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Research Report

Phospholipid and Fatty Acid Composition in Leaves and Roots of Ten Autumn Chrysanthemum Cultivars Grown at Low Temperature

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Abstract. The objective of this study was to evaluate the relationship between cold hardiness and phospholipid and fatty acid content in leaves and roots of chrysanthemum, and to explore cold resistance mechanisms of chrysanthemum in order to provide a theoretical basis for selecting and breeding a new cold-resistant cultivar. We analyzed the phospholipid and fatty acid components in leaves and roots of 10 autumn chrysanthemum cultivars including six early-flowering cultivars and four late-flowering cultivars. We determined the content of phosphatidyl ethanolamine (PE), phosphatidyl choline (PC), phosphatidyl serine (PS), phosphatidyl glycerol (PG), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), docosanoic acid (C22:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) in leaves and roots of chrysanthemum seedlings grown at 16°C and 5°C. The cultivars had different responses to low temperature that included changes in the cell membrane composition in leaves and roots. The main phospholipid in leaves and roots of chrysanthemum was PE, and the main saturated fatty acid was palmitic acid. Among unsaturated fatty acids, linolenic acid was found in leaves, whereas oleic acid and linoleic acid were present in the roots. Based on the unsaturated fatty acid content and the ratio of unsaturated to saturated fatty acids in leaves, the early-flowering cultivars 'Tan Xiang Shi Zi' and 'Tong Que Chun Shen' and the late-flowering cultivars 'Guan Dong Xin Xia' and 'Yun Long Feng Wu' were classified as strongly cold tolerant, while the early-flowering cultivar 'Jin Feng Ling' and the late-flowering cultivar 'Mo Bao' were weakly cold tolerant.

Additional key words: *Dendranthema* × *grandiflorum*, hardiness

Introduction

Chrysanthemum (*Dendranthema* × *grandiflorum*) is native to China. There are more than 40 *Dendranthema* species and 3,000 cultivars in China (Li and Chen, 2004). Chrysanthemums are among the most famous flowers in China, and *Dendranthema* is the second most important ornamental species worldwide (Liu et al., 2008; Janská A et al. 2013; Teixiera da Silva et al., 2013). The chrysanthemum industry is vulnerable because this species is sensitive to abiotic stresses. Improving the resistance of chrysanthemum plants to abiotic stresses such as heat, low temperatures, drought, and salinity is an important goal (Liu et al., 2008). Low temperature is one of main factors affecting the growth and development of chrysanthemum (Xu et al., 2009). Low temperature can lead to growth arrest and blocked flower formation (Ren et al., 2014). Therefore, it is necessary to obtain cold-tolerant germplasm and breed new cold-tolerant varieties (Xu et al., 2009; Teixiera da Silva et al., 2015). Plants have different ways to compensate for and adapt to environmental stress; cold is an abiotic stress that has been intensively studied in plants because of its negative effects on plant growth, development, and on the yield and productivity of cultivated crops. Cold is a limiting factor when cultivating plants from warm areas in different climatic areas where the temperature is less suitable for their growth (Tasseva et al. 2004; Palma et al., 2008).

For most plant species, temperature is a major determinant of the geographical distribution and the length of the growing season (Yang et al., 2005; Janská et al., 2010). In China, extreme low temperatures in winter or sudden frosts in the fall or early spring periods that correspond to the reproductive growth stage of chrysanthemum can lead to growth arrest and a block in flower formation, resulting in significant economic losses every year (Chinnusamy et al., 2007). Temperate plant species can acquire the ability to withstand

a prolonged period of sub-zero temperatures if they are previously exposed to low temperatures above 0°C (Janská et al., 2010; Chinnusamy et al., 2010; Yamaguchi-Shinozaki and Shinozaki, 2006). It is hypothesized that lipid metabolism plays an important role in the mechanism of frost- or cold-tolerance in plants (Badea and Basu, 2009).

