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Cold-resistance evaluation in 25 wild grape species

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Summary

A cold-resistance analysis of 64 accessions of 18 wild Chinese *Vitis* species and 9 accessions of 7 wild American *Vitis* species was carried out to give a cold index, relative conductivity, soluble sugars, soluble protein, malondialdehyde and proline of one-year-old shoots under appropriate freezing treatments. Subordinate function analysis results indicate that there are substantial differences in cold resistance among the various germplasms. Among the eighteen wild *Vitis* species native to China, the cold-resistance rankings (high to low) are: *V. amurensis, V. yeshanensis*, *V. adstricta*, *V. pseudoreticulata*, *V. quinquangularis*, *V. piasezkii*, *V. hancockii*, *V. ficifolia*, *V. romanetii, V. davidii*, *V. piasezkii* var. *pagnucii*, *V. bashantica, V. liubaensis*, *V. qinlingensis*, *V. davidii* var. *cyanocarpa*, *V. wilsonae*, *V. baihensis,* and *V. davidii* var. *ninqiangensis*. Among the seven wild *Vitis* species native to America, cold-resistance rankings are: *V. riparia*, *V. arizonica*, *V. rupestris*, *V. rotundifolia* and *V. californica* (all high resistance) and *V. labrusca* and *V. cinerea* (medium resistance). The most cold-resistant accession is *V. riparia* Mcadams. These results will be valuable for the selection of wild grape species for cold-resistance breeding.

Key words: wild *Vitis*, germplasm resource, cold resistance, subordinate function (SF), evaluation.

Introduction

Temperature is one of the main environmental factors influencing plant growth and thus determining the regional distribution of any particular genotype. Low temperature can cause severe injury, affecting crop productivity, quality and even survival (Alberdi and Corcuera 1991). Grapes are the world's second-largest fruit crop, in terms both of cultivated area and also in fresh-weight production. The majority of commercial grape varieties are cold-sensitive and a significant problem with their cultivation is that to overwinter safely in many cool, continental climates they must be buried beneath the soil. This practice is both inconvenient and expensive (He and Chao 1982, Hamman *et al*. 1996). Therefore, an effective strategy to resolve this problem would be highly beneficial to the wine industry in such regions. China is a major center of origin for *Vitis* species. Here are to be found 42 species and 7 distinct varieties (He 1999). Evidence suggests that China's wild *Vitis* species have no foxy flavors, that they cross readily with European grapes (*V. vinifera*) and that they are resistant to many fungal diseases (Wang *et al*. 1995). Studies reveal that *V. amurensis* is one of the most cold-resistant grape species (He 1999) but information on many other species/accessions is lacking. For this reason, it would be useful to evaluate the cold-resistance of a range of China's wild grape genotypes to assess their potential for breeding.

Cold hardiness in plants usually involves a combination of morphological, physiological and biochemical features which develop by natural selection over very long periods of time. These features are often associated so that cold hardiness can be screened for by testing for change in the relative amounts of particular biochemicals (Keller and Lynn 2007). Thus, changes in electrolyte exudation rates, and in the contents of soluble sugars, soluble proteins, and of proline, malondialdehyde (MDA) and peroxidases, are all associated with the natural adaptation of many plant species to low temperatures (Wang *et al*. 2000, John *et al*. 2010). Therefore, it is possible to use changes in these materials in plants exposed to low temperature stress as surrogates to evaluate cold hardiness in a range of grape germplasm resources (Wang *et al*. 2000, John *et al*. 2010).

Appraisal of grape germplasm depends on accurate and reliable methodologies. One of these uses fuzzy mathematics to measure the average of the subordinate functions (SF) of several different indices to evaluate cold hardiness. This method has already been applied successfully with poplar (Shi *et al*. 2003), grape (Zhang *et al.* 2007) and loosestrife (Xu *et al*. 2009). In this study, we investigate the cold resistance of 64 accessions of 18 wild Chinese *Vitis* species and of 9 accessions of 7 wild American *Vitis* species. Here, principle component analysis and the SF method were used to obtain estimates of cold-resistance based on measurements of physiological and biochemical change. The results are expected to be of considerable usefulness in breeding for cold hardiness in grapes.

Material and Methods

Plant materials: Shoots from 73 grape accessions were sampled from the grape repository of Northwest A & F University, Shaanxi, China. These included 64 accessions of 18 wild Chinese *Vitis* species and 9 accessions of 7 wild American *Vitis* species (Tab.1, Tab. 2). The two

