Original article

Establishment and application of a comprehensive assessment system for cold resistance in Denphal-group *Dendrobium* cultivars

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Summary

The objective of this study was to establish a comprehensive assessment system for cold resistance in Denphal-group *Dendrobium* cultivars, and evaluation of cold resistance of 35 Denphal-group *Dendrobium* cultivars. The concentration of soluble sugar, soluble protein, free proline, malondialdehyde, chlorophyll, and LT50 of two Denphal-group *Dendrobium* cultivars were measured under low temperature, and significance analysis, correlation analysis, principal component analysis, and weight analysis of these indicators were conducted, and then a comprehensive assessment system was constructed using the membership function and weight of indicators methods. Secondly, the cold resistance of 35 cultivars was assessed using the comprehensive assessment system. Under our experimental conditions, free proline, malondialdehyde, and chlorophyll were significantly correlated with LT50 at 0.05-level. The principal component analysis and weight analysis indicated that the most significant factor affecting cold resistance was free proline, followed by MDA and chlorophyll, indicating that the three indicators could be used to determine cold resistance in Denphal-group *Dendrobium* cultivars. The cold resistance evaluation of 35 cultivars showed that the most cold-resistant cultivar was 'Sophie Bonnie', and the most sensitive cultivar was 'More Red × Thailand'. These results demonstrated that the three indicators of free proline, MDA and chlorophyll could be used to determine cold resistance in Denphal-group *Dendrobium* cultivars.

Keywords

cold resistance, comprehensive assessment, Denphal-group *Dendrobium*, membership function method, physiological indicators

Introduction

Dendrobium is among the largest genera in flowering plants, with more than 1,200 identified species throughout the world, most of which grow as epiphytes in tropical and subtropical Asia and eastern Australia (Wang et al., 2009). Interest in *Dendrobium* species is broad, ranging from traditional medicine to ornamental horticulture. In recent years,

Significance of this study

What is already known on this subject?

- Denphal-group *Dendrobium* is original in tropical and subtropical regions; low temperature is a key limitation of the expanding cultivation area and inhibiting the industrial development. Selecting and breeding high cold tolerance cultivars is an important method to solve the problem. Comprehensive assessment systems are widely used to assess plant tolerance to abiotic stresses.

What are the new findings?

- The physiology indicators of free proline, MDA and chlorophyll were the most significant factors affecting cold resistance of Denphal-group *Dendrobium* cultivars, and could be used to determine cold resistance in Denphal-group *Dendrobium* cultivars. The most cold-resistant cultivar was 'Sophie Bonnie', and the most sensitive cultivar to low temperature was 'More Red × Thailand' of 35 detected cultivars.

What is the expected impact on horticulture?

- The comprehensive assessment system is useful for Denphal-group *Dendrobium* germplasm resources cold resistance evaluation. The high cold resistance cultivars could be a potential parent for cold resistance cultivars breeding and expanding cultivation area of Denphal-group *Dendrobium*.

Dendrobium orchids have become increasingly popular for ornamental use due to their floriferous nature and availability in a wide range of colors, sizes, and shapes. Compared with other flowering plants, *Dendrobium* flowers also have a relatively long life, lasting several weeks to months.

Although a large number of morphological variations exist in *Dendrobium*, there are two groups in the genus suited for ornamental sale: the Nobile-group and the Denphal-group. The Nobile-group produces inflorescences and flowers that are distributed along the pseudobulbs, and the Denphal-group produces one or more terminal inflorescences from the pseudobulbs (Cardoso, 2012).

Commercial cultivation of Denphal-group *Dendrobium* is relatively recent in China and some subtropical regions,

and Denphal-group cultivars breeding is started late. Thus, Denphal-group *Dendrobium* cultivars are mostly imported from Thailand or Taiwan, where temperatures are high year-round and are suitable for Denphal-group cultivar growth (Gong et al., 2007). In China and some subtropical regions, however, it becomes cold in the winter, which can cause low temperature damage to plants, including leaves yellowed rapidly, leaf and flower senescence, growth retardation, etc. (He et al., 2016). Due to these challenges, it is necessary to select and breed high cold-tolerant cultivars for China and the subtropical regions having cold winters. However, we are not aware of any research on cold resistance in Denphal-group *Dendrobium* in these regions.

The aim of this study was to establish a comprehensive assessment system for cold resistance in Denphal-group *Dendrobium* cultivars, and then to select the cultivars with high cold tolerance for cultivation and high cold tolerance cultivar breeding.

Materials and methods

Plant materials

Preliminary evaluation of cold resistance in Denphal-group *Dendrobium* cultivars in the field (under natural low temperature) found that the cultivar *Dendrobium* 'Udomsri Beauty' had excellent cold resistance, and 'Sonia Hiasakul' was sensitive to low temperatures (He et al., 2016). Based on these preliminary results, 'Udomsri Beauty' and 'Sonia Hiasakul' were selected to measure the physiological indicators of cold resistance, as well as to establish a comprehensive cold resistance assessment system. The cold resistance of 35 Denphal-group *Dendrobium* cultivars (including 'Udomsri Beauty' and 'Sonia Hiasakul') were evaluated by the comprehensive cold resistance assessment system.

Methods

Mature seedlings of 'Udomsri Beauty' and 'Sonia Hiasakul' were placed in a plant growth chamber at 15°, 10° and 5°C with 85% humidity and a 14/10 h light/dark period. The tips of fully extended leaves of three plants were collected at 0, 1, 2, 4, 8, 24 h and 2, 4, 8 16-day periods and soluble protein, soluble sugar, malondialdehyde (MDA), free proline, and chlorophyll were measured. The soluble protein concentration was prepared using the Coomassie Brilliant Blue G-250 staining method (Li, 2000), and the anthrone method was used to determine soluble sugar concentration (Li, 2000). MDA, free proline, and the chlorophyll concentrations were determined based on the methods of Li (Li, 2000). Each treatment and measurement played three replicates.

