Plant Physiology and Biochemistry 136 (2019) 204–214

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Review

Molecular identification of the magnesium transport gene family in *Brassica napus*

Lin Zhang[a], Anning Wen[a], Xiaoqi Wu[a], Xiaoling Pan[a], Nan Wu[a], Xuan Chen[a], Yan Chen[a], Dandan Mao[a,***], Liangbi Chen[a,**], Sheng Luan[a,b,*]

[a] College of Life Sciences, Hunan Normal University, Changsha, 410081, China
[b] Department of Plant and Microbial Biology, University of California, Berkeley, 94720, California, USA

ARTICLE INFO

Keywords:
Brassica napus
BnMGT
Magnesium transport
Phylogeny
Expression pattern

ABSTRACT

Magnesium (Mg^{2+}) is an essential element for plant growth. Its transport and homeostasis in plants is mainly maintained by the *MRS2/MGT* of Mg^{2+} transporters. Little is known about the *MRS2/MGT* gene family in *Brassica napus* L. (*B. napus*), one of the most important oil grains. In our present study, we identified 36 putative *MRS2/MGT* genes (*BnMGTs*) from *B. napus* and investigated their phylogeny, expression pattern and function. These *BnMGT* genes were sorted into five distinguished groups by the phylogenetic analysis, and they were clearly homologous with the *MRS2/MGT* genes in *Arabidopsis* and rice. Complementation assays using the *Salmonella typhimurium* mutant MM281 demonstrated that the *BnMGT* genes were capable of mediating Mg^{2+} uptake and transport, with varied affinities to Mg^{2+}. The expression pattern analysis showed that the expression of *BnMGTs* were tissue-specific and varied in different tissues. This work provides the molecular basis to discover the function of *BnMGT* gene family in plant growth and development.

1. Introduction

Magnesium (Mg^{2+}) is required for proper operation of numerous cellular activities related to chlorophyll synthesis, membrane stability and enzyme activation (Guo et al., 2016; Hortensteiner, 2009; Shaul, 2002; Williams and Salt, 2009). Nevertheless, the molecular mechanisms related to magnesium transport in plant remain poorly understood.

Several protein types were identified having a role in Mg^{2+} transport across the membrane. The bacterial CorA protein is a major Mg^{2+} uptake system (Knoop et al., 2005). Homologues of CorA have been identified from fungi, animals and plants. Unique topological analyses of CorA protein have shown that its N-terminal region is a large, acidic periplasmic and its C-terminal region contains two transmembrane domains (TM) (Smith et al., 1993). CorA possesses a GMN motif in the first transmembrane domain, which is indispensable to Mg^{2+} transport (Szegedy and Maguire, 1999). The CorA crystal structure analysis from *Thermotoga maritima* revealed that CorA is a pentameric cone-shaped channel (Payandeh and Pai, 2006; Lunin et al., 2006; Eshaghi et al., 2006).

In plants, the important functions of the *CorA*-like family have been identified from *Arabidopsis* and rice. In *Arabidopsis*, 10 *CorA*-like genes were discovered and annotated as *AtMRS2* or *AtMGT* gene family (Schock et al., 2000; Li et al., 2001). Using bacteria and yeast *MGT* mutants lacking Mg^{2+} transporters, it was revealed that nine of those genes participate in Mg^{2+} transport, *AtMGT8/AtMRS2-9* is a pseudogene, and their capacities for Mg^{2+} transport vary from high (*MGT1* and *MGT10*) to low (*MGT6, MGT7* and *MGT9*) and dual affinity (*MGT5*) (Li et al., 2001, 2008; Schock et al., 2000; Chen et al., 2009; Gebert et al., 2009; Mao et al., 2008). In rice, this gene family consists of nine members. Among those members, enhanced expression of the *OsMRS2-2/OsMGT1* is associated with aluminum tolerance in rice (Saito et al., 2013).