Previous research on cold tolerance in plants has revealed that fatty acids play an important role in the structure of biological membranes. A higher proportion and content of membrane lipid unsaturated fatty acids are related to stronger cold resistance (Murata and Los, 1997). It is generally believed that plants with higher unsaturated fatty acid content and a higher index of unsaturated fatty acids (IUFA) will have strong cold resistance (Murata et al., 1992). However, some researchers have also reported that an increase in unsaturated fatty acid content in cold cultivation conditions is a way to induce a normal adaptation in plants to low temperature. De La Roche et al. (1973) found that the fatty acid content of chlorophyll thylakoid membranes in wheat was not related to cold tolerance, and Zheng et al. (1994) reported that the hardiness in chrysanthemum was related to nutritional characteristics. Anderson and Gesick (2004) counted the number of chrysanthemum foot shoots in autumn to identify which cultivars had greater cold hardiness. Xu et al. (2009) compared lethal temperatures and degree of cold hardiness of eight chrysanthemum cultivars by the growth of foot shoots, a reliable chrysanthemum cold tolerance evaluation system was established. However, perevious reports about cold ressitance of chrysanthemum focus on the evaluation of hardiness and physiological traits, none of them mention if there are some changes in membrane fatty acids and phospholipids under low temperature stress.

In this study, we measured phospholipid and fatty acid compositions in the cells of roots and leaves in six early-flowering and four late-flowering cultivars of autumn chrysanthemum that were grown under normal temperature (16°C) and cold temperature (5°C). The aim of this study was to explore the physiological mechanisms of cold resistance in different chrysanthemum cultivars. The results will provide technical guidance for improving cold tolerance in chrysanthemum and enrich the theoretical foundation for breeding transgenic chrysanthemum, as well as promote the industrialization and in landscape application of chrysanthemum.

Materials and Methods

Plant Materials

Ten autumn chrysanthemum (*Dendranthema* × *grandiflorum*) cultivars were used as experimental materials. Six early-flowering cultivars included 'Tan Xiang Shi Zi' (TXSZ), 'Tai Ping De Xiao Gu' (TPDXG), 'Ri Chu Dong Fang' (RCDF), 'Tong Que Chun Shen' (TQCS), 'Zao Fen Pan' (ZFP), and 'Jin Feng Ling' (JFL); and four late-flowering cultivars included 'Guan Dong Xin Xia' (GDXX), 'Yun Long Feng Wu' (YLFW), 'Xing Guang Can Lan' (XGCL), and 'Mo Bao' (MB). Seedlings of all cultivars were obtained from the Kaifeng Chrysanthemum Institute (Kaifeng, He'nan, China). Chrysanthemum seedlings were grown in plastic pots 8 cm in diameter containing a mixture of lime:vermiculite:perlite (v:v:v=1:1:1) in a growth chamber at 25°C ± 2°C with a light cycle of 10 h light/14 h dark (photosynthetic active radiation 360 ± 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$). After 45 days, when the leaves had fully expanded, chrysanthemum plants were transferred into new chambers set at either 16°C and 5°C with a 10 h light/8 h dark photoperiod (Kazemi-Shahandashti et al., 2014). After treatment for seven days, fresh roots and leaves were collected and stored at -80℃ until use.

Phospholipid Component Analysis

Phospholipids were extracted from 0.2 g of dry leaves and 0.5 g of dry roots by chloroform- methanol extraction. Briefly, samples were immersed in mixture of chloroform: methanol (v:v=2:1) and then subjected to ultrasonic extraction at room temperature for 30 min. The extraction mixture was centrifuged at 12,000 g for 20 min into upper phase and lower phase, lower phase including phospholipids was separated with separating funnel, and then 5 mL 1% NaCl was added to the separating solution. The mixture was incubated at 4°C until it separated into two phases.

Phospholipids were separated with a high-performance liquid chromatograph (HPLC-2695, Waters) equipped with a Nucleosil 100-5 column (250 mm × 4.6 mm × 5 μm) under the following conditions: mobile phase: 100% methanol, flow rate of 0.8 $mL \cdot min^{-1}$, isocratic elution; evaporative light-scattering detector parameters: drift tube temperature 70°C, spray room temperature 30°C, light room temperature 50.0°C; 50 Psi pressure; the sample quantity was 10 μL.

The PE, PC, PS and PG standards were dissolved in 100% methanol and subjected to chromatographic analysis under the conditions described above. Compounds in samples were identified based on a comparison of the sample retention times with those of the phospholipid standards. Phospholipids were quantified with the external standard method.