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Table 1

Cold resistance results for various accessions of wild Chinese *Vitis* species

Species	Accession or cultivar	Evaluation index						The average of SF	Cold resistance level
		C1 (%)	RC (%)	SS (%)	SP (mg·g^{-1} FW)	MDA (μmol·g^{-1} FW)	PRO (μg·g^{-1} FW)		
V. amurensis Rupr.	Huaxian-47	10	56.17	10.24	2.3	4.7	30	0.75	H
	Tonghua-3	10	56.45	10.01	2.2	4.8	32	0.80	H
	Taishan-11	12	51.25	11.16	2.4	4.6	31	0.88	H
	Shuangyou	13	58.19	11.24	2.2	4.5	32	0.76	H
	Zuoshan74-1-326	20	52.16	9.86	2.1	4.7	29	0.64	M/H
	Zuoshan-1	12	54.55	10.17	2.2	4.5	30	0.71	H
	Zuoshan75097	14	53.15	11.05	2.3	4.4	32	0.78	H
	Zuoshan-2	18	52.19	10.42	2.1	4.8	31	0.67	M/H
	Heilongjiang Seedling	15	54.26	10.34	2.3	4.9	31	0.76	H
V. pseudoreticulata W.T. Wang	Guangxi-1	25	58.19	10.87	2.0	4.9	30	0.65	M/H
	Shangnan-2	29	78.21	9.97	2.1	5.1	27	0.42	M
	Guangxi-2	25	61.19	10.16	2.0	5.1	28	0.60	M/H
	Hunan-1	26	65.14	8.87	2.1	5.3	29	0.51	M
	Baihe-35-2	29	65.78	9.01	2.0	5.4	27	0.58	M
	Baihe-35-1	19	60.18	10.21	2.2	5.2	30	0.64	M/H
	Baihe-13	25	65.13	8.87	2.1	5.5	29	0.49	M
	Baihe-13-1	24	62.60	9.67	2.1	5.3	30	0.52	M
V. adstricta Hance	Taishan-1	12	53.25	10.32	2.2	4.8	32	0.64	M/H
	Taishan-2	13	56.45	9.21	2.1	5.1	31	0.60	M/H
	Anlin-1	20	57.25	10.24	2.0	5.0	31	0.62	M/H
	Anlin-3	19	58.19	9.16	2.1	5.4	29	0.58	M
V. quinquangularis Rehd.	Danfeng-2	28	60.22	9.01	2.0	5.4	30	0.47	M
	83-4-96 (♀)	18	61.45	10.24	2.1	4.9	29	0.51	M
	83-4-96 (♂)	27	63.25	9.86	2.0	5.1	28	0.45	M
	Shangnan-24	26	62.19	10.34	2.1	5.4	29	0.40	M
	Taishan-12	19	59.16	10.26	2.0	5.1	30	0.58	M
	Nanzheng-1	25	67.55	9.01	2.1	5.4	28	0.39	M/L
V. romanetii Roman.	Pingli-2	35	79.56	9.32	2.0	5.4	26	0.39	M/L
	Jiangxi-1	38	79.19	8.83	2.1	5.2	28	0.32	M/L
	Jiangxi-2	39	84.25	8.87	1.8	5.5	29	0.39	M/L
	Liuba-1	40	77.45	9.45	2.0	5.1	27	0.43	M
	Pingli-7	43	83.52	8.79	1.9	5.9	25	0.31	M/L
V. davidii Foex	Tangwei	39	76.45	9.77	2.2	5.4	29	0.35	M/L
	Fujian-4	38	73.98	9.69	2.0	4.9	28	0.38	M/L
	Lueyang-4	42	77.48	8.41	1.9	5.3	26	0.29	L
	Xuefeng	35	70.19	9.38	2.0	5.4	27	0.46	M
	Ji'nan-1	40	80.26	8.77	2.0	5.5	25	0.34	M/L
	Ji'nan-2	39	72.19	9.69	2.1	5.1	28	0.42	M
V. piasezkii Maxim.	Liuba-9	26	60.14	9.89	2.2	5.4	29	0.46	M
	Gansu-91	27	65.78	10.27	2.1	5.1	29	0.51	M
	Liuba-6	22	63.18	10.89	2.0	5.4	28	0.52	M
	Nanzheng-2	29	65.13	9.68	2.1	5.6	29	0.35	M/L
	Liuba-8	19	60.22	10.36	2.0	4.9	28	0.59	M
	Huaxian-11	27	61.45	9.53	2.1	5.1	29	0.45	M
	Baishui-40	23	63.35	9.76	2.0	5.7	28	0.34	M/L
	Meixian-6	28	62.59	10.10	2.1	5.6	26	0.32	M/L
	Liuba-7	27	63.78	9.89	2.2	5.4	28	0.42	M
V. davidii var. *cyanocarpa* Sarg.	Zhen'an-3	39	75.69	8.41	1.8	5.1	27	0.31	M/L
	Langao-5	41	84.25	8.38	1.9	5.3	25	0.24	L
V. qinlingensis P.C. He	Pinli-5	38	78.19	8.89	1.8	5.4	27	0.26	L
	Lueyang-4	42	75.60	8.41	1.8	5.2	29	0.30	M/L
V. bashanica P.C. He	Xunyang-8	36	72.03	8.46	1.9	5.5	28	0.28	L
	Baihe-41	41	78.19	8.27	1.8	5.8	30	0.33	M/L
V. hancockii Hance	Lingye (♀)	19	60.18	9.77	2.2	5.1	30	0.52	M
	Jiangxi-3 (♂)	27	65.13	9.69	2.1	5.3	29	0.48	M
V. liubaensis L.X. Niu	Langao-2	51	79.19	8.38	1.8	5.2	27	0.30	M/L
	Liuba-10	45	79.56	8.41	1.9	5.4	25	0.29	L
V. ficifolia Bunge	Weinan-3	25	77.49	9.89	2.1	5.0	27	0.43	M
	Shandong	29	78.35	9.19	2.2	5.1	28	0.40	M

