OPEN ACCESS Biotechnology

ISSN 1682-296X
DOI: 10.3923/biotech.2018.

Research Article
Cloning and Expression Analysis of a *Chalcone isomerase* (*CnCHI*) Gene from *Chamaemelum nobile*

[1,2]Lanlan Wang, [2]Xiaomeng Liu, [2]Xiangxiang Meng, [1]Guangxu Wu and [2]Feng Xu

[1]College of Life Science, Yangtze University, Jingzhou, 434025 Hubei, China
[2]College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025 Hubei, China

Abstract

Background and Objective: *Chamaemelum nobile* (*C. nobile*) is a precious natural medicinal plant, with diverse functions. *Chalcone isomerase* (*CHI*) gene is one of the key enzyme genes in the synthesis pathway of flavonoids. Information on *CHI* gene in *C. nobile* is relatively lacking. The aim of this study was to characterize a *Chalcone isomerase* (*CnCHI*) gene from *C. nobile*. **Methodology:** In this study, a *CHI* gene was cloned from *C. nobile*, namely, *CnCHI* (GenBank accession No. MF784449). The expression level of *CnCHI* gene in different tissues of *C. nobile* was speculated by analyzing the FPKM value from the transcriptome data of *C. nobile*. **Results:** The sequence analysis and homologous alignment results showed that the CnCHI protein sequence was highly homologous with other CHI protein sequences. CnCHI protein has a Chalcone 3 superfamily conserved domain, highly conserved in evolution, indicating that *CnCHI* is a member of *CHI* gene family. *CnCHI* gene had the closest relationship with *Chrysanthemum morifolium CHI* gene. The analysis results of FPKM value showed that, the *CnCHI* expression in the flowers was much higher than in the roots, stems and leaves of *C. nobile*, indicating that *CnCHI* gene was a differentially expressed gene. **Conclusion:** *CnCHI* was expressed specifically in the flowers of *C. nobile*. *CnCHI* is a key enzyme gene in the synthesis pathway of flavonoids in *C. nobile*. This finding lays the foundation for the production of natural flavonoids.

Key words: *Chamaemelum nobile*, *Chalcone isomerase*, natural flavonoids, FPKM value, Chalcone 3 superfamily

Received: Accepted: Published:

Citation: Lanlan Wang, Xiaomeng Liu, Xiangxiang Meng, Guangxu Wu and Feng Xu, 2018. Cloning and expression analysis of a *Chalcone isomerase* (*CnCHI*) gene from *Chamaemelum nobile*. Biotechnology, CC: CC-CC.

Corresponding Author: Feng Xu, College of Horticulture and Gardening, Yangtze University, Jingzhou, 434025 Hubei, China Tel: +86 13177040087

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Roman chamomile (*Chamaemelum nobile*) is a perennial herb that belongs to Asteraceae. This herb has a wide range of distribution[1]. Rome chamomile is also a precious natural medicinal plant, which can produce terpenoids and flavonoids and other medicinal ingredients. It is usually considered as a preservative, fungicide, antibiotic, disinfectant and fungicide[2], with diverse functions. In recent years, researchers have made a lot of studies on terpenoids in the volatile oil from Rome chamomile. However, researches on the key enzyme genes of flavonoids metabolic pathways were lacking.

Flavonoids are a kind of natural secondary metabolites in higher plants, with many important functions. They were closely related to the resistance of plants. They also have many kinds of pharmacological effects such as elimination of oxygen free radicals and anti-cancer. In addition, they were involved in the formation of flower color[3,4]. *Chalcone isomerase* (*CHI*), also known as chalcone flavanone isomerase[5], was one of the key enzymes in the synthesis pathway of flavonoids[6]. Studies have shown that *CHI* was an early enzyme in the metabolic pathway of flavonoids and plays an important catalytic role in the formation of flavonoids[7]. *CHI* gene is widespread in plants. At present, *CHI* gene has been cloned in many plants, such as *Arabidopsis thaliana*[8], *Saussurea medusa*[9], *Glycine max*[10], *Medicago sativa*[11], *Lilium brownii*[12] and peanut (*Arachis hypogaea*)[13]. The studies on *Ginkgo biloba*[5] showed that the activity of *CHI* and the accumulation of flavonoids in ginkgo leaves were positively correlated with the expression of *CHI* gene. The expression level of *CHI* gene not only affected the flower color but also affected the synthesis of flavonoids metabolites. *CHI* gene played an important role in regulating the accumulation of flavonoids[14]. The expression of *CHI* gene could change the flower color of *Brunfelsia acuminata*[15].