Additionally, mature seedlings of 'Udomsri Beauty' and 'Sonia Hiasakul' were placed in a plant growth chamber at 10°, 5°, 0° and -5°C with 85% humidity and a 14/10 h light/

dark period. After 12 h, tips of fully extended leaves of three plants were collected, and the relative electric conductivity (REC) of electrolyte leakage, as well as the semi-lethal temperature (LT50), were determined using the methods outlined by Li (Li, 2000). Each treatment and measurement played three replicates.

Statistics

The mean, standard deviation, significance analysis, correlation analysis, and principal component analysis were all calculated using Duncan's multiple range test (Duncan, 1955) using SPSS 20.0 (SPSS Inc., Chicago, Illinois). Indicators with a significant correlation were extracted to calculate the contribution rate (C_i) and weight (W_i) with principal component analysis. These above processes were also achieved by SPSS 20.0.

A slightly modified membership function method was used as a comprehensive evaluation of the 35 Denphal-group *Dendrobium* cultivars, as outlined by Zhang (Zhang et al., 2012) and Wang (Wang et al., 2014). If the correlation between the index and LT50 was positive, the specific membership function value was calculated using Equation 1:

$$X_{ij} = (X - X_{\min}) / (X_{\max} - X_{\min}).$$

If the correlation between the index and LT50 was negative, the specific membership function values were calculated using Equation 2:

$$X_{ij} = (X_{\max} - X) / (X_{\max} - X_{\min}),$$

where X is the value of character j for cultivar i , $X_{\min}$ is the minimum value of character j among all measurements of cultivar i , $X_{\max}$ is the maximum value of character j among all measurements of cultivar i , and X_{ij} is the membership value of cold resistance of cultivar i and character j . For each cultivar, the integrated index (I) was calculated using the multiplication and addition Equation 3:

$$I = \sum (W_i \times X_{ij}).$$

The value of 'I' denoted the level of cold resistance, with higher cultivar values indicating increased resistance.

Results

Optimum temperature and treatment period selection

The concentrations of soluble protein, soluble sugar, MDA, free proline, and chlorophyll of the two cultivars were shown in Supplemental Tables 1–5. Soluble protein tended to rise, fall, and then rise again in 'Sonia Hiasakul' and 'Udomsri Beauty', with the processing time in same temperature.

TABLE 1. Correlation coefficient matrix of *Dendrobium* 'Sonia Hiasakul' under low temperature stress.

	LT50	Soluble protein	Soluble sugar	Chlorophyll	Free proline	MDA
LT50		0.395	0.633	-0.880**	0.947**	0.958**
Soluble protein	0.449		-0.212	-0.719**	0.338	0.500
Soluble sugar	0.875**	0.523		0.357	0.744**	0.479
Chlorophyll	-0.868**	-0.485	-0.927**		-0.872**	-0.953**
Free proline	0.947**	0.610	0.906**	-0.833*		0.930**
MDA	0.925**	0.623	0.885**	-0.919**	0.954**	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 2. Correlation coefficient matrix of *Dendrobium* 'Udomsri Beauty' under low temperature stress.

	LT50	Soluble protein	Soluble sugar	Chlorophyll	Free proline	MDA
LT50		0.066	-0.371	-0.983**	0.985**	0.770**
Soluble protein	-0.556		-0.217	-0.053	0.046	0.077
Soluble sugar	0.650	-0.299		0.227	-0.370	0.072
Chlorophyll	-0.967**	0.542	-0.642		-0.891*	-0.980**
Free proline	0.885**	-0.554	0.597	-0.966*		0.758*
MDA	0.800**	-0.470	0.394	-0.917**	0.949**	

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

TABLE 3. Total variance explained of each variety of Denphal-group *Dendrobium* cultivars.

Component	Characteristic vector		
	Eigenvalue	Contributiveness (%)	Cumulative (%)
1	2.569	51.386	51.386
2	1.837	36.741	88.127
3	0.448	9.762	97.889
4	0.106	2.111	100.000

TABLE 4. Capacity and weight of cold resistance indicator of Denphal-group *Dendrobium* cultivars.

Index	Load		$F_i \times Y_1 + F_i \times Y_2$	Weight
	The first principal component	The second principal component		
Chlorophyll	0.875	0.028	0.4599	0.1599
Free proline	0.872	-0.45	0.6132	0.2132
MDA	-0.559	-0.805	0.6166	0.2144
LT50	0.570	0.672	0.5398	0.1877

The concentration of soluble sugar, MDA, and free proline increased with processing time in both cultivars, and chlorophyll decreased with processing time. All indicators showed a significant difference to control at 5°C for 8 d, thus, the critical treatment temperature and period appears to be 5°C and 8 d.

Correlation analysis of cold resistance indicators

The correlation analysis of six cold hardiness indicators (Table 1) found that for 'Sonia Hiasakul', soluble sugar, MDA, and free proline were positively correlated with LT50 ($p \leq 0.01$), and that chlorophyll was negatively correlated with LT50, soluble protein, MDA ($p \leq 0.01$) and free proline ($p \leq 0.05$). For 'Udomsri Beauty', MDA and free proline were positively correlated with LT50 ($p \leq 0.01$) and chlorophyll was negatively correlated with LT50 ($p \leq 0.01$). These results showed that the correlations between LT50 and MDA, free proline, and chlorophyll were conspicuous in both 'Sonia Hiasakul' and 'Udomsri Beauty'. Thus, using MDA, free proline and chlorophyll as indicators to assess cold resistance should provide a more accurate and robust assessment of cold resistance of Denphal-group *Dendrobium* cultivars.

