

# Bioinformatics Analysis of the Glucosyltransferase Gene (*UGT74B1*) in Cabbage (*Brassica Oleracea* Var. *Capitata*)

Bo Sun, Min Jiang, Qiao Yuan, Fen Zhang and Haoru Tang <sup>a, \*</sup>

College of Horticulture, Sichuan Agricultural University, Chengdu, China

<sup>a</sup> htang@sicau.edu.cn.

**Keywords:** UGT74B1; Brassica database; loosely related to *Prunus avium*.

**Abstract.** UGT74B1 is an important glucosyltransferase enzyme in glucosinolate biosynthesis. Here, the *Brassica oleracea* var. *capitata* *UGT74B1* (*BocUGT74B1*) gene sequence was obtained from Brassica database (BRAD), and performed for bioinformatics analysis. The *BocUGT74B1* gene mapped to chromosomes 5, and contains an open reading frame of 1,311 bp that encodes a 436-amino acid protein with a calculated molecular mass of 47.69 kD and an isoelectric point (pI) of 5.24. Subcellular localization predicted the *BocUGT74B1* gene was in the chloroplast and mitochondria. The conserved domain of the *BocUGT74B1* protein is belonged the glucosyltransferase\_GTB\_type superfamily. The UGT74B1 protein is most closely related to *Prunus avium*. The findings of the present study provide a molecular basis for the elucidation of *UGT74B1* gene function in cabbage.

## 1. Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is a member of the Brassicaceae family that is widely distributed in the world. In China, cabbage is an important vegetable crop, and consumed considerable every years. Cabbage is generally grown for its leafy head as common edible part, which are crispy, tender, and tasty [1]. Besides its good flavor, cabbage is also a rich source of nutrients, antioxidants, and anticarcinogenic compounds, including carbohydrates, vitamin C, carotenoids, and glucosinolates [1-2].

Glucosinolates are a group of sulfur- and nitrogen-containing secondary metabolites that are mainly found in the order of Brassicales and related groups of dicotyledonous angiosperms [3-4]. Glucosinolates and the hydrolytic myrosinase ( $\beta$ -thioglucoside glucohydrolase) are stored separately under normal situations, but they come into contact with each other when tissues are damaged, and then the glucosinolates are hydrolyzed into several degradation products, such as isothiocyanates and nitriles [5]. Glucosinolates and their degradation products have diverse biological functions, which contribute to human health, as well as the taste and odor of cruciferous crops. The anticancer activity of isothiocyanates has been widely studied, and the mechanism involved has been elucidated [6].

Glucosinolate metabolism in plants is modulated by numerous biotic and abiotic factors, and the regulatory network of glucosinolate metabolism has been well elucidated in *Arabidopsis* [6]. UGT74B1 is a glucosyltransferase, and essential for the S-glucosylation from thiohydroxamic acid (the product of C-S lyase) to form desulfoglucosinolates [7]. The genes encoding the UGT74B1 protein have been isolated in various plant species, including *Arabidopsis* and *Brassica napus* [6]. To date, research studies on *UGT74B1* in cabbage are limited. In the present study, the *UGT74B1* gene sequence of cabbage was obtained from web database, and then bioinformatics analysis of the *UGT74B1* gene were analyzed. The present study aimed to establish the foundation for further studies on the molecular mechanism of *UGT74B1* in cabbage.

## 2. Materials and Methods

### 2.1 Sequence Obtention of the *Bocugt74b1* Gene

The genomic DNA and mRNA sequences of *UGT74B1* gene of cabbage were downloaded and obtained from The Brassica database (BRAD) (<http://brassicadb.org>), and then used to subsequent bioinformatic analysis.

## 2.2 Bioinformatics Analysis of the Bocugt74b1 Gene

The amino acid sequence, protein molecular weight, isoelectric point, stability index, and hydrophobicity of the *BocUGT74B1* gene were analyzed and predicted by ExPASy (<http://web.expasy.org>) and NCBI (<https://www.ncbi.nlm.nih.gov/>). Subcellular localization was predicted by WoLF PSORT (<http://www.genscript.com/wolf-psort.html>). The conserved domain were predicted by NCBI (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Phylogenetic tree analysis of the UGT74B1 proteins was executed in MEGA 6.0 using the neighbor-joining (NJ) method.

## 3. Results

### 3.1 Analysis an Genomic Organization

The *Brassica* database (BRAD) was used to analyze the chromosomal localization and genomic organization of *BocUGT74B1*. The gene ID in BRAD is Bol005786. The *BocUGT74B1* gene was mapped to chromosomes 5 and has 2 exons and 1 intron (Fig. 1).