In this study, the *CnCHI* gene was cloned from *C. nobile* to investigate key enzyme genes in the synthesis pathway of flavonoids. The expression level of *CnCHI* gene in different tissues was analyzed by FPKM value of the transcriptome data of *C. nobile*. This finding lays the foundation for verifying the role of the *CnCHI* gene in the process of producing flavonoids and provides a theoretical basis for regulating the content of flavonoids.

MATERIALS AND METHODS

Materials: *Chamaemelum nobile*, were cultivated in the Laboratory of College of Horticulture and Gardening, Yangtze University. Roots, stems, leaves and flowers of *C. nobile* at different developmental stages were collected in April, 2017 and then, all samples were quickly frozen by liquid nitrogen and stored at -80°C for the cloning of *CnCHI* gene.

RNA extraction kit (MiniBEST Plant RNA Extraction kit), reverse transcription kit (PrimeScript™ 1st Strand cDNA Synthesis Kit), gel recovery kit (Agarose Gel DNA purification Kit Ver.4.0), ampicillin (AMP), pMD18-T cloning vector and *Escherichia coli* competent cell DH5α, DNA marker and other PCR reagents were purchased from TaKaRa, Dalian Bao Biotechnology Co. sequencing and synthesis of the primers were performed by Shanghai Sangon Biological Engineering company.

Cloning of *CnCHI* gene: According to the transcriptome data of *C. nobile* measured before, all the sequences of the *CHI* gene from *C. nobile* (Table 1) were identified. Through the comparative analysis, the sequence of Unigene (*CnCHI*) (Group1_Unigene_BMK.108109) with the highest similarity to other plant *CHI* genes was screened. Its specific primers *CnCHI*-up (5′-3′: ACTTAAATTACTACCATGGCAGCAC) and *CnCHI*-down (5′-3′: CCCTCAAACTCCTGCTTTACCCTAC) for amplification were designed according to this sequence.

Total RNA was extracted from *C. nobile* using MiniBEST Plant RNA Extraction kit. PCR system was 25 μL. The amplification program was 94°C for 3 min, 32 cycles of 94°C for 30 sec, 55.2°C for 30 sec, 72°C for 1 min, a final extension at 72°C for 10 min. After the PCR product was successfully tested with 1% agarose gel electrophoresis, the target fragment was recovered according to the instructions of the gel recovery kit. Then, the target gene fragment was ligated into the pMD18-T vector and transformed into *E. coli* DH5α. The single colony was picked and cultured. Screened positive clones were sent to Shanghai Sangon Biotech for sequencing. Bioinformatics and molecular evolution analysis of *CnCHI* gene. The sequences of *CnCHI* gene were spliced using DNAMAN V6 and analyzed by Vector NT I 11.5. The open reading frame (ORF) of *CnCHI* gene can be translated into protein. According to the deduced protein sequence, the highest similarity sequences were obtained by using the BLAST tool for homologous alignments. The molecular weight and theoretical isoelectric point of the CnCHI protein were predicted by using ExPASy online server ProtParam and other properties of the protein were analyzed online. The deduced protein sequences were also analyzed by CLUSTAL X2 and MEGA6 and the phylogenetic tree was constructed by Neighbor-Joining (NJ) method.

Expression analysis of *CnCHI* gene: Using the transcriptome data of *C. nobile* measured before as the reference data, the

Table 1: Predicted Unigene of *CHI* in *C. nobile* transcriptome

Sequence ID	Coding sequence/bp	Biological function annotation of Unigene prediction (plant)
Group1_Unigene_BMK.108109	708	*CHI* (*Chrysanthemum morifolium*)
Group1_Unigene_BMK.23668	267	*CHI* (*Saussurea medusa*)
Group1_Unigene_BMK.137526	546	*CHI* (*Saussurea medusa*)
Group1_Unigene_BMK.135984	645	*CHI* (*Saussurea medusa*)
Group1_Unigene_BMK.127903	486	*CHI* (*Gynura bicolor*)
Group1_Unigene_BMK.129289	633	*CHI-like* (*Fragaria vesca subsp. Vesca*)

Group1_Unigene_BMK.108109 is the *CHI* sequence selected in this study

gene annotations were analyzed. The expression of *CnCHI* gene was analyzed by the method of FPKM value (Fragments per kilobase of transcript per million mapped reads)[16-18]. In this study, *CnCHI* gene (Group1_Unigene_BMK.108109) was selected and the expression characteristics of *CnCHI* gene in different tissues of Rome chamomile were speculated by analyzing the FPKM value.