Principal component analysis of cold resistance indices

The results from principal component analysis showed that the contribution rates of the two principal components were about 65.25% and 20.76% (Table 2), and that the cumulative contribution rate reached 86.01%, accounting for 86.01% of the cold resistance of included Denphal-group *Dendrobium* cultivars. The values of the eigenvector of principal components were 3.26, 1.04, 0.50 and 0.20, respectively.

Results show that only the first and second eigenvector values in the principal components were greater than 1. Thus, the first and second principal components were extracted for subsequent studies. The weight (W_i) of LT50, MDA, free proline, and chlorophyll were calculated by the capacity (F) and contribution value (Y) of each index in the first and second principal components. Result showed that the most significant factor affecting cold resistance was free proline, followed by MDA, and lastly, by chlorophyll.

Comprehensive assessment of cold resistance of Denphal-group *Dendrobium* cultivars

A comprehensive assessment of the cold resistance of cultivars was conducted using the index membership value and index weight of each cultivar. The results showed that the 35 Denphal-group *Dendrobium* cultivars could be divided into seven classes according to membership value (Table 4). The highest value of 0.818 represented one cultivar, 'Sophie Bonnie'. The second class contained five cultivars and values ranged from 0.700 and 0.799. The third class contained six cultivars and values ranged from 0.600 and 0.699. The fourth class contained 11 cultivars and values were between 0.500 and 0.599. The fifth and sixth classes contained seven and three cultivars, respectively, and values ranged from 0.400 to 0.499 and from 0.300 to 0.399, respectively.

The comprehensive assessment showed that the 35 Denphal-group *Dendrobium* cultivars could be divided into six classes (Table 5). The strongest cold resistance class had an index value above 0.600 and contained two cultivars, 'Sophie

Bonnie' and 'Salaya Pink'. The second strongest cold resistant class had index values from 0.500 to 0.599 and contained seven cultivars. The medium cold resistance class had index values from 0.400 to 0.499 and contained thirteen cultivars. Index values from 0.300 to 0.399 contained eight cultivars and were considered to have low cold resistance. The class with weak cold resistance had index values from 0.200 to 0.299 and was composed of four cultivars. Lastly, the least cold resistance class produced index values less than 0.200 and represented only one cultivar, 'More Red × Thailand'.

Discussion

Plant adaption to cold stress is a gradual process of morphological, physiological, and biochemical variation accompanied by the accumulation of particular substances such as soluble sugars, soluble protein, free proline, unsaturated fatty acids, and membrane lipid peroxide (Gusta et al., 2003; Strand et al., 1997; Wang, 1987; Zhang et al., 2012). However, the factors affecting plant cold resistance are mul-

tifaceted, and the physiological process of plant adaptation to cold stress is extremely complex. Thus, evaluating plant cold resistance by a single index or multiple separate indicators may cause the one-sidedness and deviation. A comprehensive assessment which considers multiple indicators is better able to account for the cold resistance of plants. Such assessments have been used in other crops, such as grape (Zhang et al., 2012), herbaceous peony (Wang et al., 2014), hybrid hazelnut (Lv et al., 2008), Cotton (Wu et al., 2012), *Zenia insignis* (Liu et al., 2007), chrysanthemum (Xu et al., 2009), apricot (Ai et al., 2013), etc.

Under cold stress, soluble sugars can function as protective substances which increase the osmotic potential of the cytoplasm and lower the freezing temperature, and are positively correlated with cold hardiness (Gusta et al., 2003; Wang, 1987; Zhang et al., 2012). Our study showed that soluble sugars were accumulated in leaves of Denphal-group *Dendrobium* cultivars under low temperature, and were significantly correlated with LT50 in cold sensitive cultivar

TABLE 5. Average membership degree of cold resistance indicators of Denphal-group *Dendrobium* cultivars.