Fatty Acid Component Analysis

All samples were dried in an oven set to 105°C for 5 min, and then at 50°C for 12 h. Dried roots (0.5 g) and leaves (0.2 g) were ground into a powder and placed in conical flasks, and then a 10% (v/v) mixture of sulfuric acid:methanol (v:v=1:9) was added and heated at 70°C for 30 min. The resultant fatty acid methyl esters were extracted with 5 mL

anhydrous ether and analyzed by gas chromatography (GC-2010, Shimadzu) equipped with a DB-WAXFTLP capillary column (60 m × 1 μm × 0.53 mm ID). Initially, the column temperature was maintained at 140°C for 2 min, and then subjected to a step gradient of 140-200°C at 5°C min^{-1}, 200°C for 1 min, 200-220°C at 2°C min^{-1}, and 220°C for 20 min. Nitrogen was used as the carrier gas (flow rate, 1 $mL \cdot min^{-1}$) (Nejadsadeghi et al., 2015). The injector and detector temperatures were 225 and 275°C, respectively. Fatty acids were identified by the comparison of the sample retention times with those of fatty acid standards (Nu-Check-Prep, Elysian, MN, USA). Fatty acids were quantified with the external standard method.

Statistical Analysis

Data from four replicates per treatment were analyzed. Mean difference comparisons were conducted with analysis of variance (ANOVA) and Tukey's test at the $p < 0.05$ level.

Results

Phospholipid Composition and Contents in Leaves

Table 1 shows the changes in phospholipid composition in leaves of plants grown at 16°C and 5°C. The major phospholipid composition detected in leaves was PE. The content of PE ranged from 5.42 $mg \cdot g^{-1}$ ('Tan Xiang Shi Zi') to 37.15 $mg \cdot g^{-1}$ ('Yun Long Feng Wu') in plants grown at 16°C and from 6.58 $mg \cdot g^{-1}$ ('Tan Xiang Shi Zi') to 57.79 $mg \cdot g^{-1}$ ('Yun Long Feng Wu') in plants grown at 5°C. PE content in the 10 chrysanthemum cultivars at 5°C was higher than that at 16°C. There was very low content of PG in 'Zao Fen Pan' at 5°C and 'Yun Long Feng Wu' at 16°C, whereas no PG was detected in the other cultivars. Similarly, we did not detect PS or PC in experimental chrysanthemum cultivars. The PE content increased in 5°C treatments in all cultivars except 'Guan Dong Xin Xia'. Among the plants grown at 5°C, 'Zao Fen Pan' showed the highest PE content (20.93 $mg \cdot g^{-1}$) in the early-flowering cultivars, and 'Yun Long Feng Wu' had the highest PE content (57.79 $mg \cdot g^{-1}$) in the late-flowering cultivars.

Phospholipid Composition and Content in Roots

The major phospholipid detected in roots was PE (Table 2). The PE content ranged from 1.48 $mg \cdot g^{-1}$ ('Xing Guang Can Lan') to 12.41 $mg \cdot g^{-1}$ ('Tai Ping De Xiao Gu') in plants grown at 16°C, and from 1.36 $mg \cdot g^{-1}$ ('Guan Dong Xin Xia') to 15.57 $mg \cdot g^{-1}$ ('Tai Ping De Xiao Gu') in plants for 5°C. Low levels of PG were detected in the roots of 3 cultivars ('Tan Xiang Shi Zi', 'Guan Dong Xin Xia', 'Yun Long Feng Wu') at 5°C and 4 cultivars ('Jin Feng Ling', 'Zao Fen Pan', 'Mo Bao', 'Xing Guang Can Lan') at 16°C, Low levels of PS and PC were detected in the roots of three cultivars('Ri Chu Dong Fang', 'Tai Ping De Xiao Gu', 'Tong Que Chun Shen') at 5°C. Under 5°C conditions, the PE content in roots decreased in 'Jin Feng Ling', 'Ri Chu Dong Fang', 'Zao Fen Pan', 'Guan Dong Xin Xia', and 'Yun Long Feng Wu', but increased in the other five cultivars. Among the plants grown at 5°C, 'Tai Ping De Xiao Gu' had the highest PE content (15.57 $mg \cdot g^{-1}$) among the early-flower cultivars, and 'Mo Bao' had the highest PE content (7.42 $mg \cdot g^{-1}$) among the late-flower cultivars. Generally, the PE content in leaves was higher than that measured in roots.