RESULTS

Analysis of the *CnCHI* gene sequence: The cDNA of *C. nobile* was used as the PCR template. The primers *CnCHI*-up and *CnCHI*-down were designed for PCR amplification. The sequence analysis results showed that the cDNA sequence of *CHI* gene from *C. nobile* was 744 bp in length. It contained a 708 bp ORF, encoding a putative protein of 235 amino acids (Fig. 1), namely, *CnCHI* (GenBank accession no. MF784449).

The start codon and termination codon were indicated by the box, the primers were indicated by the underline, * was at the position of the termination codon, indicating that the protein translation was complete.

Analysis of CnCHI protein: Results of the online analysis of ExPASy-ProtParam showed that the theoretical molecular weight of the CnCHI protein was 25.16 kDa and the isoelectric point was 5.18. The analysis of phosphorylation sites using NetPhos 2.0 Server indicated that many phosphorylation sites of serine and threonine and a few phosphorylation sites of tyrosine existed. Online analysis showed that CnCHI protein had no signal peptide by SignalP 4.1 Server and had no transmembrane structure by TMHMM Server v. 2.0.

The homologous alignment results of the deduced amino acid sequence of *CnCHI* with BLAST-protein on NCBI site showed that, the CnCHI protein sequence had high homology with the CHI protein sequences of other plants, with the highest similarity of 94%. The sequence similarities of CnCHI protein and other CHI proteins were as follows: *Chrysanthemum morifolium* (ABK88309.1), *Callistephus chinensis* (CAA91921.1), *Gynura bicolor* (BAJ17665.1), *Pyrus communis* (ABQ08639.1), *Prunus cerasifera* (AKV89240.1), *Prunus avium* (AJO67975.1), *Pyrus pyrifolia* (ADP09377.1), *Malus hybrid cultivar* (ACP30360.1) and *Canarium album* (AEO36936.1) were 94, 83, 78, 75, 74, 73, 73, 72 and 71%, respectively.

Table 2: Sequence accessions of *CHI* Genes

Species	Accession number
Chrysanthemum morifolium	ABK88309.1
Callistephus chinensis	CAA91921.1
Carthamus tinctorius	ALG75881.1
Glycyrrhiza uralensis	ABM66533.1
Glycine max	AAK69432.1
Medicago sativa	AAB41480.1
Oryza sativa	AAO65886.1
Hordeum vulgare	AID60040.1
Zea mays	NP_001144002.2
Olea europaea	AHI86006.1
Osmanthus fragrans	ALL27265.1
Lonicera japonica	AGE10599.1
Prunus armeniaca	AGG18091.1
Prunus persica	AJA79076.1
Prunus salicina	AKQ09549.1
Malus domestica	AAM12893.1

The conserved functional domain of the CnCHI protein was predicted online by NCBI. AlignX of Vector NT I 11.5 was used to compare the CnCHI protein sequence with other CHI protein sequences for multiple alignments (Fig. 2). The results show that CnCHI protein sequence is highly homologous with other CHI protein sequences and has a Chalcone 3 superfamily conserved domain, highly conserved in evolution, indicating that *CnCHI* is a member of *CHI* gene family.