Cultivars	Chlorophyll	MDA	Free proline	Average membership degree
<i>Dendrobium</i> Sophie Bonnie	0.6226	0.8883	0.9421	0.8177
<i>Dendrobium</i> Salaya Pink	0.6358	0.8834	0.7860	0.7684
<i>Dendrobium</i> Enobi Purple	0.5912	1.0000	0.6520	0.7477
<i>Dendrobium</i> Siriratana × <i>D. Chao Praya</i> Gem	1.0000	0.2343	0.9623	0.7322
<i>Dendrobium</i> Burana Sundae × <i>D. Black Cat</i>	0.5673	0.7215	0.8323	0.7070
<i>Dendrobium</i> Bigibbum	0.4309	0.9987	0.6818	0.7038
<i>Dendrobium</i> Salaya × <i>D. Sriprathum</i> Red	0.4377	0.8975	0.6417	0.6590
<i>Dendrobium</i> Madame Lucy	0.5872	0.8003	0.5446	0.6440
<i>Dendrobium</i> New Stripes Delite	0.4837	0.8613	0.5587	0.6346
<i>Dendrobium</i> X1	0.2660	0.8126	0.7893	0.6226
<i>Dendrobium</i> Marilyn Monroe	0.3435	0.6620	0.8209	0.6088
<i>Dendrobium</i> Burana Green	0.6564	0.2546	0.8985	0.6032
<i>Dendrobium</i> Burana Charming × <i>D. Earsakul</i>	0.5729	0.6937	0.5273	0.5980
<i>Dendrobium</i> Lady Lisa	0.5859	0.8387	0.3656	0.5967
<i>Dendrobium</i> Udomsri Beauty	0.4954	0.6395	0.6305	0.5884
<i>Dendrobium</i> Burana Stripe	0.3527	0.7698	0.6398	0.5874
<i>Dendrobium</i> Nopporn White Diamond	0.4936	0.8201	0.3789	0.5642
<i>Dendrobium</i> Rainbow Compactum	0.3189	0.7755	0.5827	0.5591
<i>Dendrobium</i> Sirin Peach	0.3281	0.5369	0.7750	0.5467
<i>Dendrobium</i> X2	0.5224	0.7195	0.3884	0.5434
<i>Dendrobium</i> Sirin Peach	0.3300	0.6471	0.6104	0.5292
<i>Dendrobium</i> New Aridang Blue Mutation	0.5220	0.4828	0.5616	0.5221
<i>Dendrobium</i> NPR863	0.5331	0.6201	0.3525	0.5019
<i>Dendrobium</i> Angel Blue	0.4319	0.5824	0.4584	0.4909
<i>Dendrobium</i> NPR727	0.3324	0.5145	0.6046	0.4839
<i>Dendrobium</i> Popeye	0.4884	0.6901	0.2481	0.4755
<i>Dendrobium</i> Aridang	0.4537	0.5661	0.3662	0.4620
<i>Dendrobium</i> Mary Green	0.2832	0.6682	0.4241	0.4585
<i>Dendrobium</i> Liberty White	0.4105	0.6254	0.2945	0.4435
<i>Dendrobium</i> Sonia Hiasakul	0.5148	0.6559	0.1327	0.4345
<i>Dendrobium</i> NPR844	0.6562	0.4404	0.0913	0.3959
<i>Dendrobium</i> NPR699	0.5994	0.0080	0.5777	0.3950
<i>Dendrobium</i> Sunny Red	0.2730	0.3679	0.3753	0.3387
<i>Dendrobium</i> Burana Jade	0.0000	0.0114	0.7621	0.2578
<i>Dendrobium</i> More Red × <i>D. Thailand</i>	0.2966	0.0000	0.4737	0.2568

'Sonia Hiasakul', a finding which has also been reported in grape (Cao et al., 2010; Zhang et al., 2012), *Zenia insignis* (Liu et al., 2007), *Cymbidium hybridum* (Zhou et al., 2017), chewing cane cultivars (Chen et al., 2018), and peach (Feng et al., 2018). However, the soluble sugars showed no significant correlation with LT50 in the cold tolerant cultivar 'Udomsri Beauty', and was similar to herbaceous peony (Wang et al., 2014), azalea cultivars (Liu et al., 2016) and *Ilex centrochinensis* (Hu et al., 2018). This observation indicates that increases in the soluble sugar content is a low temperature stress response and can be used to evaluate cold resistance in some plants, but not all.

Soluble protein content also increases under cold stress, due to increased rates of synthesis or the degradation of high 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. The concentrations of soluble proteins are strongly associated and both tend to increase the water-retention capacity

of the protoplast (Dionne et al., 2001). Our study showed that under cold stress, soluble proteins accumulated in the leaves of cultivars, but showed no significant correlation with LT50, similar to research with azalea (Liu et al., 2016), pineapple (Lu et al., 2010), potato (Yang and Guo, 2016), *Prunus domestica* (Abula et al., 2017).

MDA is the membranous peroxidation product in the organ; when the plant organs are undergoing adverse conditions, its accumulation can reflect the response strength of the plants to adverse conditions (Tabassum et al., 2014). The accumulation of MDA is considered to reflect the physiological state of plant membrane lipids under cold stress (Imahori et al., 2008). Our study showed that MDA significantly correlated with LT50 in the Denphal-group *Dendrobium* cultivars, that is consistent with studies with most plants, including peach (Feng et al., 2018), *Ilex centrochinensis* (Hu et al., 2018), chewing cane cultivars (Chen et al., 2018), *Cymbidium hybridum* (Zhou et al., 2017), herbaceous peony (Wang et al., 2014), grape (Cao et al., 2010; Zhang et al., 2012). Moreover,

TABLE 6. Integrated index of cold resistance indicator of Denphal-group *Dendrobium* cultivars.