Tab. 1, continued

Species	Accession of cultivar	Evaluation index						The average of SF	Cold resistance level
		C1 (%)	RC (%)	SS (%)	SP (mg·g^{-1} FW)	MDA (μmol·g^{-1} FW)	PRO (μg·g^{-1} FW)		
V. yeshanensis J.C. Chen	Yanshan-1	11	56.34	10.83	2.2	4.7	29	0.72	H
V. wilsonae Veitch.	Zhengzhouwngmai	48	86.34	7.89	1.8	5.8	29	0.26	L
V. baihensis L.X. Niu	Baihe-40	45	84.16	8.41	1.9	5.9	26	0.21	L
V. davidii Foex var. *ningqiangensis* L.X. Niu	Ningqiang-6	43	79.46	8.46	1.8	5.8	27	0.29	L
V. piasezkii Maxim. var. *pagnucii* (Planch.) Rehd.	Wanxian-15	48	83.14	8.41	2.0	5.3	25	0.32	M/L
V. vinifera L. (CK)	Muscat Hamburg	86	87.14	8.62	1.9	6.0	24	0.21	L
	Red Globe	93	89.52	8.41	2.0	6.3	25	0.19	L

Table 2

Comprehensive analysis of cold resistance of wild American *Vitis* species

Species	Accession or cultivar	Evaluation index						The average of SF	Cold resistance level
		C1 (%)	RC (%)	SS (%)	SP (mg·g^{-1} FW)	MDA (μmol·g^{-1} FW)	PRO (μg·g^{-1} FW)		
V. cinerea Engelm.		16	57.25	9.86	2.0	4.5	30	0.64	M/H
V. arzonica Engelm.		10	52.16	10.16	2.3	5.1	32	0.78	H
V. rotundifolia Michx.		10	53.56	10.19	2.2	4.6	35	0.75	H
V. californica	Gold Hill#1	18	54.45	10.29	2.1	4.9	36	0.74	H
V. labrusca L.	Y157	20	56.17	10.11	2.2	4.8	34	0.69	M/H
V. riparia Michx.	*V. riparia* Beaumont	10	55.19	10.89	2.2	4.9	35	0.80	H
	V. riparia Mcadams	10	53.78	10.99	2.2	4.5	34	0.86	H
V. rupestris Scheele	A. De Serres	16	53.20	10.01	2.0	4.7	36	0.74	H
	Constantia	10	52.19	10.21	2.1	5.1	34	0.76	H
V. vinifera L. (CK)	Muscat Hamburg	86	87.14	8.62	1.9	6.0	24	0.21	L
	Red Globe	93	89.52	8.41	2.0	6.3	25	0.19	L

V. vinifera varieties Muscat of Hamburg (a wine grape) and 'Red Globe' (a table grape) were used as controls.

Selection of optimal temperature treatment: Chill treatment was performed according to the method of He and Niu (1989), with slight modification. Seven chill temperatures were used: 4 °C (control), -16 °C, -20 °C, -24 °C, -28 °C, -32 °C and -36 °C. The chill treatment lasted 10 h and both the cooling and heating rates were set at 4 °C·h^{-1}. The *V. amurensis* accession, 'Heilongjiang Seedling', hybrid 'Beichun' ('Muscat of Hamburg' × *V. amurensis*), and the *V. vinifera* cultivars 'Muscat of Hamburg' and 'Red Globe' were used to test relative conductivity after holding for 12 h at room temperature after chilling treatment. Optimal chilling temperatures were determined from the semi-lethal temperature (LT50) using a logistic equation.

Cold index: Twenty, 1-mm thick sections were cut from the middle of an internode and the area of browning of the secondary xylem was recorded with a light microscope. The level of cold injury of each shoot was scored on a ten-point scale based on the area (%) of browning in the whole cross-section: (1 = 0-3.0 %, 2 = 3.1-6.0 %, 3 = 6.1-12.0 %, 4 = 12.1-25.0 %, 5 = 25.1-50.0 %, 6 = 50.1-75.0 %, 7 = 75.1-88.0 %, 8 = 88.1-94.0 %, 9 = 94.1-97.0 % and 10 = 97.1-100 % (He and Niu 1989).