Phylogenetic analysis of *CnCHI*: In order to study the evolutionary relationships between *CnCHI* gene and other *CHI* genes, the CHI protein sequences of other species were downloaded from GenBank (Table 2). The CLUSTAL X2 and MEGA6 software were used to construct the *CHI* phylogenetic tree by NJ method (Fig. 3). *CHI* gene phylogenetic tree showed that *CHI* genes were widely present in plants. *CnCHI* gene had the closest relationship with the *CHI* gene of *C. morifolium*, followed by *C. Chinensis* and *Carthamus tinctorius*. They were all compositae plants and classified as Asteraceae. Oleaceae, Caprifoliaceae and Rosaceae, mostly woody plants, were classified as a branch alone. Therefore,

```
1       ACTTAAATTACTACCATGGCAGCACCACCTTCAGCCACCAGTCTCAACGTCGAAAACATC
1                       M  A  A  P  P  S  A  T  S  L  N  V  E  N  I
61      GTATTTTCATCGTCCGTTAAGCCTCCTGGTCGTGCCAACACTTTGTTCCTCGGTGGTGCA
16       V  F  S  S  S  V  K  P  P  G  R  A  N  T  L  F  L  G  G  A
121     GGTGTGAGAGGTATGGAAATACAAGGTAACTTTGTTAAGTTTACGGGAATTGGTGTTTAC
36       G  V  R  G  M  E  I  Q  G  N  F  V  K  F  T  G  I  G  V  Y
181     TTAGAGGATAAAGCGATTCCGTTCCTTGCTGGCAAGTGGAAGGGGAAAACAGCTGAGGAG
56       L  E  D  K  A  I  P  F  L  A  G  K  W  K  G  K  T  A  E  E
241     TTGGTTAATTCTGTTGAGTTCTTCAGGGACATCGTTACGGGCCCCTTTGAAAAGTTTACT
76       L  V  N  S  V  E  F  F  R  D  I  V  T  G  P  F  E  K  F  T
301     CAGGTGACAATGATACTACCATTAACTGGTAAGCAATACTCTGAAAAGGTGTCTGAAATG
96      Q  V  T  M  I  L  P  L  T  G  K  Q  Y  S  E  K  V  S  E  M
361     TGCGTTGGAGTTTGGAAAGCACACGGAGCATATACAGATGCAGATGGTGCAACCATCGAC
116      C  V  G  V  W  K  A  H  G  A  Y  T  D  A  D  G  A  T  I  D
421     AAGTTTCTTGAGGTTTTCAAGGACGAAAACTTCCCACCAGGCGCCTCTATTCTCTTCACA
136      K  F  L  E  V  F  K  D  E  N  F  P  P  G  A  S  I  L  F  T
481     ACCTCGCCTGATGGTTCACTAACGATCAGCTTTTCTAAAGATGGTATAATACCGGAAGCT
156      T  S  P  D  G  S  L  T  I  S  F  S  K  D  G  I  I  P  E  A
541     GCGAACATTGTGTTAGAGAACGAAAAATTGGCACAAGCAGTTATTGAGTCGGTGATCGGG
176      A  N  I  V  L  E  N  E  K  L  A  Q  A  V  I  E  S  V  I  G
601     AAGCATGGTGTTTCTCCAGCGACCAAACAAAGTTTGGCTATAAGACTTTCAGATCTTATG
196      K  H  G  V  S  P  A  T  K  Q  S  L  A  I  R  L  S  D  L  M
661     AACCATTTTGATGAGAAAGCAACTACAGATGTTGAATCAACTCTTAGCAAAAATGGTGCG
216      N  H  F  D  E  K  A  T  T  D  V  E  S  T  L  S  K  N  G  A
721     TAGGGTAAAGCAGGAGTTTGAGGG
236      *
```