Changes in Fatty Acid Composition in Leaves

By contrast, the content of unsaturated fatty acids was higher in seedlings grown at 5°C than that at 16°C, whereas saturated fatty acid content was higher in plants grown at 16°C than those grown at 5°C (Fig. 1). In leaves, the major

Table 1. Phospholipid contents ($mg \cdot g^{-1}$) in leaves of chrysanthemum cultivars in low temperature

Cultivar	Phosphatidyl glycerol		Phosphatidyl ethanolamine		Phosphatidyl serine		Phosphatidyl choline	
	5°C	16°C	5°C	16°C	5°C	16°C	5°C	16°C
TPDXG	-[z]	-	14.95±1.12d[y]	14.73±0.65d	-	-	-	-
JFL	-	-	7.64±0.31e	7.38±0.47e	-	-	-	-
TQCS	-	-	9.95±0.57de	7.45±0.51e	-	-	-	-
RCDF	-	-	10.92±0.43de	9.17±0.72e	-	-	-	-
TXSZ	-	-	6.58±0.28e	5.42±0.38f	-	-	-	-
ZFP	0.18±0.008a	-	20.93±1.64c	13.94±0.84d	-	-	-	-
MB	-	-	8.85±0.56e	6.33±0.46f	-	-	-	-
XGCL	-	-	14.37±0.86d	13.42±0.58d	-	-	-	-
GDXX	-	-	12.04±0.84d	12.97±0.82d	-	-	-	-
YLFW	-	0.1±0.004b	57.79±4.63a	37.15±2.95b	-	-	-	-

[z]- not detected.
[y]Data are means from four independent experiments ±SE. Means with different letters indicate a significant differences at $p < 0.05$.

Table 2. Phospholipid contents ($mg \cdot g^{-1}$) in roots of chrysanthemum cultivars in low temperature

Cultivar	Phosphatidyl glycerol		Phosphatidyl ethanolamine		Phosphatidyl serine		Phosphatidyl choline	
	5°C	16°C	5°C	16°C	5°C	16°C	5°C	16°C
TPDXG	-[z]	-	15.57±1.18a[y]	12.41±0.85a	-	-	0.25±0.014a	-
JFL	-	0.09±0.005b	5.19±0.36c	5.46±0.34c	-	-	-	-
TQCS	-	-	8.88±0.42b	5.64±0.31c	-	-	0.06±0.004b	-
RCDF	-	-	6.44±0.46bc	7.41±0.29b	0.14±0.005	-	-	-
TXSZ	0.03±0.002c	-	5.30±0.21c	3.89±0.23de	-	-	-	-
ZFP	-	0.25±0.018a	2.42±0.19e	2.69±0.17e	-	-	-	-
MB	-	0.25±0.016a	7.42±0.43b	5.65±0.44c	-	-	-	-
XGCL	-	0.20±0.009a	1.49±0.96f	1.48±0.09f	-	-	-	-
GDXX	0.21±0.013a	-	1.36±0.08f	1.66±0.09f	-	-	-	-
YLFW	0.19±0.008a		2.62±0.11e	4.47±0.36cd	-	-	-	-

[z]- not detected.
[y]Data are means of four independent experiments ±SE. Means with different letters indicate a significant differences at $p < 0.05$.

saturated fatty acid detected was C16:0 and the main unsaturated fatty acid was C18:3. In seedlings grown at 5°C, C16:0 content decreased in early-flowering cultivars and the largest decrease was measured in 'Zao Fen Pan'. At 5°C, increased C18:3 content varied among the early-flowering cultivars, except in 'Tan Xiang Shi Zi'. The maxium increase in C18:3 content was observed in 'Zao Fen Pan'(17.80%), the minimum in 'Ri Chu Dong Fang' (7.50%), for other three cultivars, 'Tong Que Chun Shen' increased by 11.54%, 'Jin Feng Ling' 8.97% and 'Tai Ping De Xiao Gu' 7.69%. Late-flowering cultivars showed similar fatty acid accumulation to those of early-flowering cultivars. The C16:0 content in the leaves decreased under low temperature, with the largest decrease in 'Xing Guang Can Lan'. The C18:3 content increased under low temperature, with the largest increase in 'Guan Dong Xin Xia', followed by 'Xing Guang Can Lan'.

