

Table S1

Temperatures set for semi-lethal temperature (LT₅₀) detremination (°C) on each sampling date.

Sampling date	Nov.13	Nov.27	Dec.11	Dec.25	Jan.8	Jan.22	Feb.4	Feb.19	Mar.5	Mar.18
Temperature 1	4	0	0	-4	-4	-4	-4	-5	0	0
Temperature 2	0	-3	-4	-8	-8	-8	-8	-10	-4	-4
Temperature 3	-3	-6	-8	-12	-12	-12	-12	-15	-8	-8
Temperature 4	-6	-9	-12	-16	-16	-16	-16	-20	-12	-12
Temperature 5	-9	-12	-16	-20	-20	-20	-20	-25	-16	-16
Temperature 6	-12	-15	-20	NA	NA	NA	NA	NA	NA	NA

Note: NA indicated temperature 6 was not set.

Table S2

Genes and corresponding primers used for quantitative real-time PCR.

Gene name	Gene full name	<i>Arabidopsis</i> homolog	Functional description	Forward/reverse primer sequence (5'-3')
reference gene				
<i>ACT7</i>	<i>Actin7</i>	AT5G09810	cell division, cytoskeleton organization	ACATTGTGCTCAGTGAGGT/ ACTTCCTCTCTGGTGGAGCA
Genes associated with carbohydrate metabolism process				
<i>SEX4</i>	<i>starch-excess 4</i>	AT3G52180	starch catabolic process	GTAAACGGCCATGCTTTCCG/ TTCTCTGCCCCATCCAATG
<i>MEX1</i>	<i>maltose excess 1</i>	AT5G17520	Starch metabolism process	CCCGAATAGCATTTTGCCCG/ ACAAGTCTCGCTCCTTGCTC
<i>SUT4</i>	<i>sucrose transporter 4</i>	AT1G09960	sucrose metabolic process, sucrose transport	TGCTTGGCTTTGCTACTGGA/ GGGGACTTCTTGGACTGACG
<i>AMY3</i>	<i>alpha-amylase-like 3</i>	AT1G69830	starch catabolic process	GTGGGCGAGTATTGGGACTC/ ACGTCAAAGGCACCACAAGT
<i>ISA3</i>	<i>isoamylase 3</i>	AT4G09020	starch catabolic process	TGCGTTCTCGGCAAATGAAG/ TTGTTCCGTTACGCGTGTG
<i>DPE2</i>	<i>disproportionating enzyme 2</i>	AT2G40840	starch catabolic process	GGAGAGCCCGTCTTACACAG/ TCCCAACCAGACCCGTAAGA
<i>GLT1</i>	<i>glucose transporter 1</i>	AT5G16150	response to trehalose	ATGTTGCAGCTAGTGCCCTT/ TGAAGCAGCCATTCCAGTGA
<i>SUS4</i>	<i>sucrose synthase 4</i>	AT3G43190	sucrose metabolic process	GCGAAATGGCGAGCTGTATC/ TAGGCCGTCGCAAATGTAGG
<i>GWD3</i>	<i>phosphoglucan water dikinase</i>	AT5G26570	Starch metabolism process	CTACAGAGGCCATGCTTGCT/ TCGGTCAAGCTTCCTGCATT
<i>LSF1</i>	<i>Like SEX4 1</i>	AT3G01510	starch catabolic process	AACAGCACAAAGGATGGCGTA/ GGAAAGCTCCCATATGCCCA
<i>SS3</i>	<i>starch synthase 3</i>	AT1G11720	Starch biosynthetic process	TGCTCCTGTTGCGTGTTAT/ CACCGTCGTAGCCTTATCGG
<i>BAM3</i>	<i>beta-amylase 3</i>	AT4G17090	maltose biosynthetic process, response to cold	TGGCGAGTTCCAGTGCTATG/ TCCCGTAGTCCGAGTTCCAT
<i>GBSS</i>	<i>granule bound starch synthase</i>	AT1G32900	starch biosynthetic process	ACCGAGTCTTGACAGTGAGC/ TCGTTTGCTGGATCCCACTC
<i>BAM1</i>	<i>beta-amylase 1</i>	AT5G65700	starch catabolic process	TTCCATTGCATTGCGGTGCG/ TTGTAGACGATTCCGGCCC
<i>SNRK1.1</i>	<i>snf1-related protein kinase 1.1</i>	AT3G01090	sugar mediated signaling pathway, abscisic acid-activated signaling pathway	GTTCCACTGGGCATCCTGAA/ TCCTCCCTCGTCTTGTAAGC
Genes associated with phytohormone signaling pathway				
<i>TIR1</i>	<i>transport inhibitor response 1</i>	AT3G62980	response to auxin	CGCTTCCGGCTCTGTATCAT/ GTCAGCAGAGGCCCAATAG