Fig. 1: Nucleotide sequence and deduced amino acid sequence of *CnCHI*

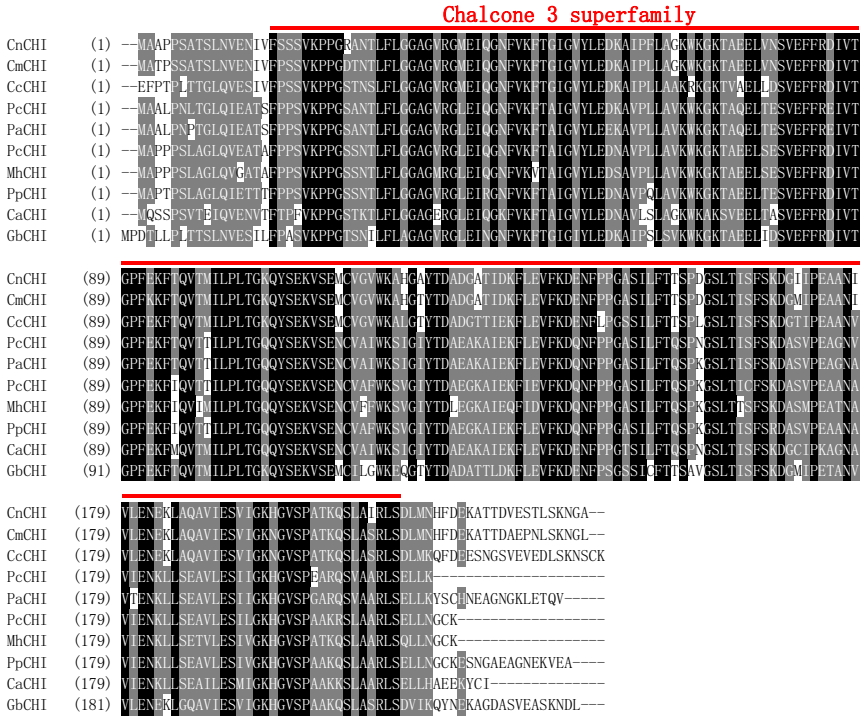

Fig. 2: Similarity analysis of *CnCHI*-coding protein and other known CHI proteins

CnCHI: Chamaemelum nobile, *CmCHI*: Chrysanthemum morifolium, *CcCHI*: Callistephus chinensis, *PcCHI*: Prunus cerasifera, *PaCHI*: Prunus avium, *PcCHI*: Pyrus communis, *MhCHI*: Malus hybrid cultivar, *PpCHI*: Pyrus pyrifolia, *CaCHI*: Canarium album, *GbCHI*: Gynura bicolor

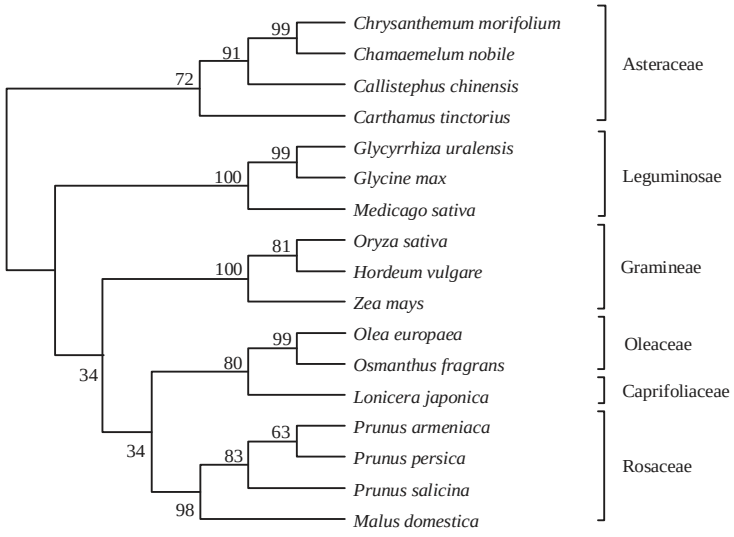

Fig. 3: Phylogenetic tree of *CHI* using Neighbor-Joining method

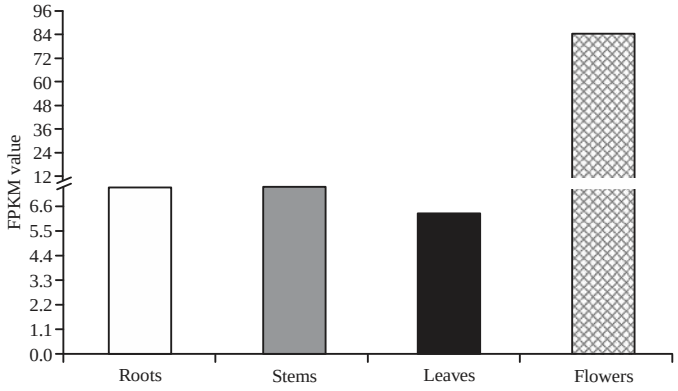

Fig. 4: FPKM value of *CnCHI* gene in different tissues of *C. nobile*

woody plants were separated from the herbaceous plants such as Asteraceae, Leguminosae and Gramineae. The genetic relationship of *CHI* genes between *C. nobile* and the woody plants was the farthest. Maybe, *C. nobile* is a herb and has a closer relationship with Gramineae and Leguminosae.