Cold index % (CI) is the standard of cold injury severity and can be calculated

$$\text{Cold Index (\%)} = \frac{\sum_{i=1}^{10} n_i \times i}{10 \sum n_i} \times 100 \quad (1)$$

where i is the cold injury level (1, 2…10) and n_i is the number of shoot whose cold injury level was i.

Relative conductivity: The epidermis of the shoots was first removed and these were then washed in distilled water. After drying with filter paper, shoots were cut into 2-5 mm thick slices (He and Chao 1982, He and Niu 1989). Next, 1.0 g samples of each material were placed in graduated test tubes with 20 mL of deionized water. After resting at room temperature for 25 min, the tubes were then evacuated for 15 min to remove air from the tissues. After the vacuum had been released the samples rested for a further 15 min at room temperature before conductivity (C1) was measured using a DDS-307 Conductivity Meter (Shanghai Precision & Scientific Instrument Co., Ltd., China). After boiling for 30 min and resting at room temperature for 5 h, conductivity (C2) was again measured. Relative conductivity % (RC) was calculated as:

$$RC = C1/C2 \times 100 \quad (2)$$

Soluble sugars: For each material and for each treatment, a 0.2 g internode sample was held for 20 min in 20 mL of distilled water in a lidded conical flask in a boiling water bath. When cooled, the liquid was filtered into a 100 mL volumetric flask and the volume adjusted to the graduation. Then 1 mL of this solution was placed in a glass tube and 4 mL of anthranone solution (2 g anthranone dissolved in 1000 mL 80 % H_2SO_4) added. After 10 min heating in a water bath at 100 °C, the colored solution was cooled and absorption was measured at 620 nm in a model 721 spectrophotometer (Shanghai Precision & Scientific Instrument Co., Ltd., China) (WANG *et al*. 1998). The soluble sugar content (*SS*) was obtained from a standard curve as:

$$SS = (C_s \times V_1)/ (FW \times 10^6) \times 100 \quad (3)$$

where C_s is the content of soluble sugar read from the standard curve, V_1 is the total volume of extraction solution (mL) and *FW* is the sample weight (mg).

Soluble proteins: Samples (0.2 g) of each material were ground with 5 mL of distilled water and a small quantity of quartz sand in a mortar close to 0 °C (ice). The homogenate was then transferred to a centrifuge tube and spun at 4,000 rev·min^{-1} for 10 min. Next, 1 mL of the supernatant liquid was added to 5 mL 10 % of G-250 coomassie blue (w/v). After 5 min, absorption was measured at 595 nm in the model 721 spectrophotometer. The soluble protein content (*SP*) (mg·g^{-1}) was obtained from a standard curve (SUN and HU 2008) as:

$$SP = (C_p \times V_1)/ (A \times FW \times 1000) \quad (4)$$

where C_p is the soluble protein content read from the standard curve, V_1 (mL) the total volume of extraction solution, *A* (mL) the volume of test solution and *FW* is the sample weight (g).

Malondialdehyde content: Malondialdehyde (MDA) content was measured as described by SUN and HU (2008). For each material and treatment, a 0.2 g sample was mixed with 5 mL trichloroacetic acid and a small quantity of quartz sand in a mortar. After grinding to homogenate in an ice bath, the material was transferred to a centrifuge tube and spun at 4,000 rpm for 10 min. A volume of 2 mL of the supernatant liquid was then transferred to a test tube (2 mL of distilled water was added for the controls) and shaken. Next, 2 mL 0.6 % 2-Thiobarbituric acid (TBA) was added and the tube placed in a boiling water bath for exactly 10 min with this timing being started when small bubbles appeared in the test tubes. Test tubes were placed in cold water for rapid cooling and then centrifuged for 15 min at 3,000 rpm. Absorption values (A) were measured at three wavelengths: 450 nm, 532 nm and 600 nm using the model 721 spectrophotometer. In the controls, 2 mL of distilled water and 2 mL of TBA were used. The MDA content was calculated as:

$$MDA \text{ (nmol·g}^{-1} FW) = 6.452 \times (A_{532} - A_{600}) - 0.559 \times A_{450} \times V_1/(V_2 \times FW) \quad (5)$$

where V_1 is the total volume of extracting solution (mL), V_2 is the volume of test solution (mL) and *FW* is the sample weight (g).

Free proline: To determine free proline (PRO), a 0.2 g sample of each cold-treated material was heated with 5 mL 3 % aqueous sulfosalicylic acid in a conical flask for 10 min in a boiling water bath with constant shaking. After cooling, the material was filtered using Watman No.50 filter paper into a clean test tube and 2 mL of the filtrate was heated with 2 mL glacial acetic acid and 2 mL acidic ninhydrin for 30 min on a boiling water bath. The mixture was again cooled and 4 mL of toluene was added and allowed to extract for 30 s and the supernatant was transferred to a centrifuge tube and spun for 5 min at 3,000 rpm. Absorption of the red supernatant was measured at 520 nm in the model 721 spectrophotometer (ZHANG 2002). PRO was calculated from the standard curve as,

$$PRO \text{ (μg·g}^{-1}) = (C \times V_1/A)/FW \quad (6)$$

where *C* is the free proline content read from the standard curve (μg); V_1 is the total volume of extracting solution (mL), *A* is the volume of sample solution (mL) and the unit of *FW* is g.