Brassica napus L. is one of the most important oil grains. Many metabolic processes in *B. napus* are affected by Mg^{2+} deficiency. For example, chlorophyll synthesis, photosynthesis, carbon fixation *etc*. are affected by Mg^{2+}-limited and ultimately resulting in yield loss. Though the roles of *MRS2/MGT* transporters have been studied extensively in *Arabidopsis* and rice, information about this gene family in *B. napus* is largely undefined. Here, we conducted a detailed identification and systematic analysis of the gene structure, chromosomal locations, conserved motifs, phylogenetic relationships, duplication events and expression patterns, as well as functional analyses of the *MGT* family in *B.*

---

[*] Corresponding author. College of Life Sciences, Hunan Normal University, Changsha, 410081, China.
[**] Corresponding author.
[***] Corresponding author.
E-mail addresses: mdd0303@163.com (D. Mao), chenliangbi@126.com (L. Chen), sluan@berkeley.edu (S. Luan).

https://doi.org/10.1016/j.plaphy.2019.01.017
Received 14 October 2018; Received in revised form 14 January 2019; Accepted 14 January 2019

Table 1
List of *CorA/MRS2/MGT* genes in the *B. napus* genome.

Gene name[a]	Accession number[b]	Chr	Protein	Mass (kDa)	pl	TM domains	Predicted location[c]
			Length (no.of amino acids)				
BnMGT1.1a	BnaA07g35210D	A07	410	46.33	4.85	2	Plas
BnMGT1.2a	BnaA02g19370D	A02	406	45.96	5.57	2	Plas
BnMGT1.1c	BnaC02g25960D	C02	405	45.89	5.57	2	Plas
BnMGT1.2c	BnaC06g40130D	C06	410	46.45	4.90	2	Plas
BnMGT2a	BnaA06g10710D	A06	420	47.44	5.21	2	Plas
BnMGT2c	BnaC05g12220D	C05	418	47.51	5.99	2	Plas
BnMGT3a	BnaA06g39860D	A06	421	46.91	4.95	2	Nucl
BnMGT3.1c	BnaC06g06680D	C06	421	46.94	4.94	2	Nucl
BnMGT3.2c	BnaC07g22430D	C07	421	46.97	4.94	2	Nucl
BnMGT4.1a	BnaA01g25990D	A01	456	50.32	5.54	2	Golg
BnMGT4.2a	BnaAnng21960D	Ann	441	49.16	5.18	2	Chlo
BnMGT4.1c	BnaC01g33220D	C01	455	50.26	5.65	2	Golg
BnMGT4.2c	BnaCnng33870D	Cnn	730	82.88	6.53	2	Nucl
BnMGT5a	BnaA04g07170D	A04	378	42.91	8.14	2	Chlo
BnMGT5c	BnaC04g29360D	C04	371	42.16	6.41	2	Golg
BnMGT6.1a	BnaA09g55390D	A09	429	48.18	5.19	2	Chlo
BnMGT6.2a	BnaA05g21170D	A05	420	47.18	4.92	2	Chlo
BnMGT6.1c	BnaC05g34100D	C05	419	47.03	5.04	2	Chlo
BnMGT6.2c	BnaCnng47920D	Cnn	429	48.08	5.17	2	Chlo
BnMGT7a	BnaA02g00130D	A02	389	44.00	4.77	2	Chlo
BnMGT7.1c	BnaCnng45320D	Cnn	389	43.93	4.81	2	Chlo
BnMGT7.2c	BnaCnng45330D	Cnn	366	41.66	4.76	2	Chlo
BnMGT9.1a	BnaA09g06890D	A09	384	43.12	5.03	2	Chlo
BnMGT9.2a	BnaA06g23540D	A06	378	42.16	4.90	2	Chlo
BnMGT9.3a	BnaA02g34270D	A02	384	42.83	5.16	2	Chlo/ER
BnMGT9.1c	BnaC09g06390D	C09	370	41.67	5.05	2	Chlo
BnMGT9.2c	BnaCnng30470D	Cnn	378	42.15	4.90	2	Chlo
BnMGT9.3c	BnaC02g43190D	C02	375	42.11	5.22	2	ER
BnMGT10.1a	BnaA10g13720D	A10	465	51.64	5.70	2	Plas
BnMGT10.2a	BnaA07g04620D	A07	436	49.56	7.69	2	Plas
BnMGT10.3a	BnaA04g24710D	A04	505	57.55	5.69	2	Plas
BnMGT10.4a	BnaA08g17680D	A08	535	60.23	6.04	2	Plas
BnMGT10.1c	BnaC09g36230D	C09	458	50.84	5.81	2	Plas
BnMGT10.2c	BnaC04g48600D	C04	487	55.24	5.74	2	Plas/ER
BnMGT10.3c	BnaC03g59500D	C03	535	60.22	6.04	2	Plas
BnMGT10.4c	BnaCnng00560D	Cnn	436	49.57	8.15	2	Plas

