, soluble sugar content, and free proline content of the cultivars in the following year.

Relative electrical conductivity: Fresh roots weighing 0.2 g each were collected from the three *Phlox* cultivars, cut into small pieces, and mixed in 15 mL of distilled water for 24 h. The conductivity of the samples (RC1) were determined using a DDS-11A conductivity meter. The roots were then boiled for 30 min and cooled under room temperature. A control tube was prepared parallel to each test sample, in which distilled water substituted for fresh roots. The conductivity of the boiled roots (RC2) and control ground (RC0) was determined (Richardson, 1991) using the following formula:

Relative electrical conductivity = $(RC_1 - RC_0) / (RC_2 - RC_0)$

Extraction and analysis of soluble sugar: Extraction and analysis of soluble sugar from the three *Phlox* cultivars were performed as previously described (Leon *et al*., 2001). Briefly, dried roots from the three cultivars were ground into powder with a mortar and pestle. Soluble sugars were extracted with 100 °C water thrice, and the extract was separated each time by filtration. Combined extracts were used to estimate the glucose content via Anthrone colorimetric method.

Analysis of free proline: Free proline was determined colorimetrically as described by Bates *et al*. (1973). Roots weighing 0.2 g each were ground in liquid nitrogen and extracted with 5 mL of 3% sulfosalicylic acid. After filtration through Whatman No. 1 filter paper, 2.5 mL of the extract was used for acid ninhydrin reaction. Ninhydrin-proline complexes were extracted in 1,2-dichloroethane. Relative fluorescence intensity was measured using a spectrophotometer at 650 nm and compared with a standard curve obtained with pure proline.

Statistical analysis: Each plant was considered as an experimental unit. Data were analyzed using SPSS and reported as mean ± standard error of the mean. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSION

Relative electrical conductivity of three *Phlox* cultivars under field conditions: When plant tissues are damaged, the function and structure of the membranes are destroyed, and the permeability is increased. The effusion of salts or organic matter from the cells alters the conductivity. A higher permeability indicates more injury, and increased effusion will further enhance the relative conductivity. As shown in Figure 1, the relative electrical conductivity in the *Phlox* species increased significantly when the temperature was decreased. As a result of adaptation to low temperature, the relative electrical conductivity showed a declining trend. At the same time and temperature, the three cultivars showed some differences (Figure 1). Cold temperature caused more tissue damage on *P. paniculata* ‘Rosea’ than on *P. paniculata* ‘Aida’ and *P. paniculata* ‘Purpurea.

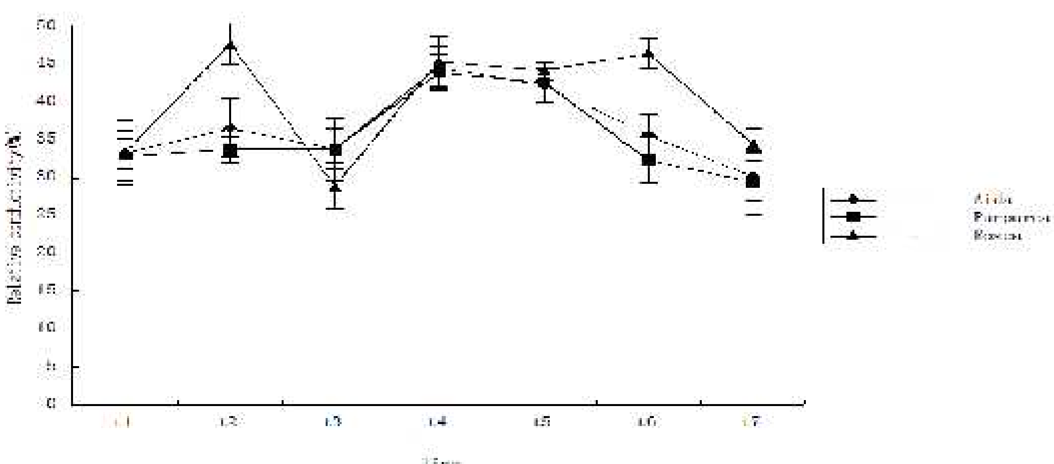

Figure 1. Relative electrical conductivity of there *Phlox* cultivars under field conditions

Soluble sugar content of three *Phlox* cultivars under field conditions: In autumn cold acclimation, the plants’ soluble sugar content increased, thereby improving the ability of the plant cells to resist adverse environmental conditions. The cold resistance of the plants is closely related to the total soluble sugar content. Carbohydrate, an important energy source for plants to survive during winter, is the main substrate of respiration and the source of new buds (Aebi and Bergmeyer, 1974). Plants containing more carbohydrates may demonstrate stronger cold endurance and increased abilities for new buds. The soluble sugar content of the *Phlox* species rapidly accumulated during autumn when the temperature decreased and reached the maximum values upon winter dormancy (Figure 2). With decreasing temperatures, soluble sugar consumption also decreased. This study revealed that the decreased soluble sugar content in *P. paniculata* 'Rosea' was more significant than that in *P. paniculata* 'Aida' and *P. paniculata* 'Purpurea,' indicating that *P. paniculata* 'Aida' and *P. paniculata* 'Purpurea' were more adaptable to cold stress.