Expression analysis of *CnCHI*: The FPKM value of the transcriptome data of *C. nobile* was used to predict the expression level of *CnCHI* gene in roots, stems, leaves and flowers of *C. nobile*. The analysis results of FPKM value were shown in Fig. 4. *CnCHI* was expressed in the roots, stems, leaves and flowers. The highest *CnCHI* expression was in the flowers and the *CnCHI* expression level in the flowers was significantly higher than in the roots, stems and leaves. Furthermore, the *CnCHI* expression levels in the roots, stems and leaves were not significantly different. The *CnCHI* expression level in the flowers of *C. nobile* was very high and specific, indicating that *CnCHI* gene was a differentially expressed gene.

DISCUSSION

Phylogenetic analysis showed that *CnCHI* gene is a member of *CHI* gene family. CnCHI protein sequence was highly homologous with CHI protein sequences of other plants, with the highest similarity of 94%. The FPKM value indicated that the expression of *CnCHI* gene was the highest in the flowers and the lowest in the leaves. The expression of *CHI* gene directly affects the amount of flavonoids metabolites and also directly affects the synthesis of pigments in flowers. *CnCHI* gene may also play a crucial role in the synthesis of flavonoids. As reported, the

expression level of *G. uralensis CHI* gene affected the accumulation of flavonoids[19]. *B. acuminata CHI* gene may be associated with the formation of flower color of *B. acuminata*[15], mainly because the flower pigments, like anthocyanin, belong to flavonoids. The *MpCHI* (*Millettia pinnata*) gene could directly regulate its response to salt stress by altering its mRNA level or protein level[20]. Studies have also shown that *GbCHI* (*G. biloba*) gene was the key gene to regulate the accumulation of flavonoids in ginkgo leaves[5].

In different tissues of different plants, the expression characteristics of *CHI* gene may be different. In this study, *CnCHI* expression level in the flowers was significantly higher than in the leaves. However, the highest expression of *GbCHI* gene was in mature leaves of *G. biloba*. The reason is that its flavonoids are mainly synthesized in ginkgo leaves. Thus, to clarify the function and mechanism of *CnCHI* gene in the generation process of flavonoids in *C. nobile*, the function verification and transgenic experiments were needed to be implemented in next plan. This study will provide a theoretical basis for the production of natural flavonoids.

CONCLUSION

It is concluded that, *Chalcone isomerase* gene from *C. nobile* (*CnCHI*) was cloned at the first time. *CnCHI* contained a 708 bp ORF and encoded a deduced protein of 235 amino acids. *CnCHI* is highly conserved in evolution and is a member of CHI gene family. The *CnCHI* expression in the flowers of *C. nobile* was very high and specific. The characteristics of *CnCHI* were clarified. This finding lays the foundation for investigating the key enzyme genes in flavonoids metabolic pathways in *C. nobile*.

SIGNIFICANCE STATEMENT

This study discovers a *Chalcone isomerase* gene from *C. nobile* that can be beneficial for the production of natural flavonoids. The *CnCHI* is a key enzyme gene in the synthesis pathway of flavonoids, which is useful for increasing flavonoid content of *C. nobile*. This study will help the researcher to uncover the critical areas of key enzyme genes in the metabolic pathway of flavonoids from *C. nobile* that many researchers were not able to explore. The study may be helpful for potential application of natural flavonoids.

ACKNOWLEDGMENT

This study was supported by National Natural Science Foundation of China (No. 31400603).