[a] Name assigned to Brassica napus *CorA/MRS2/MGT* genes in this research.
[b] Accession numbers was obtained from *Brassica napus* genome database.
[c] WoLF PSORT predictions.

napus. Our results will aid further functional identification of Mg^{2+} transport-related genes in *B. napus* and enhance our understanding of the functions of Mg^{2+} transporters in plants.

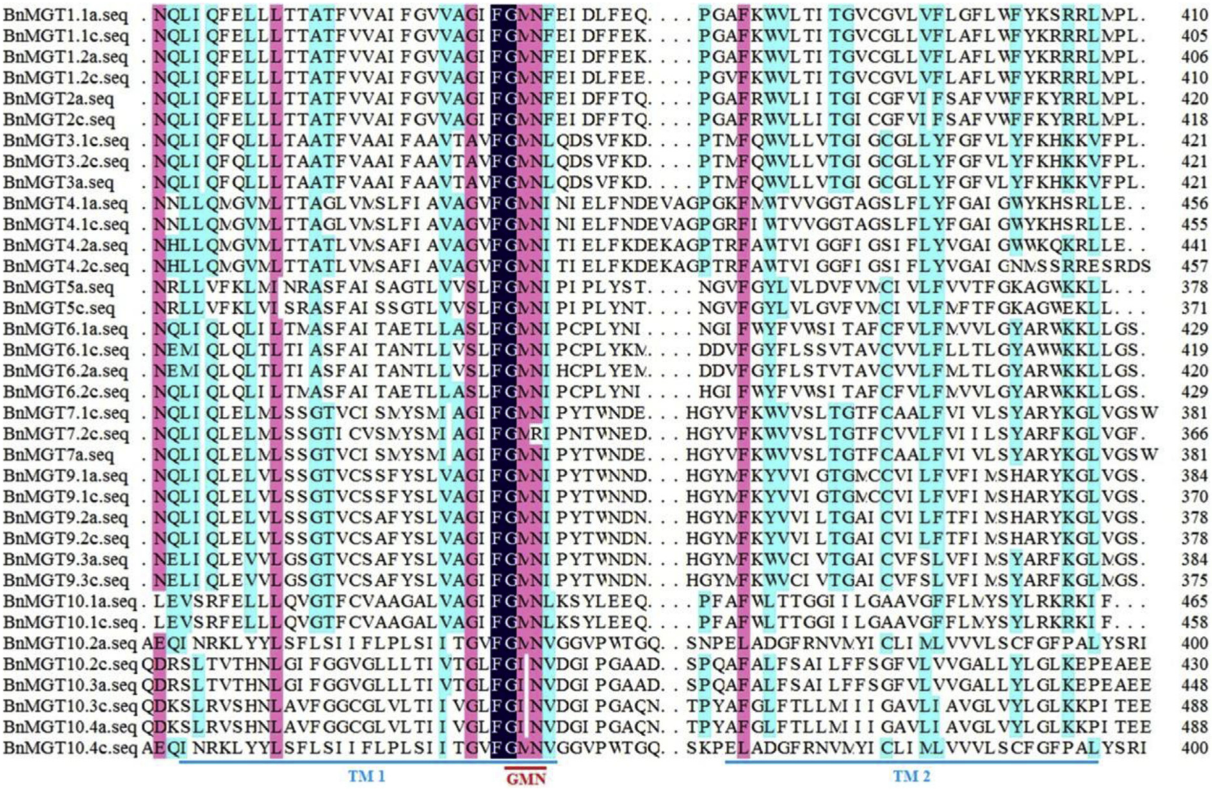
2. Materials and methods

2.1. Identification and annotation of MRS2/MGT genes in B. napus

By using the TAIR (https://www.arabidopsis.org/) and TIGR (http://rice.plantbiology.msu.edu) databases, we obtained the sequences of 10 *Arabidopsis thaliana* and nine rice MRS2/MGT proteins. The candidate *MRS2/MGT* genes in *B. napus* were identified by using protein BLAST (https://www.ncbi.nlm.nih.gov/) to search the *Brassica* database (http://brassicadb.org/brad/) and using *Arabidopsis* and rice MRS2/MGT protein sequences as queries and an E-value $\leq 1 \times 10^{-20}$ as a threshold. The HMM profiles of the CorA domains (PF01544), which contains two transmembrane domains and a conserved GMN domain downloaded from the Pfam database (http://pfam.xfam.org/) were applied to search for the CorA-type sequences in the *B. napus* genome (http://www.genoscope.cns.fr/brassicanapus/) by performing local HMMER search program. All candidate protein sequences searched by HMMER were used as queries and placed in the Interpro online program (http://www.ebi.ac.uk/interpro/) to verify the existence of the CorA domain. *Brassica rapa* (*B.rapa*) and *Brasscia oleracea* (*B.oleracea*) genome databases were also used to identify the CorA/MRS2/MGT proteins (Supplementary Tables S1 and S2) by using the local HHMER program. *MRS2/MGT* gene accession numbers were extracted from the *B. napus* genom edatabase (http://www.genoscope.cns.fr/brassicanapus/). We designated names of all putative *MRS2/MGT* genes in *B. napus* according to the homologous gene IDs in *Arabidopsis* and the chromosomal location in the *Brassica* A genome or C genome. For one *MRS2/MGT* gene in *Arabidopsis*, the orthologous *MRS2/MGT* genes in *B. napus* were named alphabetically. The possible TM domains of the BnMGT proteins were predicted with TMHMM Server 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Horton et al., 2007). The subcellular localization of BnMGT proteins were predicted with the WoLF PSORT online tool (https://www.genscript.com/wolf-psort.html) (Zhao et al., 2012).