Free proline content of three *Phlox* cultivars under field conditions: Free proline plays multiple roles in plant anti-freeze and regeneration during winter. It can reduce the freezing point, serve as a source of nitrogen and carbon for root survival after the leaves fall, and promote sprout regeneration during spring. Free proline also functions in osmotic adjustment, which can protect protein from low temperature damage. Hence, free proline plays an important role in freezing temperature adaption. As shown in Figure 3, the free proline content of the *Phlox* species reached the maximum values before winter dormancy; with decreasing temperature, free proline consumption also decreased. The free proline content of *P. paniculata* 'Purpurea' was higher than those in *P. paniculata* 'Aida' and *P. paniculata* 'Rosea.' However, no difference existed in the recovery growth among the three *Phlox* cultivars under field conditions in the following year (data not shown).

Comparison of relative conductivity and state of growth under cold stress in three *Phlox* cultivars: The relative electrical conductivity of the *Phlox* species increased with decreasing temperature and extended duration, which indicated that the level of plant damage increased with decreasing temperature (Table 2). The growth recovery of the *Phlox* cultivars in the following year under different conditions (Table 3) showed that the survival rate decreased, although the plant endured −35 °C for a short period. Under −40 °C for any time period, none of the plants survived.

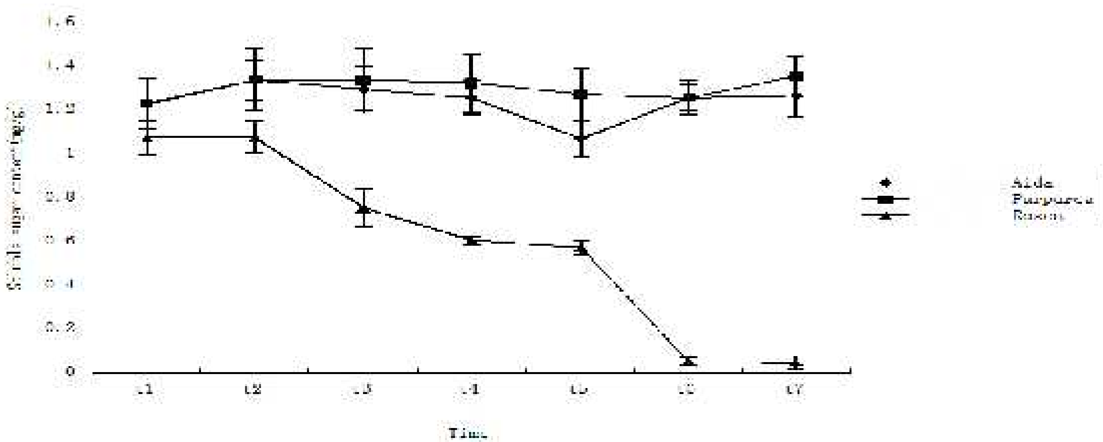

Figure 2. Soluble sugar content of three *Phlox* cultivars under field conditions (mg/g)

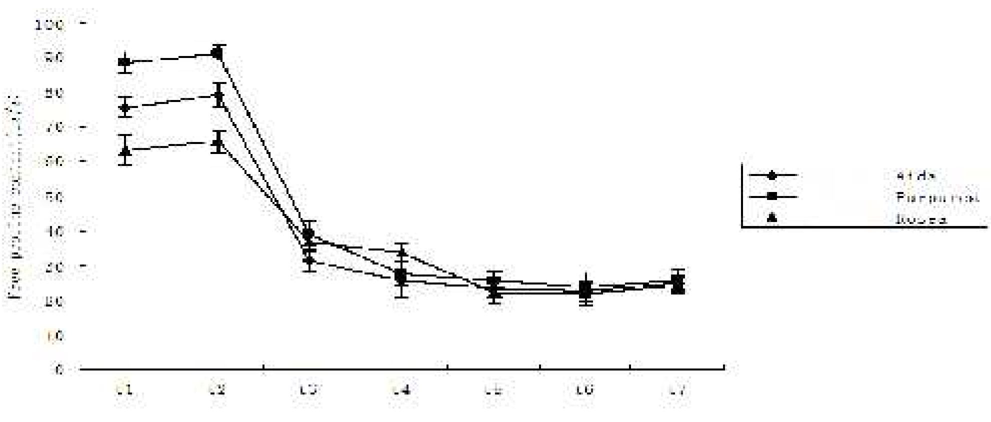

Figure 3. Free proline content of three *Phlox* cultivars under field conditions (μg/g)

Table 2. Comparison of relative conductivity under cold stress in three *Phlox* cultivars（%）