<i>AMT1</i>	<i>a-methyl tryptophan resistant 1</i>	AT5G05730	auxin biosynthetic process	TGCCGACCCATTGAAGTGT/ AGAATCTCAGGGCTTGACGC
<i>IAA27</i>	<i>indole-3-acetic acid inducible 27</i>	AT4G29080	response to auxin	CCTCGGCACTTGAGAAGATGT/ CCAGGGTACGTCACCAACAA
<i>GH3.2</i>	<i>auxin upregulated 3</i>	AT4G37390	auxin homeostasis, response to auxin	TCTGCCGTTCATCCGCTTC/ GGTGACGGAATCTCGGACAG
<i>ABA1</i>	<i>ABA deficient 1</i>	AT5G67030	abscisic acid biosynthetic process, sugar mediated signaling pathway	GGAAAGCTCTGAGACAGGGG/ GGAAAGCTCTGAGACAGGGG
<i>ABA2</i>	<i>ABA deficient 2</i>	AT1G52340	abscisic acid biosynthetic process, proline biosynthetic process	AGATTCGGGATTTCGCGTCAA/ CCTCGAACTCCTCGTTCGTC
<i>AAO3</i>	<i>abscisic aldehyde oxidase 3</i>	AT2G27150	abscisic acid biosynthetic process	TCCCCTGCGTTCAAGAAGTC/ GGCTCACCAACCGGAAAGTA
<i>NCED3</i>	<i>nine-cis-epoxycarotenoid dioxygenase 3</i>	AT3G14440	abscisic acid biosynthetic process	GCCGGGCTCATCTACTTCAA/ CTGCCGTCGAAATCGTACCT
<i>PUB44</i>	<i>Arabidopsis thaliana plant u-box 44</i>	AT1G20780	negative regulation of abscisic acid biosynthetic process	TTCCACCGCTCATGAAGGAC/ AAGTCAGCACCCGAAGACAC
<i>PYL8</i>	<i>PYR1-LIKE 8</i>	AT5G53160	positive regulation of abscisic acid-activated signaling pathway	AGACCAGGGACGCTAGTGAT/ TCTATGGCTAGACGCTCGGA
<i>PP2C5</i>	<i>phosphatase 2C5</i>	AT2G40180	abscisic acid-activated signaling pathway	AGGTGGGAAAGAAGCTGCAA/ TGCTGCCTCTAGGGATGACA
<i>SnRK2.4</i>	<i>snf1-related protein kinase 2.4</i>	AT1G10940	abscisic acid signaling pathway	GCCGAGGTTGAAGATTGCG/ CCAGCTTGCCGTCGTATTCT
<i>ABF1</i>	<i>abscisic acid responsive element-binding factor 1</i>	AT1G49720	abscisic acid-activated signaling pathway	AAGGCCTACCAGCTGAGTCT/ CGCAATCCTCTTCTCCGTT
<i>ABF4</i>	<i>abscisic acid responsive element-binding factor 4</i>	AT3G19290	abscisic acid-activated signaling pathway	TGGTGAGCAGTGGATTGGTG/ AGGTACTGGTGACAACGACG
<i>AREB3</i>	<i>ABA-responsive element binding protein 3</i>	AT3G56850	abscisic acid-activated signaling pathway	TCGTGCGGTTCCCCAAATAA/ ATCTTATCCGTGTCCCCCGA
<i>SnRK2.6</i>	<i>snf1-related protein kinase 2.6</i>	AT4G33950	abscisic acid-activated signaling pathway	AATAGCGGAGGCCACAATCC/ TGGCATTGACTATCTCGCCG
<i>HOS1</i>	<i>high expression of osmotically responsive genes 1</i>	AT2G39810	response to cold	TTCTACGATGCATTGGGCGT/ AACCGGCCACTTTCAATCCT
<i>ICE1</i>	<i>inducer of CBF Expression 1</i>	AT3G26744	abscisic acid-activated signaling pathway; response to cold, freezing	CGACATCCTTACCAAGCCCA/ CCATCAAGTGCCCTCATCGT
<i>COI1</i>	<i>coronative insensitive 1</i>	AT2G39940	jasmonic acid mediated signaling pathway	TTGGAAGAGTTTGGTGGGGG/ ACAGGCATCTCGTTTGTCCT
<i>JAZ2</i>	<i>jasmonate-zim-domain protein 2</i>	AT1G74950	response to jasmonic acid	AGCCAACCCAAGTCACTGTT/ TATGGTGCCCTGGCAATTGAT
<i>MYC2</i>	<i>jasmonate insensitive 1</i>	AT1G32640	Phytohormone (JA) signaling	TCGATTTCGGAGAACGAGAGC/ CGCTTCCGTGCCCATATTC
<i>CBF2</i>	<i>C-repeat binding factor 2</i>	AT4G25470	cold acclimation; response to cold; regulation of transcription	TTGGGAGAGAATTCGCGGG/ ATCCCTCCGCCATATCCTT
<i>CBF4</i>	<i>C-repeat binding factor 4</i>	AT5G51990	regulation of transcription	GCCGTCTAGTTCGTCGGTAG/ CGTCTCCCTGCATCATAGC
<i>COR27</i>	<i>cold regulated gene 27</i>	AT5G42900	response to cold	TGGTTGCATGACAGAGGCAT/ CCACAGACGGAACCACTTGA
<i>GA20ox2</i>	<i>gibberellin 20-oxidase</i>	AT5G51810	gibberellin biosynthetic process, response to gibberellin	TCATTCAAACCTCGGCGAA/ GAGTCGTGCCCTCGAAGAA
<i>GID1c</i>	<i>GA insensitive dwarf1C</i>	AT5G27320	gibberellin mediated signaling pathway	TCCGCTTACGATGACGGATG/ ACATTATGCGCGATGTTGCC
<i>GAI</i>	<i>gibberellic acid insensitive</i>	AT1G14920	negative regulation of seed germination, jasmonic acid mediated signaling pathway,	AGCAGGACCAGTTGATGTCG/ CATGCTCGCCTGCTTGAAC