Standardization: As the various indicators had different dimensions, it was necessary to standardize these to the dimensionless form (TANG and FENG 2007):

$$xi' = \frac{xi - \overline{xi}}{di} \quad (7)$$

where, for a raw data sample, $\overline{xi}$ is the average, *di* is the standard deviation and *xi* is a particular raw value.

The standardized data for the substances measured were analyzed with DPS (Data Processing System) and processed using the subordinative function (SF) (XU *et al*. 2009) to evaluate the level of cold hardiness of the range of wild grape germplasm examined here.

Membership function method: Evaluation of cold hardiness is based on the evaluation of the various SF indices in the form (SHI *et al*. 2003),

$$Uij = \frac{Xij - Xj \min}{Xj \max - Xj \min} \quad (8)$$

(positive correlation)

$$Uij = 1 - \frac{Xij - Xj \min}{Xj \max - Xj \min} \quad (9)$$

(negative correlation)

$$\overline{Ui} = \frac{1}{n} \sum_{j=1}^{n} Xij \quad (10)$$

Here, *i* is a particular accession, *j* is a particular index, *Xij* is the testing value of the index *j* of accession *i*, *Xjmin* is the minimum value of index *j* for all accessions, *Xjmax* is the maximum value of index *j* of all accessions, *Uij* is the SF value of accession *i*, and index *j* that relates to cold hardiness and $\overline{Ui}$ is the average SF of that accession.

Using the above forms, the SF values of the six different indices were combined and their averages calculated (HE and NIU 1989, ZHANG *et al.* 2007). Based on the aver-

Table 3

The relative conductivity of various species/accessions under different low temperature treatments

Accession or cultivar	Low temperatures treatment						
	4 °C (control)	-16 °C	-20 °C	-24 °C	-28 °C	-32 °C	-36 °C
Heilongjiang Seedling	41.31Bb	40.68Bb	46.83Cc	54.26Bb	62.59Cc	89.41Bb	85.69Bb
Beichun	43.98Bb	42.89ABb	47.67BCc	64.15Bb	79.45Bb	83.52Bb	86.19Bb
Muscat Hamburg	58.14Aa	52.43Aa	54.47Bb	82.36Aa	86.76Bb	98.45Aa	100.76Aa
Red Globe	50.22ABab	48.41ABab	67.14Aa	89.15Aa	98.52Aa	102.78Aa	104.78Aa

age SF, each germplasm accession was then rated as having a cold hardiness resistance varying between: High (H) (SF average = 0.70-1.00), medium/high (M/H) (SF average = 0.60-0.69), medium (M) (SF average = 0.40-0.59), medium/low (M/L) (average SF = 0.30-0.39) and Low (L) (SF average = 0.00-0.29).

Results

Optimum temperature selection: All values of the four materials tested at -16 °C showed a slight decrease compared to the controls (Tab. 3). With decreasing treatment temperatures, the rate of conductivity change with temperature interval changed. At -20 °C, 'Red Globe' was about 16.9 % higher than the control while 'Heilongjiang Seedling', 'Beichun' and 'Muscat of Hamburg' were about 5.5 %, 3.7 %, -3.7 % higher than the controls respectively, this emphasizes 'Red Globe's low resistance to cold compared with the other materials. At -24 °C, -32 °C and -36 °C, there were significant differences (P = 0.01) between 'Heilongjiang Seedling', 'Beichun' and the European cultivars. There was no significant difference between 'Heilongjiang Seedling' and 'Beichun' or between 'Muscat of Hamburg' and 'Red Globe'. However, at -28 °C, there was no significant difference between 'Beichun' and 'Muscat of Hamburg' while there was a highly significant difference (P = 0.01) between 'Heilongjiang Seedling' and 'Beichun'. Taken together, -24 °C, -32 °C and -36 °C were good temperatures for distinguishing between species resistant to cold from those that lack cold resistance. Taken into consideration the LT_{50} values of the four materials (Tab. 4), all were below -32 °C. So, the critical treatment temperature was -24 °C.

Correlation analysis of cold hardiness indices: The correlation analysis of six cold hardiness indices with DPS, indicated that the average SF value correlated positively with relative conductivity, cold index and MDA content and correlated negatively with soluble sugar content, soluble protein and free proline (Tab. 5). The correlation between the average SF and the soluble sugar and soluble protein contents was conspicuous. So, to some degree, these six indices can all be considered a single index of cold hardiness. However, correlations among these indices were present so that the test data information will have a superposition component and thus produces an inexact result. Hence, using the average all six SF values as a surrogate to assess cold hardiness (rather than just one or two of them) should provide a more accurate and robust assessment of cold resistance.