2.2. Chromosomal location and duplication of MGT genes in B. napus

Characterization of the chromosomal location of each *BnMGT* gene was acquired from the *B. napus* genome database. Based on the start and stop codon position information of each *BnMGT* gene on the chromosomes, all the *BnMGT* genes were located on the A and C genome chromosomes. Chromosomal location images of *BnMGT* genes were generated using the MGC2 online program (http://mg2c.iask.in/mg2c_v2.0/). Genes located surrounding and parted by less than five genes can be as tandem duplications. The segmental duplications were analyzed by the Maher's study (Maher et al., 2006).



```
BnMGT1.1a.seq  .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDLFEQ....PGAFKWVLTITGVCGVLVFLGFLWFYKSRRLMPL.  410
BnMGT1.1c.seq  .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDFFEK....PGAFKWVLTITGVCGLLVFLAFLWFYKRRRLMPL.  405
BnMGT1.2a.seq  .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDFFEK....PGAFKWVLTITGVCGLLVFLAFLWFYKRRRLMPL.  406
BnMGT1.2c.seq  .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDLFEE....PGVFKWVLTITGVCGVLVFLAFLWFYKRRRLMPL.  410
BnMGT2a.seq    .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDFFTQ....PGAFRWVLIITGICGFVIFSAFVWFFKYRRLMPL.  420
BnMGT2c.seq    .NQLIQFELLLTTATFVVAIFGVVAGIFGMNFEIDFFTQ....PGAFRWVLLITGICGFVIFSAFVWFFKYRRLMPL.  418
BnMGT3.1c.seq  .NQLIQFQLLLTAATFVAAIFAAVTAVFGMNLQDSVFKD....PTMFQWVLLVTGIGCGLLYFGFVLYFKHKKVFPL.  421
BnMGT3.2c.seq  .NQLIQFQLLLTAATFVAAIFAAVTAVFGMNLQDSVFKD....PTMFQWVLLVTGIGCGLLYFGFVLYFKHKKVFPL.  421
BnMGT3a.seq    .NQLIQFQLLLTAATFVAAIFAAVTAVFGMNLQDSVFKD....PTMFQWVLLVTGIGCGLLYFGFVLYFKHKKVFPL.  421
BnMGT4.1a.seq  .NNLLQMGVMLTTAGLVMSLFIAVAGVFGMNINIELFNDEVAGPGKFMWTVVGGTAGSLFLYFGAIGWYKHSRLLE..  456
BnMGT4.1c.seq  .NNLLQMGVMLTTAGLVMSLFIAVAGVFGMNINIELFNDEVAGPGRFIWTVVGGTAGSLFLYFGAIGWYKHSRLLE..  455
BnMGT4.2a.seq  .NHLLQMGVMLTTATLVMSAFIAVAGVFGMNITIELFKDEKAGPTRFAWTVIGGFIGSIFLYVGAIGWWKQKRLLE..  441
BnMGT4.2c.seq  .NHLLQMGVMLTTATLVMSAFIAVAGVFGMNITIELFKDEKAGPTRFAWTVIGGFIGSIFLYVGAIGNMSSRRESRDS  457
BnMGT5a.seq    .NRLLVFKLMNRASFAISAGTLVVSLFGMNIPIPLYST....NGVFGYLVLDVFVMCIVLFVVTFGKAGWKKLL...  378