Changes in Fatty Acid Composition in Roots

The fatty acid composition and content in roots of the 10 chrysanthemum cultivars are shown in Table 3. The fatty acids measured in roots included C14:0, C16:0, C18:0, C22:0, C18:1, C18:2, and C18:3. Although all cultivars had common fatty acid components, the relative proportions of these components differed among the cultivars. The main saturated fatty acid in roots of early-flower cultivars was C16:0. The proportion of palmitic acid to total fatty acids ranged from 27.87% to 33.79% in plants grown at 16°C, and from 22.53% to 34.90% in those grown at 5°C. These proportions were much higher than those measured in leaves. The main unsaturated fatty acid in chrysanthemum roots was C18:2. The proportion of linoleic acid ranged from 30.10% to 40.68% at 16°C, and from 34.54% to 38.83% at 5°C. C18:1 also accounted for a considerable proportion of the unsaturated fatty acids, which ranged from 14.02% to 23.04% at 16°C, and from 16.34% to 27.44% at 5°C. The main saturated fatty acid in roots of late-flowering cultivars was the same as early-flowering cultivars. The proportion of palmitic acid ranged from 22.04% to 34.38% at 16°C, and from 25.57% to 34.95% at 5°C. C18:1 and C18:2 were the main unsaturated fatty acids present and accounted for about 50% of total fatty acids.

Discussion

Although there is ample research on plant lipids, little information is known about the physiological basis for the response to low temperature in chrysanthemum (Suh et al., 2015). Abiotic stresses affect the properties of the plasma membrane; therefore, membrane attributes and membrane damage are useful indexes for stress damage in plants (Kazemi-Shahandashti et al., 2014). Lipids are an important component of the membrane in higher plants and play critical roles in maintaining membrane fluidity, formation of the chloroplast ultrastructure, light absorption for photosynthesis, the transfer and conversion of light energy, and the synthesis of ATP (Ohlrogge and Browse, 1995). Leakage points may result from cold-induced changes in the membrane lipid phase or from membrane damage, especially from lipid damage (Campos et al., 2003). Phospholipids are one of the main components of membrane lipids and relate closely to cold hardiness in plants. In the present study, the main phospholipid in the leaves and roots of 10 chrysanthemum cultivars was PE, while other phospholipids were barely detectable. The PE content in leaves of most chrysanthemum cultivars increased at low temperature, although 'Guan Dong Xin Xia' was the exception. In the roots, changes in PE content in low temperature conditions differed among the cultivars. In general, the PE content was higher in the leaves than in the roots,