Table 4

Semi-lethal temperatures (LT_{50}) of different accessions or cultivars

Accession or cultivar	Equation of semi-lethal temperature	LT_{50} (°C)
Hailongjiang Seedling	Y = 100.17/ (1+8.71e-0.064X)	-31.12
Beichun	Y = 96.24/ (1+10.17e-0.093X)	-26.78
Muscat Hamburg	Y = 94.15/ (1+10.42e-0.081X)	-23.78
Red Globe	Y = 93.78/ (1+16.48e-0.096X)	-21.78

Principle component analysis of cold resistance indices: The results from principle component analysis (PCA) show that the contribution rates of the three principle components Y_1, Y_2, Y_3 were about 62 %, 28 %, and 7 %, respectively (Tab. 6). The cumulative contribution rate reached about 98.7 % which represents about 98.7 % of all the cold hardiness information of this grape germplasm. The value of the eigenvector of each principle component Y_1, Y_2, Y_3 is listed. X_1-X_6 refers to the six indices (factors) and according to their values arise the following equations:

$$Y_1=0.956X1-0.963X2+0.451X3-0.369X4-0.896X5+0.256X6 \quad (11)$$

$$Y_2=-0.101X1+0.321X2+0.817X3+0.774X4+0.014X5+0.246X6 \quad (12)$$

$$Y_3=-0.245X1+0.140X2-0.458X3+0.025X4-0.016X5+0.587X6 \quad (13)$$

For Y_1, of the six indices, the eigenvectors CI, RC, MDA had the larger moduli and the total contribution rate reached 62 %; for Y_2, SS and SP had the highest absolute values and the total rate was 28 %; for Y_3, SS and PRO had the highest absolute values and the total rate achieved was about 7 %. Overall, it is possible to obtain cold hardiness information using the average of all six indices CI, RC, SS, SP, MDA and PRO.

Overall evaluation and analysis: By testing the six indices of different species or germplasm lines, the results show significant differences among the 64 materials tested from 18 species of wild Chinese *Vitis*. As is shown in Tab. 7, *V. amurensis* and *V. yeshanensis* have high cold resistance with average SF values of 0.75 and

Table 5

Correlation analysis of cold resistance indices of different grape resources

Index	X_1	X_2	X_3	X_4	X_5	X_6	X_7
X_1	1.000						
X_2	0.870**	1.000					
X_3	-0.788**	-0.974**	1.000				
X_4	-0.736**	-0.863**	0.877**	1.000			
X_5	-0.832**	0.728**	-0.869**	-0.922**	1.000		
X_6	-0.629*	-0.749**	0.648*	0.871**	-0.704*	1.000	
X_7	-0.721**	-0.824**	0.874**	0.715**	-0.727**	0.742**	1.000

Significant differences: *P < 0.05; **P < 0.01; X_1-X_6: RC, CI, SS, SP, MDA and PRO, respenctively; X_7: represents the average SF.

Table 6

The eigenvalue, contribution rate and cumulative contribution rate of differnt grape resources

Index	Eigenvectors		
	Y_1	Y_2	Y_3
X1	0.956	-0.101	-0.245
X2	-0.963	0.321	0.140
X3	0.451	0.817	-0.458
X4	-0.369	0.774	0.025
X5	-0.896	0.014	-0.016
X6	0.259	0.246	0.587
Eigenvalues	5.719	2.558	0.631
Contribution rate (%)	62.292	28.445	6.961
Cumulative contribution rate (%)	62.292	91.737	98.698

X_1-X_6 represent RC, C1, SS, SP, MDA and PRO, respectively; Y_1-Y_3 represent the three principal components, respectively.

0.72 respectively; *V. adstricta* has medium/high cold resistance with an SF value of 0.6; *V. pseudoreticulata*, *V. quinquangularis*, *V. piasezkii*, *V. hancockii* and *V. ficifolia* all have medium cold resistance with average SF values between 0.42 and 0.57; *V. romanetii*, *V. davidii*, *V. piasezkii* var. *pagnucii*, *V. bashantica* and *V. liubaensis* have medium/low cold resistance with SF values from 0.30 to 0.39, while *V. qinlingensis*, *V. davidii* var. *cyanocarpa*, *V. wilsonae*, *V. baihensis and V. davidii* var. *ninqiangensis* have low cold resistance with SF values from 0.21 to 0.29. Among the low cold resistance types, *V. baihensis* was the least resistant with a subordinative level of only 0.21 and similar to the controls. At -24 °C, the RC values of *V. romanetii* and *V. piasezkii* var. *pagnucii* were similar to the controls even though these two germplasms belong to low cold resistance type. The SS of *V. piasezkii* was similar to that of *V. amurensis* even though their cold resistance levels were very different. This suggests that an index based on a single character would not be a reliable indicator of cold resistance.