BnMGT5c.seq    .NRLLVFKLVISRASFAISSGTLVVSLFGMNIPIPLYNT....NGVFGYLVLGVFVMCIVLFMFTFGKAGWEKLL...  371
BnMGT6.1a.seq  .NQLIQLQLILTMASFAITAETLLASLFGMNIPCPLYNI....NGIFWYFVWSITAFCFVLFMVVLGYARWKKLLGS.  429
BnMGT6.1c.seq  .NEMIQLQLTLTIASFAITANTLLVSLFGMNIPCPLYKM....DDVFGYFLSSVTAVCVVLFLLTLGYAWWKKLLGS.  419
BnMGT6.2a.seq  .NEMIQLQLTLTIASFAITANTLLVSLFGMNIHCPLYEM....DDVFGYFLSTVTAVCVVLFMLTLGYARWKKLLGS.  420
BnMGT6.2c.seq  .NQLIQLQLILTMASFAITAETLLASLFGMNIPCPLYNI....HGIFWYFVWSITAFCFVLFMVVLGYARWKKLLGS.  429
BnMGT7.1c.seq  .NQLIQLELMLSSGTVCISMYSMIAGIFGMNIPYTWNDE...HGYVFKWVVSLTGTFCAALFVIVLSYARYKGLVGSW  381
BnMGT7.2c.seq  .NQLIQLELMLSSGTICVSMYSMIAGIFGMRIPNTWNED...HGYVFKWVVSLTGTFCVVLFVIILSYARFKGLVGF.  366
BnMGT7a.seq    .NQLIQLELMLSSGTVCISMYSMIAGIFGMNIPYTWNDE...HGYVFKWVVSLTGTFCAALFVIVLSYARYKGLVGSW  381
BnMGT9.1a.seq  .NQLIQLELVLSSGTVCSSFYSLVAGIFGMNIPYTWNND...HGYMFKYVVIGTGMCCVILFVFIMSHARYKGLVGS.  384
BnMGT9.1c.seq  .NQLIQLELVLSSGTVCSSFYSLVAGIFGMNIPYTWNND...HGYMFKYVVIGTGMCCVILFVFIMSHARYKGLVGS.  370
BnMGT9.2a.seq  .NQLIQLELVLSSGTVCSAFYSLVAGIFGMNIPYTWNDN...HGYMFKYVVILTGAICVILFTFIMSHARYKGLVGS.  378
BnMGT9.2c.seq  .NQLIQLELVLSSGTVCSAFYSLVAGIFGMNIPYTWNDN...HGYMFKYVVILTGAICVILFTFIMSHARYKGLVGS.  378
BnMGT9.3a.seq  .NELIQLEVVLGSGTVCSAFYSLVAGIFGMNIPYTWNDN...HGYMFKWVCIVTGAICVFSLVFIMSYARFKGLMGS.  384
BnMGT9.3c.seq  .NELIQLEVVLGSGTVCSAFYSLVAGIFGMNIPYTWNDN...HGYMFKWVCIVTGAICVFSLVFIMSYARFKGLMGS.  375
BnMGT10.1a.seq .LEVSRFELLLQVGTFCVAAGALVAGIFGMNLKSYLEEQ....PFAFWLTTGGIILGAAVGFFLMYSYLRKRKIF...  465
BnMGT10.1c.seq .LEVSRFELLLQVGTFCVAAGALVAGIFGMNLKSYLEEQ....PFAFWLTTGGIILGAAVGFFLMYSYLRKRKIF...  458
BnMGT10.2a.seq AEQINRKLYYLSFLSIIFLPLSIITGVFGMNVGGVPWTGQ..SNPELADGFRNVMYICLIMLVVVLSCFGFPALYSRI  400
BnMGT10.2c.seq QDRSLTVTHNLGIFGGVGLLLTIVTGLFGINVDGIPGAAD..SPQAFALFSAILFFSGFVLVVGALLYLGLKEPEAEE  430
BnMGT10.3a.seq QDRSLTVTHNLGIFGGVGLLLTIVTGLFGINVDGIPGAAD..SPQAFALFSAILFFSGFVLVVGALLYLGLKEPEAEE  448
BnMGT10.3c.seq QDKSLRVSHNLAVFGGCGLVLTIIVGLFGINVDGIPGACN..TPYAFGLFTLLMIIGAVLIAVGLVYLGLKKPITEE  488
BnMGT10.4a.seq QDKSLRVSHNLAVFGGCGLVLTIIVGLFGINVDGIPGACN..TPYAFGLFTLLMIIGAVLIAVGLVYLGLKKPITEE  488
BnMGT10.4c.seq AEQINRKLYYLSFLSIIFLPLSIITGVFGMNVGGVPWTGQ..SKPELADGFRNVMYICLIMLVVVLSCFGFPALYSRI  400
```