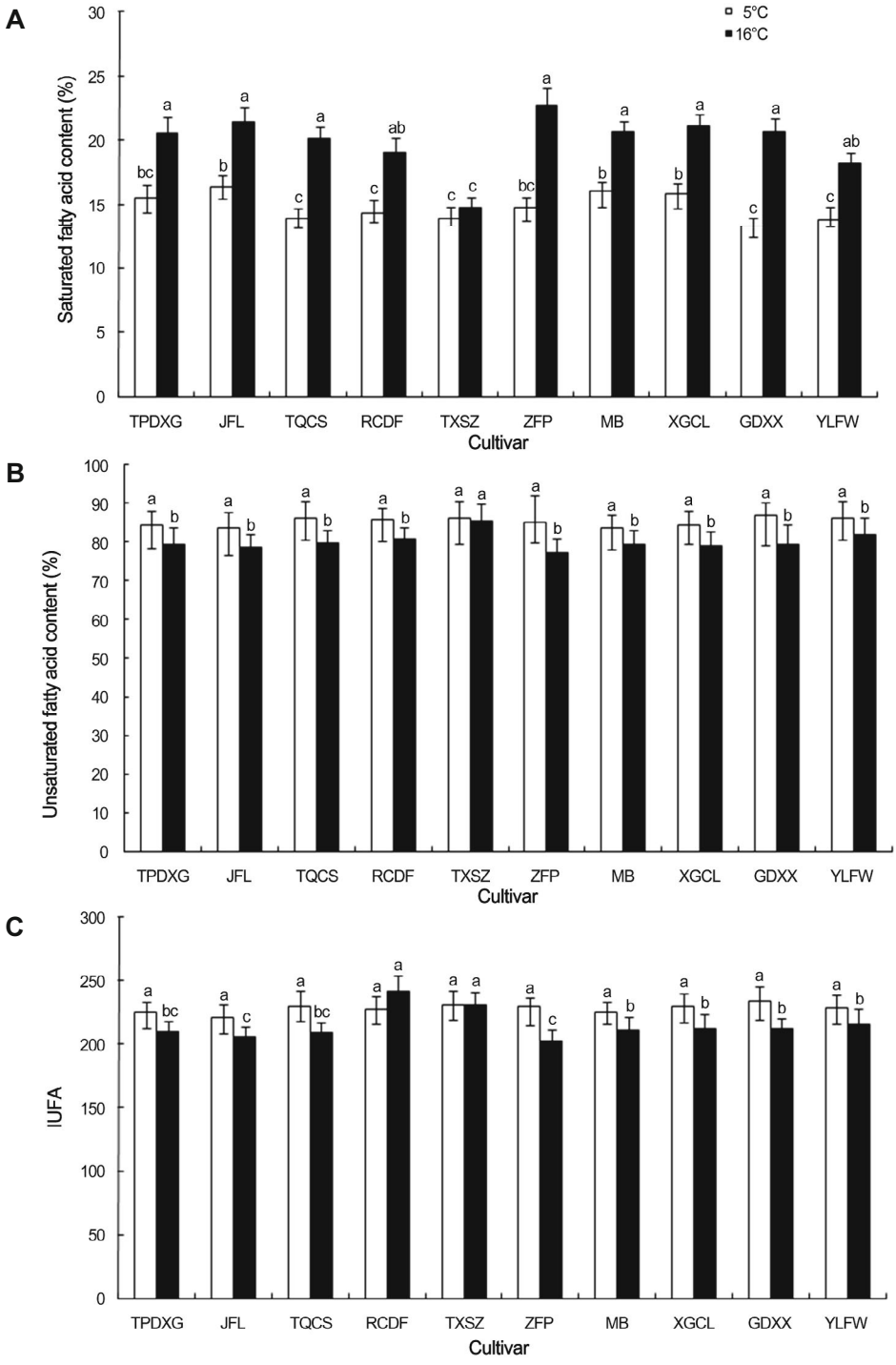

Fig. 1. Changes in saturated fatty acid content (A), unsaturated fatty acid content (B), and IUFA (Index of unsaturated fatty acids) (C) in leaves of 10 chrysanthemum cultivars at 5°C and 16°C. Fatty acids component and content were isolated and analyzed as described under Material and methods. IUFA was calculated as described under Material and methods. Results are the means of four replicate experiments ± SE (Standard error). The same letters with error bars within each graph panel are not significantly different at $p < 0.05$.

and this characteristic was similar between early-flower and late-flower cultivars.

The main fatty acid composition in chrysanthemum leaves and roots was C14:0, C16:0, C18:0, C22:0, C18:1, C18:2, and C18:3. The total fatty acid and unsaturated fatty acid contents were higher in leaves than in roots. The detectable fatty acid components were the same among all chrysanthemum cultivars, but their relative proportions differed between each other. The level of saturation of plasma membrane fatty acid is a key determinant of low temperature stress(Huang and

Table 3. Fatty acid composition and content in roots of chrysanthemum cultivars in low temperature