Overall evaluation of cold resistance: There was variation in cold resistance not only among the different wild Chinese *Vitis* species but also among the different accessions of the same species (Tab. 1). For example, the various accessions of *V. amurensis* had different levels of cold resistance even though this species has the highest cold resistance. Meanwhile, 'Taishan-11' has high cold resistance with an average SF value of 0.88 while 'Zuoshan-2' and 'Zuoshan74-1-326' have only medium/high cold resistance with SF values of 0.62 and 0.64, respectively. This occurs particularly in *V. romanetii* and *V. davidii*. For example, 'Liuba-1' of *V. romanetii* has medium cold resistance, higher than the other four accessions which are medium/low. Also, 'Lueyang-4' of *V. davidii* had low cold resistance while accessions 'Ji'nan-1' and 'Xuefeng' had medium resistance. As is shown in Tab. 1, this situation also occurred with some other species but the differences were not so large. Variation in cold resistance clearly exists between the species examined here, even among accessions of *V. amurensis*, *V. yeshanensis* and *V. adstricta* which are considered important germplasm resources for cold resistance breeding.

Evaluation of wild American species: Analysis of the six indices of the American species showed that the average SF ranged from 0.64 to 0.86, with these showing either high (*V. riparia*, *V. arizonica*, *V. rotundifolia*, *V. rupestris* and *V. californica*) or medium/high (*V. labrusca* and *V. cinerea*) cold resistance (Tab. 2). *V. riparia* Mcadams was the most cold resistant of the American species with an average SF of 0.86, which is similar to the Chinese *V. amurensis*. The least cold resistant species was *V. cinerea* with average SF of 0.64, and below *V. amurensis* and *V. yeshanensis*. On the basis of their overall cold resistance from high to low, the American species can be ranked: *V. riparia* Mcadams > *V. riparia* Beaumont > *V. arizonica* > *V. rotundifolia* = *V. rupestris* (Constantia > A.De Serres) > *V. californica*> *V. labrusca* > *V. cinerea*.

Discussion

Indices and appraisal method: Adaption to cold stress is a gradual process of morphological, physiological and biochemical change accompanied by the accumulation of particular substances such as soluble sugars, free proline, soluble protein, unsaturated fatty acids and membrane lipid peroxide (WOLF and COOK 1991, STRAND *et al.* 1997, GUSTA *et al.* 2003). Some of these changes damage the cytomembrane, inducing changes in permeability and a loss of some water. The more serious the damage, the more rapid the loss of electrolytes and the

Table 7

Comprehensive analysis of cold resistance of Chinese *Vitis* species

Species	No. of clones	Evaluation index						The average of SF	Cold resistance level
		C1 (%)	RC (%)	SS (%)	SP (mg·g^{-1} FW)	MDA (μmol·g^{-1} FW)	PRO (μg·g^{-1} FW)		
V. amurensis Rupr.	9	13.78	54.27	10.50	2.11	4.67	30.89	0.75	H
V. yeshanensis J.C. Chen	1	11.00	56.34	10.83	2.20	4.70	29.00	0.72	H
V. adstricta Hanse	4	16.00	56.29	9.73	2.10	5.20	30.75	0.60	M/H
V. pseudoreticulata W.T. Wang	8	24.71	62.60	9.67	2.17	5.24	29.20	0.57	M
V. hancockii Hance	2	23.00	62.67	9.72	2.15	5.20	29.50	0.50	M
V. quingquangularis Rehd.	6	23.83	62.30	9.79	2.03	5.22	29.00	0.47	M
V. piasezkii Maxim.	9	25.33	62.85	10.04	2.09	5.36	28.56	0.44	M
V. ficifolia Bunge	2	27.00	77.92	9.54	2.15	5.25	27.50	0.42	M
V. romanetii Roman.	5	39.00	80.36	9.21	1.98	5.37	27.00	0.38	M/L
V. davidii Foex	6	38.83	75.09	9.29	2.03	5.27	27.17	0.37	M/L
V. piasezkii Maxim. var. *Pagmucii* (Planch.) Rehd.	1	48.00	83.14	8.41	2.00	5.30	25.00	0.32	M/L
V. bashantica P.C.H	2	38.50	75.11	8.37	1.85	5.65	29.00	0.31	M/L
V. liubaensis L.X. Niu	2	48.00	79.37	8.40	1.90	5.30	26.00	0.30	M/L
V. davidii Foex var. *ninqiangensis* L.X. Niu	1	43.00	79.46	8.46	1.80	5.80	27.00	0.29	L
V. qinlingensis P.C.H	2	40.00	76.90	8.65	1.80	5.30	28.00	0.28	L
V. davidii var. *cyanocarpa* Sarg.	2	40.00	79.97	8.40	1.85	5.20	26.00	0.28	L
V. wilsonae Veitch.									
V. baihensis L.X. Niu	1	48.00	86.34	7.89	1.80	5.80	29.00	0.26	L
V. vinifera L. (CK)	1	45.00	84.16	8.41	1.90	5.90	26.00	0.21	L
	2	90.01	88.33	8.52	2.00	6.20	25.00	0.20	L