Fig. 1. Protein sequences alignment of BnMGT family members. The alignment of the 36 sequences was executed and generated with DNAMAN software. Two TM domains were shown in azury lines. The GMN motifs were marked with pink.

2.3. Gene structure, conserved domain and phylogenetic analysis

ClustalW program implemented in MEGA7.0 (Kumar et al., 2016) was used for sequence alignment and polymorphism analysis (Larkin et al., 2007). Two TM domains and the GMN motifs were marked according to the TMHMM analysis and the sequences alignment using the DNAMAN software. The phylogenetic tree was drawed with the neighbor-joining way by MEGA 7.0 and iTOL4.0 online program (http://itol.embl.de/). The Exon and intron structure of each *BnMGT* gene were constructed with MEME (http://meme-suite.org/tools/meme) in the way of blasting the coding sequence and complete genomic sequence. Meanwhile, the conserved motifs were analyzed and searched by the MEME program (http://meme-suite.org/). All the identified motif logos were produced by the Weblogo3.0 online program (http://weblogo.threeplusone.com/).

2.4. Plant materials and growth conditions

Plant samples used for expression profile analysis and RNA extraction were collected from *B. napus* 'Zhongshuang 11' (ZS11). Rape seeds were supplied by the Hunan Normal University. The crop was cultivated in a test field of the Hunan Normal University. To analyze the expression pattern of *BnMGT* genes in different tissues, four different tissue samples (root, stem, leaf, and flower) were collected from plants at the blooming stage, and capsule and embryo were collected at 14 days after flowering. Three-leaf-stage seedlings were cultivated in the hydroponic system under long-day conditions which was in line with the setting of 16 h light, 8 h dark and 5000 Lux illumination intensity at 25 °C. All samples were collected and stored in the −80 °C.