Cultivars	Chlorophyll	MDA	Free proline	Integrated index
<i>Dendrobium</i> Sophie Bonnie	0.1412	0.2491	0.2525	0.6428
<i>Dendrobium</i> Salaya Pink	0.1442	0.2478	0.2107	0.6026
<i>Dendrobium</i> Enobi Purple	0.1341	0.2805	0.1747	0.5893
<i>Dendrobium</i> Bigibbum	0.0977	0.2801	0.1827	0.5606
<i>Dendrobium</i> Burana Sundae × <i>D.</i> Black Cat	0.1286	0.2024	0.2231	0.5541
<i>Dendrobium</i> Siriratana × <i>D.</i> Chao Praya Gem	0.2268	0.0657	0.2579	0.5504
<i>Dendrobium</i> Salaya × <i>D.</i> Sriprathum Red	0.0993	0.2517	0.1720	0.5230
<i>Dendrobium</i> Madame Lucy	0.1332	0.2245	0.1459	0.5036
<i>Dendrobium</i> New Stripes Delite	0.1097	0.2416	0.1497	0.5010
<i>Dendrobium</i> X1	0.0603	0.2279	0.2115	0.4998
<i>Dendrobium</i> Marilyn Monroe	0.0779	0.1857	0.2200	0.4836
<i>Dendrobium</i> Burana Stripe	0.0800	0.2159	0.1715	0.4674
<i>Dendrobium</i> Lady Lisa	0.1328	0.2352	0.0980	0.4660
<i>Dendrobium</i> Burana Charming × <i>D.</i> Earsakul	0.1299	0.1946	0.1413	0.4658
<i>Dendrobium</i> Burana Green	0.1488	0.0714	0.2408	0.4611
<i>Dendrobium</i> Udomsri Beauty	0.1123	0.1794	0.1690	0.4607
<i>Dendrobium</i> Rainbow Compactum	0.0723	0.2175	0.1562	0.4460
<i>Dendrobium</i> Nopporn White Diamond	0.1119	0.2300	0.1016	0.4435
<i>Dendrobium</i> Sirin Peach	0.0744	0.1506	0.2077	0.4327
<i>Dendrobium</i> X2	0.1185	0.2018	0.1041	0.4243
<i>Dendrobium</i> Nopporn Pink	0.0748	0.1815	0.1636	0.4199
<i>Dendrobium</i> New Aridang Blue Mutation	0.1184	0.1354	0.1505	0.4043
<i>Dendrobium</i> NPR863	0.1209	0.1739	0.0945	0.3893
<i>Dendrobium</i> Angel Blue	0.0979	0.1633	0.1229	0.3842
<i>Dendrobium</i> NPR727	0.0754	0.1443	0.1620	0.3817
<i>Dendrobium</i> Popeye	0.1107	0.1935	0.0665	0.3708
<i>Dendrobium</i> Mary Green	0.0642	0.1874	0.1137	0.3653
<i>Dendrobium</i> Aridang	0.1029	0.1588	0.0981	0.3598
<i>Dendrobium</i> Liberty White	0.0931	0.1754	0.0789	0.3474
<i>Dendrobium</i> Sonia Hiasakul	0.1167	0.1840	0.0356	0.3363
<i>Dendrobium</i> NPR844	0.1488	0.1235	0.0245	0.2968
<i>Dendrobium</i> NPR699	0.1359	0.0022	0.1548	0.2930
<i>Dendrobium</i> Sunny Red	0.0619	0.1032	0.1006	0.2657
<i>Dendrobium</i> Burana Jade	0.0000	0.0032	0.2043	0.2075
<i>Dendrobium</i> More Red × <i>D.</i> Thailand	0.0673	0.0000	0.1270	0.1942

weight analysis showed that MDA was the second most significant factor affecting cold resistance, suggesting that it can be used as an effective indicator for the cold resistance evaluation of Denphal-group *Dendrobium* cultivars.

Free proline can help maintain osmotic equilibrium between the symplast and apoplast in plant cells, and thus, proline aids in preventing low-temperature damage by maintaining the functional integrity of the membrane (Dionne et al., 2001). Therefore, under cold stress, cold-resistant plants would accumulate free proline more than the less resistant ones. In our study, the content of free proline increased under low temperature and was significantly correlated with LT50. We showed that free proline was the most significant factor affecting cold resistance in cultivars, a conclusion that has also been reached in experiments with peach (Feng et al., 2018), grape (Cao et al., 2010; Zhang et al., 2012), herbaceous peony (Wang et al., 2014). Our research indicated that free proline responds to low temperatures, and was an effective indicator for cold resistance evaluation of Denphal-group *Dendrobium* cultivars.

Chloroplasts are extremely sensitive to low temperature reactions and under low temperature, the chloroplasts ultrastructure is damaged, causing photosynthetic pigment degradation and a decline in the chlorophyll content. The variation of chlorophyll content is an important indicator of crop tolerance to cold. Our research showed that the chlorophyll content of Denphal-group *Dendrobium* cultivars declines under low temperatures. Moreover, chlorophyll content was significantly correlated with the LT50, a relationship that had also been shown in flue-cured tobacco varieties (Chen et al., 2008) and *Ilex centrochinensis* (Hu et al., 2018). In addition, the principal component analysis and the weight analysis we conducted shows that chlorophyll was the third most significant factor affecting cold resistance in Denphal-group *Dendrobium* cultivars. Therefore, chlorophyll also could be used as an effective indicator for cold resistance evaluation of Denphal-group *Dendrobium* cultivars.

In conclusion, our results showed that free proline, MDA, and chlorophyll are important indicators of cold resistance in Denphal-group *Dendrobium* cultivars. Furthermore, we showed that soluble sugar and soluble proteins respond to cold stress, but due to variation among cultivars, were not suitable to be used as a cold resistance evaluation indicator for Denphal-group *Dendrobium* cultivars.

We provided evidence of variation in cold tolerance among Denphal-group *Dendrobium* cultivars. Our data offered a valuable resource for cold resistance breeding in Denphal-group *Dendrobium*, and our results indicated that the cultivar 'Sophie Bonnie' had the most cold resistance, followed by 'Salaya Pink', 'Enobi Purple', and 'Bigibbum'. The hybrids 'Burana Sundae × Black Cat', 'Siriratana' × 'Chao Praya Gem', and 'Salaya × Sriprathum Red' also had high cold resistance. These cultivars were the important resources for Denphal-group *Dendrobium* cold resistance cultivar breeding.

Conclusion

Under our experimental conditions, free proline, malondialdehyde, and chlorophyll were significantly correlated with LT50 at 0.05 level, and were the most significant physiology indicators affecting cold resistance between soluble sugar, soluble protein, free proline, malondialdehyde, chlorophyll. Within 35 cultivars, the most cold-resistant cultivar was 'Sophie Bonnie', and the most sensitive cultivar was 'More Red × Thailand'.

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL INFORMATION – TABLE S1. Soluble protein concentration ($\mu\text{g g}^{-1}$) of leaves of Denphal-group *Dendrobium* cultivars exposed to different temperatures and different periods.