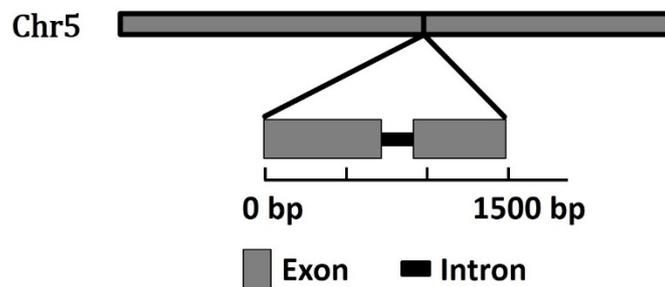


Fig. 1 Chromosomal location and genomic structure of BocUGT74B1.

### 3.2 Protein Physical and Chemical Properties Analysis

Sequence analysis indicated that the *BocUGT74B1* gene contained a 1,311-bp open reading frame (ORF), which encoded a 436-amino acids protein with a calculated molecular mass of 47.69 kD and an isoelectric point (pI) of 5.24. The amino acid types and proportions of the *BocUGT74B1* gene was shown in Figure 2, the highest number of amino acid is Serine (Ser), whereas the lowest number is Cysteine (Cys) and Tyrosine (Tyr). Its predicted formula was  $C_{2134}H_{3323}N_{559}O_{648}S_{16}$ . Its total average hydrophilicity index was -0.081, liposoluble index was 85.14, and instability index in solution was 34.6.

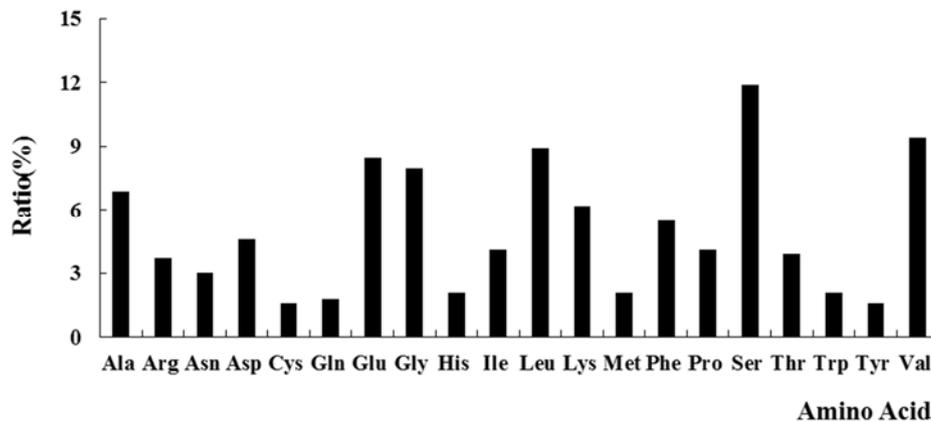


Fig. 2 Amino acid composition of BocUGT74B1

### 3.3 Subcellular Localization and Conserved Domain Analysis

Subcellular localization of the *BocUGT74B1* gene was predicted by WoLF PSORT to be in the chloroplast and mitochondria. The analysis using Conserved Domain Database (CDD) demonstrated that the amino acid sequence of the BocUGT74B1 protein belonged the glucosyltransferase\_GTB\_type superfamily.



Fig. 3 Conserved domains analysis of BocUGT74B1

### 3.4 Homology and Phylogenetic Tree Analysis

A phylogenetic tree was constructed to illustrate the relationship among the UGT74B1 proteins of cabbage and 18 other higher plant species (Fig. 4). A total of two major clusters were identified, one cluster includes Cruciferae and Rosaceae, while the other cluster includes Malvaceae, Sterculiaceae, Solanaceae, Caricaceae, Rosaceae, Cruciferae, Capparaceae, and Rhinolophidae. Sequence alignment indicated that the BocUGT74B1 protein is more closely related to *Prunus avium*.