REFERENCES

1. Ma, C.M., L. Winsor and M. Daneshtalab, 2007. Quantification of spiroether isomers and Herniarin of different parts of *Matricaria matricarioides* and flowers of *Chamaemelum nobile*. Phytochem. Anal., 18: 42-49.
2. Guimaraes, R., L. Barros, M. Duenas, R.C. Calhelha and A.M. Carvalho *et al*., 2013. Nutrients, phytochemicals and bioactivity of wild Roman chamomile: A comparison between the herb and its preparations. Food Chem., 136: 718-725.
3. Liu, D. and F. Wu, 2014. Cloning of *Chalcone isomerase* gene from potato onion and plant expression vector construction. Biotechnology Bulletin, Horticulture College, Northeast Agricultural University, pp: 88-93.
4. Zhang, D.Q., X.F. Tan and X.H. Wang, 2007. Gene characteristics and transgenic application of *Chalcone synthase* and *Chalcone isomerase*. J. Central South Univ. For. Technol., 2: 87-91.
5. Cheng, H., L. Li, S. Cheng, F. Cao, Y. Wang and H. Yuan, 2011. Molecular cloning and function assay of a *Chalcone isomerase* gene (*GbCHI*) from *Ginkgo biloba*. Plant Cell Rep., 30: 49-62.
6. Zhou, F., Y. Wang and Y. Chen, 2008. Advances in molecular biology of *Chalcone isomerase* in plants. J. Hebei Normal Univ. Sci. Technol., 22: 73-75.
7. Kang, J.H., J. McRoberts, F. Shi, J.E. Moreno, A.D. Jones and G.A. Howe, 2014. The flavonoid biosynthetic enzyme *Chalcone isomerase* modulates terpenoid production in glandular trichomes of tomato. Plant Physiol., 164: 1161-1174.
8. Jiang, W., Q. Yin, R. Wu, G. Zheng, J. Liu, R.A. Dixon and Y. Pang, 2015. Role of a *Chalcone isomerase*-like protein in flavonoid biosynthesis in *Arabidopsis thaliana*. J. Exp. Bot., 66: 7165-7179.
9. Zhao and Cheng, 2007. Overexpression of the *Saussurea medusa Chalcone isomerase* gene in *Saussurea involucrate* hairy root cultures enhances their biosynthesis of apigenin. Chinese Biol. Abstr., 10: 20-20.
10. Dastmalchi, M. and S. Dhaubhadel, 2015. Soybean *Chalcone isomerase*: Evolution of the fold and the differential expression and localization of the gene family. Planta, 241: 507-523.
11. Yan, Y., L. Huang and M.A. Koffas, 2007. Biosynthesis of 5-Deoxyflavanones in microorganisms. Biotechnol. J., 2: 1250-1262.
12. Dou, X., L. Lang, F. Bao, Y. Kong, H. Shang, J. Bai and N. Wang, 2015. Cloning and expression analysis of *Chalcone isomerase* gene LhCHI in oriental hybrid lily (*Lilium* spp.). J. Northeast For. Univ., 43: 6-11.
13. Zhang, Y., H. Xia, M. Yuan, C. Zhao and A. Li and X. Wang, 2012. Cloning and expression analysis of peanut (*Arachis hypogaea* L.) CHI gene. Elect. J. Biotechnol., Vol. 5.

14. Han, E.H., J.S. Lee, J.W. Lee, I.S. Chung and Y.H. Lee, 2011. Transgenic lettuce expressing *Chalcone isomerase*gene of chinese cabbage increased levels of flavonoids and polyphenols. Korean J. Horticult. Sci. Technol., 29: 467-473.
15. Cao, Y. and D. Qiu, 2012. Cloning and sequence analysis of *Chalcone isomerase* gene (CHI) from *Brunfelsia acuminate* flowers. J. Trop. Subtrop. Bot., 20: 475-481.
16. Yan, J., X. Meng and F. Xu, 2017. Molecular cloning and expression analysis of a new superoxide dismutase gene in *Chamaemelum nobile*. Chinese Trad. Herbal Drugs, 48: 1851-1859.
17. Mortazavi, A., B.A. Williams, K. McCue, L. Schaeffer and B. Wold, 2008. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature Methods, 5: 621-628.
18. Sabet, J.A., L.K. Park, L.K. Iyer, A.K. Tai and G.Y. Koh *et al*., 2016. Paternal B vitamin intake is a determinant of growth, hepatic lipid metabolism and intestinal tumor volume in female Apc1638N mouse offspring. Plos One, Vol. 11. 10.1371/journal.pone.0151579.
19. Zhang, H.C., J.M. Liu, H.Y. Lu and S.L. Gao, 2009. Enhanced flavonoid production in hairy root cultures of *Glycyrrhiza uralensis*fisch by combining the over-expression of *Chalcone isomerase* gene with the elicitation treatment. Plant Cell Rep., 28: 1205-1213.
20. Wang, H., T. Hu, J. Huang, X. Lu, B. Huang and Y. Zheng, 2013. The expression of *Millettia pinnata Chalcone isomerase* in Saccharomyces cerevisiae salt-sensitive mutants enhances salt-tolerance. Int. J. Mol. Sci., 14: 8775-8786.