2.5. Cloning of BnMGT genes

Total RNA was extracted via Trizol extraction method from different tissues at the blooming stage or from seedlings in the three-leaf stage according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized from RNA by reverse transcription PCR (RT-PCR) with a Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA after reverse transcription was used as template for real-time quantitative RT-PCR or amplification of the *BnMGT* coding sequence.

The opening reading frames (ORFs) of the 36 BnMGT genes were identified based on the *B. napus* genome database (http://www.genoscope.cns.fr/brassicanapus/). Clones of *BnMGT* genes were obtained by amplification using specific primers (Supplementary Table S3). Total RNA was extracted from seedling samples and used for RT-PCR. The amplification reactions were operated in a 25 μL reaction system in a Bio-Rad C 1000 thermo cycler. The PCR products were purified with a Gel DNA Purification Kit (Sangon Biotech, Shanghai, China). The purified CDS sequences were linked with the pMD18-T vector (TAKARA, Otsu, Japan) and identified by sequencing.

2.6. Functional complementation analysis of BnMGT genes

To explore the Mg^{2+} transport activities of *BnMGT* members, the opening reading frames (ORFs) from a representative of each clade were cloned into a pTrc99A vector using specific primers (Supplementary Table S4). Empty pTrc99A vector and the eight plasmid-linked ORFs were transformed into *Salmonella typhimurium* MM281 cells by electroporation. The transformants were verified by PCR amplification and sequencing. MM281 strains and MM281 strains transformed with an empty pTrc99A vector were used as negative controls. The specific operation process were as described in previous study (Li et al., 2001).