<i>SPY</i>	<i>spindly</i>	AT3G11540	gibberellic acid mediated signaling pathway, regulation of reactive oxygen species metabolic process	AGGGATGATGGCTTGCAGAC/ CAGGCAGACGAACCAACTCT
Genes associated with stress response process				
<i>GST8</i>	<i>glutathione transferase 8</i>	AT1G78380	response to oxidative stress	CACCTATGAGACCAACGCCA/ CCTTGTGGGGATCAGAGAGC
<i>GSTL2</i>	<i>glutathione transferase lambda 2</i>	AT3G55040	response to stress	GTGGATATCGCATACGCTCCA/ GCCGAGTTGATGTGTAGGCA
<i>GPX6</i>	<i>glutathione peroxidase 6</i>	AT4G11600	response to oxidative stress	TTCCCGTGCAACCAGTTTGG/ GGTGAAGCCTTGTCCCCATT
<i>P5CS2</i>	<i>delta1-pyrroline-5-carboxylate synthase 2</i>	AT3G55610	L-proline biosynthetic process;	AAGCAACGTAGAGGGAGCAG/ GCATTGGACACAACGAGGTC
<i>P5CR</i>	<i>delta 1-pyrroline-5-carboxylate reductase</i>	AT5G14800	proline biosynthetic process	TCGAGATCCTCAACGACAGC/ CAGACCACTCCTGCAAGTCT
<i>PRXR1</i>	<i>PRXR1</i>	AT4G21960	response to oxidative stress	AAGCTGGTGCATCGTCTGTA/ GTCCAGTATCATCGGGGTCC
<i>APX1</i>	<i>ascorbate peroxidase 1</i>	AT1G07890	oxidation-reduction process	CTACCTGATGCGACCGAAGG/ ATGTCCATGCTCCCTCGAAC
<i>FER2</i>	<i>FERRITIN 2</i>	AT3G11050	response to abscisic acid, response to oxidative stress	AGCTTGCTGACTTTGTGGAGA/ ATCAAAATGCCAGACCCCGT
<i>TAPX</i>	<i>thylakoidal ascorbate peroxidase</i>	AT1G77490	cold acclimation, hydrogen peroxide mediated signaling pathway	ATCCTCCAGAGGGTGTCTCC/ TGTTACACGGTTGAAAAGC
<i>MDAR1</i>	<i>monodehydroascorbate reductase 1</i>	AT3G52880	oxidation-reduction process	TGCCGTGGGATTGTATTCTGA/ TTTCCGCCAACTCCTACCAC