Cultivars	Temperature (°C)	Time									
		0 h	1 h	2 h	4 h	8 h	1 d	2 d	4 d	8 d	16 d
<i>Dendrobium</i> Sonia Hiasakul	15	214.29±7.82 abc/a	153.05±3.14 cde/a	231.88±7.37 a/a	151.58±6.41 de/b	131.06±4.83 d/b	120.86±5.01 d/a	159.56±7.53 bcde/ab	204.30±2.88 ab/a	181.13±7.07b cd/a	246.80±10.84 a/a
	10	214.29±7.82 bcd/a	161.64±4.96 cdef/a	211.18±3.28 bc/a	230.08±5.87 b/a	185.38±2.69 bcde/a	142.87±2.16 ef/a	124.28±7.42 f/b	146.03±5.04 ef/b	158.60±7.91 def/a	299.86±9.69 a/a
	5	214.29±7.82 ab/a	158.40±5.39 abc/a	204.28±8.32 ab/a	149.07±7.99 abc/b	135.81±4.45 bc/b	125.59±5.20 c/a	173.20±3.08 abc/a	173.43±7.82 abc/a	171.45±4.75 abc/a	216.20±7.45 a/ab
<i>Dendrobium</i> Udomsri Beauty	15	192.22±7.63 b/a	196.34±3.35 b/a	251.64±11.36 ab/ab	235.09±5.94 ab/a	267.60±1.85 ab/bc	242.32±8.39 ab/a	269.54±4.30 ab/ab	278.22±6.49 ab/a	231.47±7.54 ab/ab	306.53±4.25 a/a
	10	192.22±7.63 c/a	244.61±3.29 abc/a	277.50±6.10 ab/a	217.76±10.99 bc/a	297.89±7.98 a/ab	223.44±5.66 bc/a	246.99±10.08 abc/ab	277.12±6.23 ab/a	244.94±7.46 abc/a	260.84±6.20 abc/ab
	5	192.22±7.63 c/a	242.71±5.80 bc/a	294.84±5.34 abc/a	213.12±6.74 bc/a	371.88±5.72 a/a	287.76±8.64 abc/a	304.23±4.91 ab/a	295.40±8.88 abc/a	229.83±7.18 bc/ab	294.22±4.53 abc/a

Different letters in front of ' ' indicate significant differences between differences treatment time at the same temperature according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ (within a column) while different letters behind ' ' indicate significant differences between temperatures within the same treatment time according to DMRT at $P \leq 0.05$ (within a row).

SUPPLEMENTAL INFORMATION – TABLE S2. Soluble Sugar concentration (%) of leaves of Denphal-group *Dendrobium* cultivars exposed to different temperatures and different periods.

Cultivars	Temperature (°C)	Time									
		0 h	1 h	2 h	4 h	8 h	1 d	2 d	4 d	8 d	16 d
<i>Dendrobium</i> Sonia Hiasakul	15	0.414±0.077 bc/a	0.479±0.056 bc/a	0.500±0.007 bc/ab	0.354±0.004 c/b	0.448±0.051 bc/b	0.479±0.021 bc/b	0.490±0.050 bc/bc	0.526±0.049 bc/b	0.601±0.044 ab/bc	0.763±0.031 a/a
	10	0.414±0.077 c/a	0.438±0.065 bc/a	0.569±0.018 abc/ab	0.555±0.013 abc/b	0.599±0.015 abc/ab	0.618±0.010 abc/ab	0.584±0.029 abc/b	0.642±0.026 abc/ab	0.675±0.039 ab/b	0.781±0.017 a/a
	5	0.414±0.077 b/a	0.406±0.051 b/a	0.669±0.073 b/a	0.838±0.017 a/a	0.879±0.040 a/a	0.972±0.035 a/a	0.943±0.055 a/a	0.838±0.019 a/a	0.907±0.089 a/a	0.998±0.037 a/a
<i>Dendrobium</i> Udomsri Beauty	15	0.385±0.024 d/a	0.259±0.053 d/b	0.374±0.030 d/a	0.288±0.022 d/b	0.258±0.037 d/c	0.291±0.026 d/c	0.509±0.047 c/a	0.586±0.031 c/a	0.908±0.137 b/a	1.197±0.133 a/b
	10	0.385±0.024 f/a	0.361±0.045 f/a	0.423±0.018 ef/a	0.554±0.098 cd/b	0.606±0.047 bc/a	0.514±0.019 cde/a	0.496±0.023 de/a	0.674±0.104 b/a	0.433±0.084 ef/b	0.939±0.033 a/a
	5	0.385±0.024 ab/a	0.390±0.045 ab/a	0.355±0.072 ab/a	0.488±0.078 a/b	0.373±0.013 ab/b	0.279±0.064 b/c	0.352±0.027 ab/b	0.321±0.028 ab/b	0.269±0.031 b/c	0.383±0.056 ab/c

Different letters in front of ' ' indicate significant differences between differences treatment time at the same temperature according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ (within a column) while different letters behind ' ' indicate significant differences between temperatures within the same treatment time according to DMRT at $P \leq 0.05$ (within a row).

SUPPLEMENTAL INFORMATION – TABLE S3. Chlorophyll concentration ($\mu\text{g g}^{-1}$) of leaves of Denphal-group *Dendrobium* cultivars exposed to different temperatures and different periods.

