

Cloning and Functional Characterization of Chalcone Isomerase Genes Involved in Anthocyanin Biosynthesis in *Clivia miniata*

Running title: Characterization of *CHI* in *Clivia miniata*

Yue Liu¹, Xinxin Xue², Chunli Zhao^{1*}, Jia Zhang³, Meng Liu³, Xiangyu Li³, Yueqing Li^{3*}, Xiang Gao³

¹ College of Horticulture, Jilin Agricultural University, Changchun, China

² Department of Botany, University of British Columbia, Vancouver, Canada

³ Key Laboratory of Molecular Epigenetics of MOE and Institute of Genetics & Cytology, Northeast Normal University, Changchun, China

*Corresponding authors: To whom correspondence should be addressed. E-mail address: zcl8368@163.com; liyq339@126.com; Tel.:+86 431 85099360

Table S1 Information of *CmCHI1*, *CmCHI2* and *CmCHI3*

Candidate transcripts	Protein sequence length	Top <i>Arabidopsis</i> BLAST match	Top BLAST match excluding <i>Arabidopsis</i>	Homology (%)	GenBank number of the homology genes used in Blast
<i>CmCHI1</i>	237 residues	AAA32766.1 chalcone isomerase <i>Arabidopsis thaliana</i>	AIA59796.1 chalcone isomerase <i>Lycoris radiata</i>	59a,87b	AAA32766.1
<i>CmCHI2</i>	218 residues	AAA32766.1 chalcone isomerase <i>Arabidopsis thaliana</i>	AEO36936.1 chalcone isomerase <i>Canarium album</i>	51a,59b	AAA32766.1
<i>CmCHI3</i>	210 residues	AAA32766.1 chalcone isomerase <i>Arabidopsis thaliana</i>	AIU39024.1 chalcone isomerase <i>2 Narcissus tazetta subsp. chinensis</i>	83a,88b	AAA32766.1

a% Similarity to *Arabidopsis thaliana*.

b% Highest similarity to other plant species.

Table S2 Primers used in the study

		Forward(5'-3')	Reverse(5'-3')
Full length			
cDNA	<i>CmCHI1</i>	ATGGGAGAAACATCGACGAC	TTGTGTGACATTAACGTACT
sequence	<i>CmCHI2</i>	ATGGCCCAAGAGTCGTCCGT	TTGAAGAGCTTTTTAAGACT
cloning	<i>CmCHI3</i>	ATGGGTTCTGAGATGGTGATG	CTTTACGATAGTCAACGAATC
qRT-PCR	<i>CmCHI1</i>	CCCTAGTGGCTCACTCACGATT	ATTTAGCAGCAGGCGATACACC
	<i>CmCHI2</i>	CGTGGATGATTCAGCAGTTTCC	GGCCCTTTGACAATATCAAGATAGA A
	<i>CmCHI3</i>	TGAGAACTGGAAAGGCAAGAA	TGTGGGAGCTGAAATAAGAGCA G
	<i>CmActin</i>	GCATCACACCTTCTACAA	CATTGTAGAAGGTGTGATG
Protein	<i>CmCHI1</i>	TGGCTGATATCGGATCCATGGGA	CGACGGAGCTCGAATTCTCATGCAAT GAAACATCGACG
expression	<i>CmCHI2</i>	TGGCTGATATCGGATCCATGGCC	CGACGGAGCTCGAATTCTCAGAATTT CAAGAGTCGTCC
	<i>CmCHI3</i>	TGGCTGATATCGGATCCATGGGT	CGACGGAGCTCGAATTCCTAAGCAAC TCTGAGATGGTG
Transgenic	<i>CmCHI1</i>	ACGGGGGACTCTAGAGGATCCA	GATCGGGGAAATTCGAGCTCTCATG TGGGAGAAACATCG
plants	<i>CmCHI2</i>	ACGGGGGACTCTAGAGGATCCA	GATCGGGGAAATTCGAGCTCTCAGA TGGCCCAAGAGTCG
	<i>CmCHI3</i>	ACGGGGGACTCTAGAGGATCCA	GATCGGGGAAATTCGAGCTCCTAAG TGGGTTCTGAGATG
Subcellular	<i>GFP(35S:GFP)</i>	CTGATTACGCTCATATGATGGT	AGGATTCAATCTTAAGTTACTTGTA GAGCAAGGGCGAG
localization	<i>(GFP)CmCHI1</i>	GACGAGCTGTACAAGCATATGG	CAACAGGATTCAATCTTAAGTCATG GAGAAACATCG
	<i>(GFP)CmCHI2</i>	GACGAGCTGTACAAGCATATGG	CAACAGGATTCAATCTTAAGTCAGA CCCAAGAGTCG
	<i>(GFP)CmCHI3</i>	GACGAGCTGTACAAGCATATGG	CAACAGGATTCAATCTTAAGCTAAG GTTCTGAGATG