Cultivar	Treatment	Fatty acid content (mol %)									
		C14:0	C16:0	C18:0	C22:0	C18:1	C18:2	C18:3	Saturated fatty acid[z]	Unsaturated fatty acid[y]	IUFA[x]
TPDXG	5°C	1.30±0.06e[w]	22.53±1.84c	2.63±0.15b	1.46±0.08cd	27.44±1.86a	34.76±2.76b	9.88±0.76a	27.91±1.64d	72.09±4.89a	126.61±8.43a
	16°C	1.69±0.09cd	30.79±1.79a	2.55±0.12bc	1.96±0.07c	14.02±0.91c	40.68±2.89a	8.30±0.52bc	36.99±2.27b	63.00±4.36bc	120.30±7.58a
JFL	5°C	1.99±0.10bc	31.86±2.12a	2.61±0.14b	1.19±0.06d	19.58±1.23b	35.70±2.12b	7.07±0.41c	37.65±2.18b	62.35±3.21c	112.19±6.45b
	16°C	1.36±0.07d	32.80±2.25a	2.04±0.12cd	1.21±0.07d	15.15±1.12c	39.34±1.98a	8.10±0.54bc	37.41±1.93b	62.59±3.48c	118.13±5.39b
TQCS	5°C	1.93±0.10c	30.25±2.46a	2.81±0.16b	1.39±0.05cd	19.55±1.13b	35.47±1.74b	8.60±0.39b	36.38±1.85b	63.62±3.45bc	116.29±6.73b
	16°C	2.61±0.15b	31.41±1.87a	2.92±0.18b	1.25±0.04d	21.52±1.27b	31.56±1.65b	8.74±0.53b	38.18±1.97b	61.82±4.52c	110.85±6.82b
RCDF	5°C	1.42±0.08d	27.51±1.76b	1.59±0.08d	1.64±0.07c	19.79±1.35b	38.83±1.83a	9.23±0.49b	32.16±1.42c	67.84±3.91b	125.12±5.93a
	16°C	1.80±0.07c	28.57±1.69b	2.72±0.18b	1.15±0.06d	23.04±1.56b	35.15±1.96b	7.56±0.32c	34.25±1.56bc	65.75±3.47b	116.03±7.34b
TXSZ	5°C	1.47±0.08d	28.88±1.34b	1.57±0.09d	3.19±0.18b	16.34±0.87c	37.20±1.84a	11.35±0.64a	35.10±2.16b	64.90±4.32b	124.81±6.42a
	16°C	1.40±0.06d	27.87±1.67b	2.31±0.15c	1.79±0.09c	21.28±1.45b	35.88±2.17b	9.47±0.62b	33.37±1.94c	66.63±4.58b	121.44±4.98ab
ZFP	5°C	1.78±0.08c	34.90±1.68a	2.53±0.16bc	0.13±0.06e	16.97±1.27bc	34.54±1.62b	9.16±0.31b	39.33±2.51b	60.67±3.53c	113.51±5.43b
	16°C	1.70±0.06cd	33.79±2.48a	4.06±0.31a	1.08±0.07d	18.41±1.17b	30.10±1.73bc	10.86±0.64a	40.64±2.89b	59.36±3.25c	111.18±6.35b
MB	5°C	1.56±0.04d	32.28±2.12a	3.64±0.26a	3.76±0.28b	16.34±0.94bc	33.44±2.91b	8.76±0.51b	41.25±3.14b	58.75±2.96c	110.14±7.56b
	16°C	3.27±0.21a	33.71±2.56a	4.31±0.37a	6.78±0.42a	20.21±1.08b	24.97±1.42c	6.78±0.48cd	48.07±3.26a	51.93±2.71d	90.42±4.85c
XGCL	5°C	2.38±0.14b	26.00±1.44bc	3.10±0.21ab	1.87±.012c	21.38±1.25b	37.45±1.92a	7.83±0.36c	33.33±2.13c	66.67±3.79b	119.78±6.71b
	16°C	2.42±0.18b	22.04±1.42c	3.10±0.19ab	1.88±0.11c	31.67±2.39a	30.37±1.56bc	8.51±0.47b	29.45±1.26d	70.55±4.38a	117.92±7.89b
GDXX	5°C	1.35±0.04d	34.95±1.65a	2.05±0.17cd	0.88±0.03d	15.06±0.43c	37.13±1.87ab	8.58±0.46b	39.22±1.89b	60.78±3.26c	115.07±6.38b
	16°C	1.53±0.05d	34.38±1.86a	2.38±0.13c	0.99±0.04d	13.22±0.45c	39.25±2.46a	8.24±0.59bc	39.28±2.32b	60.72±3.47c	116.45±5.21b
YLFW	5°C	1.43±0.06d	25.57±1.62bc	2.72±0.15b	1.81±0.09c	25.47±1.54ab	36.67±2.12ab	6.72±0.31cd	31.05±1.71cc	68.95±4.19b	119.17±4.76b
	16°C	2.16±0.17b	25.64±1.53bc	2.31±0.11c	1.76±0.07c	29.20±1.86a	32.38±1.96b	6.04±0.32d	32.38±1.92cd	67.62±3.82b	112.08±6.38b

[z]Saturated fatty acid content = C14:0% + C16:0% + C18:0% + C22:0%, [y]unsaturated fatty acid content = C18:1% + C18:2% + C18:3%.
[x]IUFA (Index of unsaturated fatty acids) = C18:1% × 1 + C18:2% × 2 + C18:3% × 3.
[w]Data are means from four independent experiments ±SE. Means with different letters indicate a significant differences at $p < 0.05$.