Cultivars	Time										
	Temperature (°C)	0 h	1 h	2 h	4 h	8 h	1 d	2 d	4 d	8 d	16 d
<i>Dendrobium</i> Sonia Hiasakul	15	0.464±0.030 a/a	0.401±0.093 ab/a	0.396±0.028 ab/b	0.376±0.016 b/a	0.377±0.016 b/ab	0.371±0.070 b/a	0.360±0.072 b/a	0.373±0.004 b/a	0.353±0.013 b/a	0.348±0.029 b/a
	10	0.464±0.030 a/a	0.381±0.077 ab/a	0.408±0.029 ab/ab	0.385±0.040 ab/a	0.351±0.076 b/b	0.370±0.044 b/a	0.368±0.019 b/a	0.355±0.034 b/a	0.357±0.021 b/a	0.316±0.065 b/b
	5	0.464±0.030 a/a	0.396±0.042 abc/a	0.373±0.031 bcd/b	0.405±0.018 abc/a	0.393±0.069 abc/ab	0.396±0.067 abc/a	0.368±0.019 bcd/a	0.331±0.029 bcd/a	0.302±0.030 cd/b	0.253±0.054 d/c
<i>Dendrobium</i> Udomsri Beauty	15	0.389±0.059 a/a	0.376±0.069 a/a	0.380±0.070 a/a	0.397±0.096 a/a	0.380±0.073 a/a	0.381±0.056 a/a	0.390±0.039 a/a	0.394±0.023 a/a	0.368±0.045 a/ab	0.320±0.043 a/a
	10	0.389±0.059 ab/a	0.401±0.047 ab/a	0.397±0.058 ab/a	0.408±0.050 a/a	0.375±0.015 ab/a	0.388±0.056 ab/a	0.363±0.051 ab/a	0.361±0.009 ab/ab	0.339±0.033 ab/ab	0.315±0.036 b/a
	5	0.389±0.059 abcd/a	0.458±0.028 a/a	0.434±0.061 ab/a	0.440±0.099 ab/a	0.434±0.036 ab/a	0.401±0.078 abc/a	0.346±0.037 bcd/a	0.316±0.037 cd/b	0.294±0.010 d/b	0.141±0.011 e/b

Different letters in front of 'i' indicate significant differences between differences treatment time at the same temperature according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ (within a column) while different letters behind 'j' indicate significant differences between temperatures within the same treatment time according to DMRT at $P \leq 0.05$ (within a row).

SUPPLEMENTAL INFORMATION – TABLE S4. Free Proline concentration ($\mu\text{g g}^{-1}$) of leaves of Denphal-group *Dendrobium* cultivars exposed to different temperatures and different periods.

Cultivars	Time										
	Temperature (°C)	0 h	1 h	2 h	4 h	8 h	1 d	2 d	4 d	8 d	16 d
<i>Dendrobium</i> Sonia Hiasakul	15	6.20±0.08 d/a	7.80±0.45 cd/a	8.44±0.79 bcd/b	9.90±0.21 bcd/ab	10.05±0.88 bcd/b	10.67±0.48 bcd/b	10.33±0.34 bcd/b	11.13±0.47 abc/b	12.48±0.66 ab/c	15.01±0.56 a/b
	10	6.20±0.08 e/a	8.32±0.27 de/a	11.75±0.56 cd/a	11.61±0.68 cd/a	14.77±0.57 bc/a	15.53±0.29 bc/a	14.80±0.47 bc/a	18.27±0.64 b/a	22.60±0.52 a/a	25.02±0.58 a/a
	5	6.20±0.08 g/a	8.55±0.43 f/a	7.88±0.33 f/b	9.94±0.99 f/ab	12.09±0.59 e/ab	14.58±0.51 cd/a	13.94±0.60 de/a	16.36±0.82 bcd/a	18.28±0.76 b/b	23.21±0.27 a/a
<i>Dendrobium</i> Udomsri Beauty	15	4.34±0.33 e/a	5.97±0.55 de/a	6.69±0.59 d/a	7.33±0.61 cd/a	8.64±0.14 bc/a	8.62±0.30 bc/a	8.67±0.39 bcd/b	9.66±0.87 b/b	10.05±0.71 b/c	13.71±0.61 a/a
	10	4.34±0.33 d/a	4.99±0.41 cd/ab	5.21±0.36 cd/b	6.65±0.77 cd/a	7.09±0.67 cd/a	8.53±0.48 c/a	11.08±0.49 b/a	16.27±0.61 a/a	17.38±0.27 a/b	18.77±0.30 a/a
	5	4.34±0.33 c/a	5.39±0.78 c/ab	5.32±0.33 c/b	5.40±0.58 c/b	6.88±0.33 bc/ab	7.21±0.22 bc/a	8.19±0.54 bcd/b	11.83±0.72 b/b	20.44±0.25 a/a	22.21±1.14 a/a

Different letters in front of 'i' indicate significant differences between differences treatment time at the same temperature according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ (within a column) while different letters behind 'j' indicate significant differences between temperatures within the same treatment time according to DMRT at $P \leq 0.05$ (within a row).

SUPPLEMENTAL INFORMATION – TABLE S5. MDA concentration ($\mu\text{g g}^{-1}$) of leaves of Denphal-group *Dendrobium* cultivars exposed to different temperatures and different periods.