Cultivars	-35℃			-40℃		
	3h	6h	9h	3h	6h	9h
Phlox paniculata 'Aida'	52.12±2.15	59.47±3.12	66.67±4.23	63.12±2.27	69.15±2.46	75.28±3.19
Phlox paniculata 'Purpurea'	52.59±1.33	55.23±2.23*	59.14±3.37*	55.16±2.79**	62.39±2.18**	60.36±2.22**
Phlox paniculata 'Rosea'	54.13±1.79	60.59±2.03	64.58±2.89	55.36±3.18**	62.35±3.17**	69.28±3.56*

Means ± S.E.M. n=5 for the roots of *Phlox* cultivars
** P<0.01, * P<0.05

Table 3.The growth recovery of the *Phlox* cultivars in the following year under different conditions

Treatments / Cultivars	-35 ℃			-40 ℃		
	3h	6h	9h	3h	6h	9h
Phlox paniculata 'Aida'	+ + - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Phlox paniculata 'Purpurea'	+ + + - -	+ - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Phlox paniculata 'Rosea'	+ + - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -

Note：( ) recovery growth and hudded the leaves in the spring; ( ) without bud, died.

Comparison of relative electrical conductivity using different proof measures: Earth covering, use of straw curtain, and frost-proof water were used as chill-proof measures in winter, which influenced the relative electrical conductivity of the three *Phlox* cultivars (Table 4). For *P. paniculata* 'Rosea,' the relative electrical conductivity after the chill-proof measures was higher than those under the fields in winter. Among the three chill-proof measures, earth covering was the most feasible for *Phlox* species. The relative electrical conductivity of *P. paniculata* 'Purpurea' was noticeably lower than those of *P. paniculata* 'Aida' and *P. paniculata* 'Rosea' under similar chill-proof measures.

Table 4. Comparison of relative electrical conductivity using different proof measures in Phloxs （%）

Variety	Field	Earth covering	Frostpro of water	Straw curtain
Phlox paniculata 'Aida'	35.64±3.23	34.19±3.32	37.63±2.13	35.55±4.17
Phlox paniculata 'Purpurea'	32.39±1.77	30.07±1.19*	30.97±2.05	31.39±3.12
Phlox paniculata 'Rosea'	46.35±5.11	33.19±2.13*	34.36±3.09*	38.63±3.11*

Means ± S.E.M. n=5 for the roots of *Phlox* cultivars
* P<0.05

Comparison of soluble sugar content using different proof measures: Comparison of field conditions during winter showed that earth covering, use of straw curtain, and frost-proof water increased the soluble sugar content of the *Phlox* cultivars (Table 5). The percentage of soluble sugar content in the plants using earth covering measure was 11.1%, 14.0%, and 40.4% for *P. paniculata* cultivars 'Aida,' 'Purpurea,' and 'Rosea,' respectively, indicating good results.

Comparison of free proline content using different proof measures: Comparison of field conditions during winter showed that earth covering, use of straw curtain, and frost-proof water increased the soluble sugar content of the *Phlox* cultivars (Table 6). However, *P. paniculata* 'Rosea' exhibited higher free proline content than those of *P. paniculata* 'Aida' and *P. paniculata* 'Purpurea.'

Table 5. Comparison of soluble sugar content using different proof measures in *Phlox* cultivars（mg/g)

Variety	Field	Earth covering	Frostproof water	Straw curtain
Phlox paniculata 'Aida'	1.596±0.32	1.773±0.34*	1.756±0.52*	1.663±0.14*
Phlox paniculata 'Purpurea'	1.694±0.17	1.932±0.46*	1.863±0.42*	1.724±0.19*
Phlox paniculata 'Rosea'	1.051±0.29	1.476±0.09*	1.316±0.56*	1.256±0.52*

Means ± S.E.M. n=5 for the roots of *Phlox* cultivars
* P<0.05

Table 6. Comparison of free proline content using different proof measures in *Phlox* cultivars (μg/g)

Variety	Field	Earth covering	Frostproof water	Straw curtain
Phlox paniculata'Aida'	22.56±3.09	31.21±2.89*	29.65±3.57*	30.39±4.67*
Phlox paniculata'Purpurea'	20.85±2.17	32.09±2.78*	31.45±2.34*	31.08±2.49*
Phlox paniculata 'Rosea'	21.86±3.13	27.89±3.56*	27.36±2.18*	22.18±2.88*

Means ± S.E.M. n=5 for the roots of *Phlox* cultivars * P<0.05.

The different chill-proof measures are common methods for plants during winters in the north. Earth covering, use of straw curtain, and frost-proof water were used in winter under field conditions. The most feasible chill-proof measure for *Phlox* species was earth covering, which demonstrated great advantage against freezing temperatures. The straw curtain and frost-proof water are also commonly used methods in the north but require higher costs, making them impractical for large areas.

Conclusion: In summary, the results of relative electrical conductivity, soluble sugar content, and free proline content indicated that *P. paniculata* 'Purpurea' exhibited good cold resistance, and the most feasible chill-proof measure for *Phlox* species is earth covering. Moreover, *P. paniculata* 'Purpurea' suited among wild plants in Harbin Province.

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