consequent change in RC. This allows measurements of electrolyte loss rate and RC to be used as indicators of a plant's level of cold hardiness. Under cold stress, polysaccharides are hydrolyzed to soluble sugars which increase the osmotic potential of the cytoplasm and lower the freezing temperature. Soluble sugars can function as protective substances and their concentration is positively correlated with cold hardiness (Quamme 1974, Wang 1987, Gusta *et al.* 2003). Soluble protein content also increases under cold stress, due to increased rates of synthesis or to degradation of higher molecular weight material (Wang 1987). As soluble protein can increase the proportion of bound intracellular water, there is an associated reduction in the availability of free water. Meanwhile, the soluble protein can regulate the expression of cold resistance genes, also enhancing a plant's cold tolerance (Puvisac 1982, Stepokus 1982, Dionne *et al.* 2001). Concentrations of free amino acid and soluble proteins are strongly associated and both tend to increase the water-retention capacity of the protoplast (Lyons 1973, Guy 1990, Jalilir 1998, Dionne *et al.* 2001).

Thus, under low temperature stress, the free proline and total amino acid content of grape tissues are expected to increase, and this response is likely to be particularly rapid in the cold-resistant varieties (Wang *et al.* 1996). Free proline can help maintain osmotic equilibrium between the symplast and apoplast and thus aid in resisting low-temperature damage by maintaining the functional integrity of the membrane (Dionne *et al.* 2001). Thus, under cold stress, cold-resistant grape varieties accumulate free proline more than the less resistant ones (Dionne *et al.* 2001). Therefore, soluble protein and free proline contents can both be used as indicators the degree of winter hardiness.

The hardiness diversity and ecological suitability in interspecies and intraspecies: Grape germplasm resources in Eurasia and North America are abundant and are to be found over a wide range of temperate climates, including frigid-temperate, temperate, subtropical and tropical climates. A number of distinct population centers now exist and hybrid populations have arisen where these main centers abut. With the diversity of climates, a range of stress resistances have evolved including resistance to low temperatures. Within the eastern Asian population, those grapes native to China have developed several cold-hardy genotypes by natural selection (He 1999). In this study, the results of 64 accessions of 18 wild Chinese *Vitis* species and 9 accessions of 7 wild American *Vitis* species demonstrate this. Thus, *V. amurensis* and *V. yeshanensis*, deriving from northeast and northern areas of China, possess marked cold-resistance having average SF values of over 0.70 while other species native to China also have relatively high SF values. Grape species originating from N America also possess extraordinary cold resistance, in particular, *V. riparia* with the very high SF value of 0.83. Meanwhile, European cultivars are far behind with average SF values of only 0.21.

Cold resistance in plants involves a complex process of physiological change (Foodlad *et al.* 2003) in which climatic and ecological factors play an important role. Generally, species/accessions from the north are more cold-hardy than those from the south with accessions from similar latitudes usually being little different in this regard (He and Niu 1989, Zhang *et al.* 2007). For example, *V. amurensis* from northeastern and northern China is more cold resistant than *V. qinlingensis*, *V. bashanica* and *V. davidii* var. *cyanocarpa* from further south, while *V. amurensis* accessions 'Zuoshan-1', 'Zuoshan-2', 'Zuoshan74-1-326' and 'Zuoshan75097', all from Jilin province, are similar to one another in cold hardiness. Because air temperature decreases with altitude (the lapse rate is 6.5 °C per 1,000 m) cold hardiness is also found to increase with altitude. Latitude also

has a significant effect on cold-resistance so that the cold-resistance of different accessions of *V. amurensis* vary with both altitude and latitude. Thus, at the same altitude, the cold resistance of 'Taishan-11' (high latitudes, Mount Taishan, Shandong province) is greater than of 'Huaxian-47' (lower latitudes, Shaanxi Province), even greater than the cold hardiness of 'Heilongjiang Seedling' and 'Shuangyou' (higher altitude and low latitude, northeast China). For *V. adstricta*, 'Taishan-1' and 'Taishan-2' (high altitude and high latitude, Mount Taishan, Shandong province) cold hardiness is greater than of 'Anlin-1' and 'Anlin-3' (low altitude and low latitude, Anhui province). It is reasonable to conclude that both latitude and altitude are involved in the evolution of cold resistance.

Conclusion

Here, we provide evidence for a wide variation in cold resistance in wild grape species with the data offering a valuable resource for cold-resistance breeding in grapes. Our results indicate that the wild Chinese species *V. amurensis* is the most cold-resistant, next comes *V. yeshanensis*, and *V. adstricta* and then *V. yeshanensis*. The accessions 'Taishan-11', 'Tonghua-3', 'Zuoshan75097', 'Huaxian-47' and 'Heilongjiang Seedling' of *V. amurensis* and the accession 'Yanshan-1' of *V. yeshanensis* also have high cold resistance. These species/accessions all have high potential importance to grape breeding as sources of cold resistance.

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