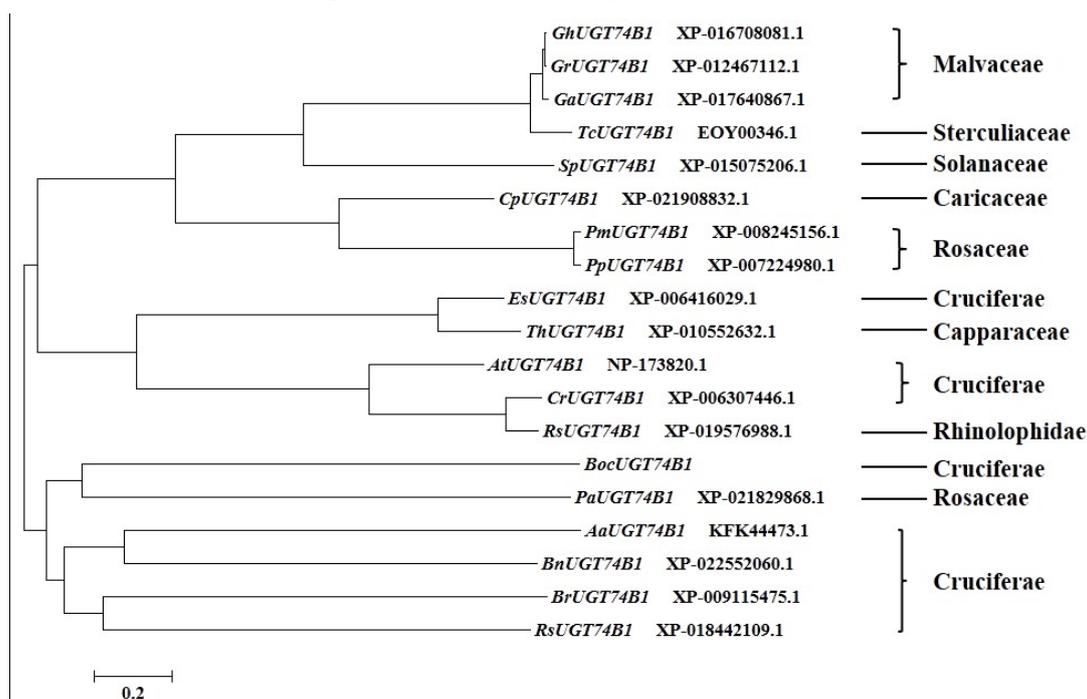


Fig. 4 Phylogenetic tree analysis of BocUGT74B1 and UGT74B1 proteins of other species

## 4. Discussion

The present study analyzed the *BocUGT74B1* gene of cabbage. UGT74B1 enzyme is encoded by a single-copy gene in *Arabidopsis thaliana* [7]. While, the *UGT74B1* gene occurred as a single copy in cabbage, indicating that the enzyme may have undergone similar evolutionary patterns. Previous studies have shown that the UGT74B1 protein is relatively conserved in plants [7]. The findings of the present study show that UGT74B1 from cabbage is highly conserved in plants, similar to that observed in earlier reports. The findings of the present study may serve as a foundation for future studies on the functions of UGT74B1 in glucosinolate metabolism in cabbage.

## Acknowledgements

Correspondence author: Haoru Tang, Tel: +86 28 86291949, E-mail addresses: htang@sicau.edu.cn. This work was supported by National Natural Science Foundation of China (31500247), key project of Department of Education of Sichuan Province (14ZA0016), and National Student Innovation Training Program (201710626030).

## References

- [1]. Wennberg, M., J. Ekvall, K. Olsson, and M. Nyman. 2006. "Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. *capitata*) during blanching and treatment with acetic acid," *Food Chem.*, 95: 226-236.

- [2]. Rokayya, S., C.J. Li, Y. Zhao, Y. Li, and C. H. Sun. 2014. "Cabbage (*Brassica oleracea* L. var. *capitata*) phytochemicals with antioxidant and anti-inflammatory potential," *Asian Pac. J. Cancer Prev.*, 14: 6657-6662.
- [3]. Hansen, B. G., R. E. Kerwin, J. A. Ober, V. M. Lambrix, T. Mitchell-Olds, J. Gershenzon, B. A. Halkier, and D. J. Kliebenstein. 2008. "A novel 2-oxoacid-dependent dioxygenase involved in the formation of the goiterogenic 2-hydroxybut-3-enyl glucosinolate and generalist insect resistance in *Arabidopsis*," *Plant Physiol.*, 148(4): 2096-2108.
- [4]. Yan, X., and S. Chen. 2007. "Regulation of plant glucosinolate metabolism," *Planta*, 226(6): 1343-1352.
- [5]. Andréasson, E., L. B. Jørgensen, A.-S. Höglund, L. Rask, and J. Meijer, 2001. "Different myrosinase and idioblast distribution in *Arabidopsis* and *Brassica napus*," *Plant Physiol.*, 127(4): 1750-1763.
- [6]. Benkeblia, N. 2017. *Phytonutritional Improvement of Crops*. John Wiley & Sons, Inc., pp. 407-733.
- [7]. Grubb, C. D., B. J. Zipp, J. Ludwig - Müller, M. N. Masuno, T. F. Molinski, and S. Abel. 2004. "Arabidopsis glucosyltransferase UGT74B1 functions in glucosinolate biosynthesis and auxin homeostasis," *Plant J.*, 40(6): 893-908.