Cultivars	Temperature (°C)	Time									
		0 h	1 h	2 h	4 h	8 h	1 d	2 d	4 d	8 d	16 d
<i>Dendrobium</i> Sonia Hiasakul	15	2.89±0.42 b/a	4.67±0.23 a/a	4.96±0.41 a/a	5.38±0.84 a/a	5.66±0.27 a/a	5.91±0.35 a/a	5.42±0.33 a/a	6.11±0.45 a/a	6.13±0.99 a/a	6.42±0.98 a/a
	10	2.89±0.42 d/a	4.10±0.04 c/ab	4.22±0.13 c/a	4.09±0.17 c/b	4.55±0.52 c/ab	5.11±0.29 bc/b	5.83±0.68 ab/a	5.96±0.55 ab/a	5.74±0.15 ab/a	6.33±0.35 a/a
	5	2.89±0.42 d/a	3.54±0.75 cd/bc	3.93±0.22 cd/b	3.81±0.24 cd/bc	4.16±0.30 c/ab	4.29±0.58 c/c	4.50±0.04 bc/ab	5.49±0.74 ab/a	5.98±1.05 a/a	6.25±0.91 a/a
<i>Dendrobium</i> Udomsri Beauty	15	3.87±0.33 abc/a	3.00±0.80 bc/bc	4.12±0.17 ab/b	3.81±0.32 abc/b	3.85±0.33 abc/a	2.72±0.19 c/c	4.49±0.33 a/b	4.13±0.33 ab/b	5.12±0.43 a/b	5.15±0.81 a/b
	10	3.87±0.33 cd/a	5.71±0.61 a/a	4.56±0.95 bc/ab	4.04±0.19 cd/b	4.21±0.28 cd/a	2.61±0.45 e/c	3.38±0.24 de/c	3.58±0.13 d/b	5.26±0.54 ab/b	5.27±0.22 ab/b
	5	3.87±0.33 c/a	5.27±0.18 b/ab	5.50±0.94 b/a	5.70±0.32 b/a	3.90±0.57 c/a	5.71±0.20 b/a	5.60±0.041 b/a	5.44±0.32 b/a	6.13±0.50 b/a	14.03±0.84 a/a

Different letters in front of ' ' indicate significant differences between differences treatment time at the same temperature according to Duncan's multiple range test (DMRT) at $P \leq 0.05$ (within a column) while different letters behind ' ' indicate significant differences between temperatures within the same treatment time according to DMRT at $P \leq 0.05$ (within a row).

SUPPLEMENTAL INFORMATION – TABLE S6. Logistic equation fitting variance parameters and LT50 of *Dendrobium* accessions.

Cultivars	Parameter equation		LT50	R2
	a	b		
<i>Dendrobium</i> Sophie Bonnie	1.5487	-0.2139	-2.0452	0.961*
<i>Dendrobium</i> Salaya Pink	1.2655	-0.2064	-1.1408	0.951*
<i>Dendrobium</i> Enobi Purple	1.1757	-0.1535	-1.0545	0.972*
<i>Dendrobium</i> Bigibbum	1.2382	-0.2135	-1.0006	0.967*
<i>Dendrobium</i> Salaya × <i>D. Sripuratum</i> Red	1.1903	-0.1773	-0.9823	0.899*
<i>Dendrobium</i> Burana Sundae × <i>D. Black Cat</i>	1.1314	-0.1579	-0.7819	0.980**
<i>Dendrobium</i> X1	0.9861	-0.2126	0.0656	0.981**
<i>Dendrobium</i> Madame Lucy	0.9698	-0.2396	0.1279	0.983**
<i>Dendrobium</i> New Stripes Delite	0.8909	-0.2473	0.4674	0.995**
<i>Dendrobium</i> Burana Charming × <i>D. Earsakul</i>	0.9123	-0.1875	0.4895	0.976*
<i>Dendrobium</i> Burana Stripe	0.8033	-0.2077	1.0547	0.945*
<i>Dendrobium</i> Burana Green	0.7939	-0.2048	1.1269	0.913*
<i>Dendrobium</i> NPR727	0.7405	-0.2230	1.3472	0.987**
<i>Dendrobium</i> New Aridang Blue Mutation	0.6587	-0.1941	2.1508	0.949*
<i>Dendrobium</i> Angel Blue	0.5643	-0.2567	2.2287	0.937*
<i>Dendrobium</i> NPR863	0.5869	-0.2309	2.3075	0.99**
<i>Dendrobium</i> X2	0.5428	-0.2518	2.4267	0.960*
<i>Dendrobium</i> Pop Eye	0.5511	-0.2422	2.4610	0.999**
<i>Dendrobium</i> Aridang	0.4495	-0.2990	2.6747	0.938*
<i>Dendrobium</i> Mary Green	0.3202	-0.3810	2.9887	0.989**
<i>Dendrobium</i> Nopporn Pink	0.4501	-0.2659	3.0019	0.974*
<i>Dendrobium</i> Siriratana × <i>D. Chao Praya Gem</i>	0.3723	-0.3262	3.0290	0.973*
<i>Dendrobium</i> Liberty White	0.3881	-0.2967	3.1897	0.975*
<i>Dendrobium</i> NPR844	0.3433	-0.3342	3.1989	0.957*
<i>Dendrobium</i> Rainbow Compactum	0.2975	-0.3647	3.3242	0.992**
<i>Dendrobium</i> Udomsri Beauty	0.2530	-0.3990	3.4470	0.902**
<i>Dendrobium</i> Lady Lisa	0.1832	-0.4525	3.7513	0.993**
<i>Dendrobium</i> More Red × <i>D. Thailand</i>	0.3032	-0.2700	4.4206	0.961*
<i>Dendrobium</i> Marilyn Monroe	0.2759	-0.2894	4.4498	0.936*
<i>Dendrobium</i> Sirin Peach	0.2154	-0.3333	4.6060	0.970*
<i>Dendrobium</i> Sunny Red	0.2319	-0.3139	4.6562	0.837*
<i>Dendrobium</i> Burana Jade	0.1118	-0.4371	5.0138	0.919*
<i>Dendrobium</i> Nopporn White Diamond	0.1431	-0.3716	5.2307	0.978*
<i>Dendrobium</i> Sonia Hiasakul	0.2140	-0.2540	6.0710	0.9438*
<i>Dendrobium</i> NPR699	0.1670	-0.2806	6.3785	0.957*