Table S3
Changes of relative chlorophyll content (RCC), leaf width (LW), No. of functional leaves (NFL) and fresh weight (FW) in evergreen and deciduous irises during natural cold acclimation and deacclimation.

Sampling stage	Deciduous iris				Evergreen iris			
	RCC	LW (mm)	FW (g)	NFL	RCC	LW (mm)	FW (g)	NFL
Nov.13	47.27±1.74a	21.20±0.62a	1.33±0.09b	6.60±0.55a	59.70±1.45a	23.93±0.40b	1.83±0.06b	4.80±0.45fg
Nov.27	36.97±2.39c	17.20±0.36b	0.96±0.04b	5.60±0.55b	56.63±1.11b	22.37±0.40bc	1.56±0.04c	4.40±0.55g
Dec.11	31.70±1.61d	14.73±0.25c	0.31±0.04c	4.40±0.55c	52.33±1.84c	18.70±6.15d	1.32±0.09d	5.00±0.71efg
Dec.25	NA	NA	NA	NA	48.87±1.19d	13.27±0.59e	0.67±0.08e	5.40±0.55def
Jan.8	NA	NA	NA	NA	47.13±1.56d	12.23±0.25e	0.34±0.06f	5.20±0.45ef
Jan.22	NA	NA	NA	NA	39.10±1.25g	11.87±0.40e	0.25±0.02fg	5.60±0.55cde
Feb.5	NA	NA	NA	NA	43.17±0.91f	12.80±0.30e	0.12±0.01g	6.00±0.71bcd
Feb.19	NA	NA	NA	NA	46.53±0.97de	20.20±0.36cd	0.68±0.06e	6.20±0.45abc
Mar.4	NA	NA	NA	NA	44.17±2.21ef	23.00±0.62bc	1.65±0.21c	6.40±0.55ab
Mar.18	43.50±1.75b	14.57±0.60c	3.04±0.46a	3.40±0.55d	51.57±1.66c	27.53±0.93a	2.98±0.20a	6.80±0.45a

Note: Data are means ± (standard error) of ten independent biological replicates with different letters indicating significant differences between treatments according to Duncan's multiple range test at $p < 0.05$. NA indicated no plant material for determination.

Table S4
Comparison in characteristics of leaf microstructure between evergreen and deciduous irises.

Species	Characteristics of leaf structure					
	Leaf thickness (um)	Epidermal cell size		No. of layer of mesophyll cell with chloroplast	Stomatal apparatus size	
		Length (um)	Width (um)		Major axis (um)	Minor axis (um)
Evergreen iris	622.9	27.5	22.5	7	27.2	27.2
Deciduous iris	585.3	12.5	11	7	20	16.7
						Stomatal apparatus densities (entries/mm ²)
						267
						286.07