Li, 2004). Lyons et al. (1970) suggested that membrane fluidity and membrane lipid phase changes are closely related to cold resistance in plants. To some extent, the effect of low temperature on plant growth and development depends on the ratio of unsaturated to saturated fatty acids. The degree of the unsaturation of membrane lipids can be adjusted to improve membrane fluidity under low temperature. These results showed that under cold stress, the unsaturated fatty acid content increased and saturated fatty acid content decreased and the ratio of saturated to unsaturated fatty acids and IUFA increased in chrysanthemum leaves. In the roots, under low temperature, there was an increase in unsaturated fatty acid content and in the ratio of unsaturated to saturated fatty acids in these cultivars 'Tai Ping De Xiao Gu', 'Ri Chu Dong Fang ', 'Tong Que Chun Shen', 'Zao Fen Pan', 'Mo Bao', 'Yun Long Feng Wu', and 'Guan Dong Xin Xia'. The other cultivars showed a decrease in fatty acids under the same conditions. Under cold stress, the saturated fatty acid content decreased in most cultivars but not in 'Jin Feng Ling', 'Tan Xiang Shi Zi', and 'Xing Guang Can Lan'. The response of fatty acids to low temperature are different in chrysanthemum cultivars with different cold resistance, these differences in metabolism mechanism of unsaturated fatty acids might be related to the resistance of cold stress.

In research on mulberry and *Nothofagus* (Yoshida, 1984; Lag et al., 1991), cold stress resulted in changes in the composition of membrane fatty acids. The proportion of unsaturated fatty acids increased, as did the content of unsaturated fatty acids such as C18:2 and C18:3. In this study, the main unsaturated fatty acids composition showing increased in contents were C18:3 in leaves and C18:2 and C18:1 in roots. Changes in C16:0 content affected the overall saturated fatty acid content in leaves in roots. Changes in C16:0 quantity affected the saturated fatty acid content in leaves and roots. Changes in contents of C18:2 and C18:3 are evident under low temperature in the leaves and roots, membrane lipids metabolism may be sensitive to low temperature, these fatty acids might serve as indicators of cold tolerance in chrysanthemum.

There are three ways in which plants can maintain membrane fluidity as the temperature decreases (Latsague et al., 1992). The amount of lipids with more unsaturated fatty

acids can increase the overall unsaturation degree of fatty acids; changes in the ratios of fatty acids in lipids can increase the content of unsaturated fatty acids. Of course, both of these changes can occur simultaneously (Murata and Yamaya, 1984; Yang et al., 1986; Latsague et al., 1992). In this paper, there were some changes in unsaturated fatty acids content in leaves and roots of the 10 chrysanthemum cultivars grown at 5°C in comparison with those at 16°C, especially in 'Tan Xiang Shi Zi', 'Tong Que Chun Shen', 'Guan Dong Xin Xia', and 'Yun Long Feng Wu'. There was no significant difference in the content of unsaturated fatty acids in neither the leaves nor the roots between the 10 chrysanthemum cultivars. Changes in the unsaturated fatty acids content in leaves and roots at low temperature showed that there might be difference in cold resistance between the chrysanthemum cultivars. During low temperature acclimation of chrysanthemum, the levels of unsaturated fatty acids increased in leaves, there are different changing trend in roots. PE content showed the largest change between the higher and lower temperatures, while PG, PC, and PS were barely detected in either condition. The main saturated fatty acid was C16:0, the main unsaturated fatty acids were C18:3 in leaves and C18:1 and C18:2 in roots. Based on these results, it could be concluded that chrysanthemum leaves and roots can adapt to low temperatures by increasing the content of unsaturated fatty acids, but that there is a difference in fatty acid components between the tissues, suggesting potentially different mechanisms for low temperature resistance in leaves and roots.

Cold-stressed plants initiate a series of responses that lead to the synthesis and accumulation of unsaturated fatty acids that help to maintain cold hardiness. The greater increase in unsaturated fatty acids was observed in the late-flower cultivars 'Guan Dong Xin Xia' and 'Yun Long Feng Wu', but was lower in the early flower cultivar 'Jin Feng Ling'. The level of membrane unsaturated lipids in leaves and roots of chrysanthemum increased at low temperature; there was a significant difference in unsaturated fatty acids and PE content among the 10 chrysanthemum cultivars tested. The main unsaturated fatty acid components are different in leaves and roots of chrysanthemum plants, furthermore, the content of these components increase under cold stress, capability of resistance to low temperature are improved, this suggests that there are different low temperature resistance mechanisms in chrysanthemum.

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