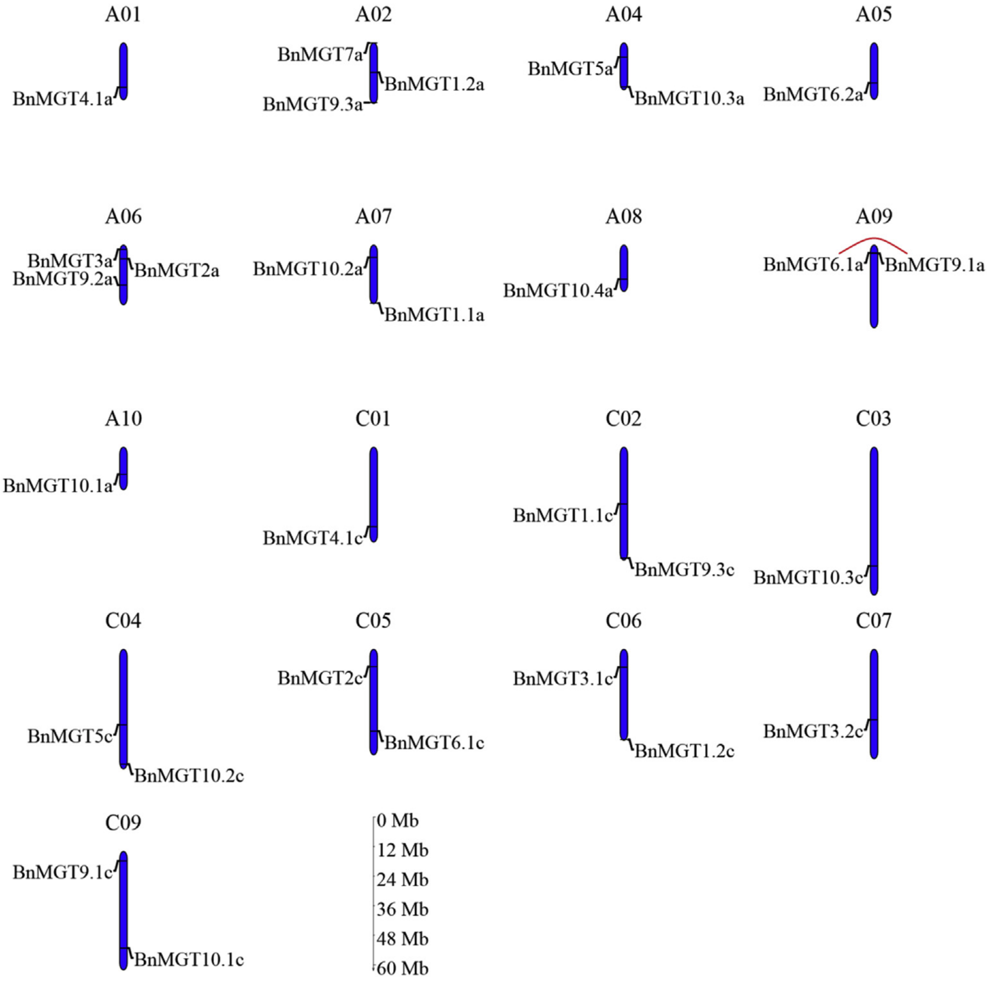


Fig. 2. Chromosomal localizations and gene duplication of *BnMGT* genes. The 36 BnMGT genes were widely mapped to 17 of the 19 *B. napus* chromosomes. Two tandem duplication genes were shown with red line, the scale bar is showed in the figure.

2.7. Real-time quantitative PCR analysis of BnMGT genes

The cDNA was synthesized from RNA by reverse transcription PCR (RT-PCR) with a Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time quantitative PCR (qRT-PCR) with three biological replicates operated by Applied Biosystems Quant Studio (Thermo Fisher Scientific, USA) were performed by using PowerUp SYBR Green Master Mix in a 20 μL reaction system. Specific primers for Real-time quantitative PCR are showed in Supplementary Table S7, *BnUBC21* was used as the inner control gene.

3. Results

3.1. Identification of MRS2/MGT family genes in Brassica napus

Using 10 AtMGT protein sequences in *Arabidopsis* and 9 OsMGT protein sequences in rice as queries for TBLASTN search, we identified 30 putative MRS2/MGT-type genes in the *B. napus* genome. HMMER search of the *B. napus* protein database with CorA-domain PF01544 as a query identified other six different MRS2/MGT-type protein sequences in the *B. napus* genome. All the putative sequences were identified by Interpro online program to verify the CorA domain. Finally, 36*MRS2/MGT*-type genes, named *BnMGT*, were identified from *B. napus* (Table 1; Fig. 1). In addition, based on the order of closest orthologues of *Arabidopsis*, the homologues of *AtMGT8* were not found as shown in Table 1.

These BnMGT proteins were 366–730 amino acids long, all the BnMGT proteins have two TM domains at the C-terminal region according to the TMHMM analysis. The blast search of all BnMGT protein sequences using DNAMAN multiple sequence alignment identified a conserved GMN motif, as a representative feature of CorA/MRS2/MGT-type proteins, in 31 BnMGT proteins. This motif was altered to GMR (Gly-Met-Arg) in BnMGT7.2c and GIN (Gly-Ile-Asn) in BnMGT10.2c, BnMGT10.3a, BnMGT10.3c, and BnMGT10.4a (Fig. 1), these alterations were proposed to be associated with cation selectivity (Sponder et al., 2013). These results confirmed that the identified genes belonged to the *BnMGT* family.

3.2. Chromosomal location, duplication patterns and gene structure

Chromosome location and gene duplication of *BnMGT* genes were determined based on the start and stop codon position information of each *BnMGT* gene on the chromosomes *B. napus* (AACC, 2n = 38) is one of the three diploid species of *Brassica*. It is an allotetraploid hybrid species of *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18). All the *BnMGT* genes were located on chromosomes of the A and C genome, 29 BnMGT genes located on A or C genome were mapped to corresponding chromosome positions as shown in Fig. 2 according to the position of the start and stop codons (Supplementary Table S6), whereas the additional seven *BnMGT* genes could not be mapped accurately to any *B. napus* chromosome (Table 1; Fig. 2). These 29 BnMGT genes were distributed on *Brassica* chromosomes unevenly. Except for chromosomes A3 and C8, all the chromosomes harbored at least one *BnMGT* gene. There were 17 and 19 BnMGT genes located on the genome A and

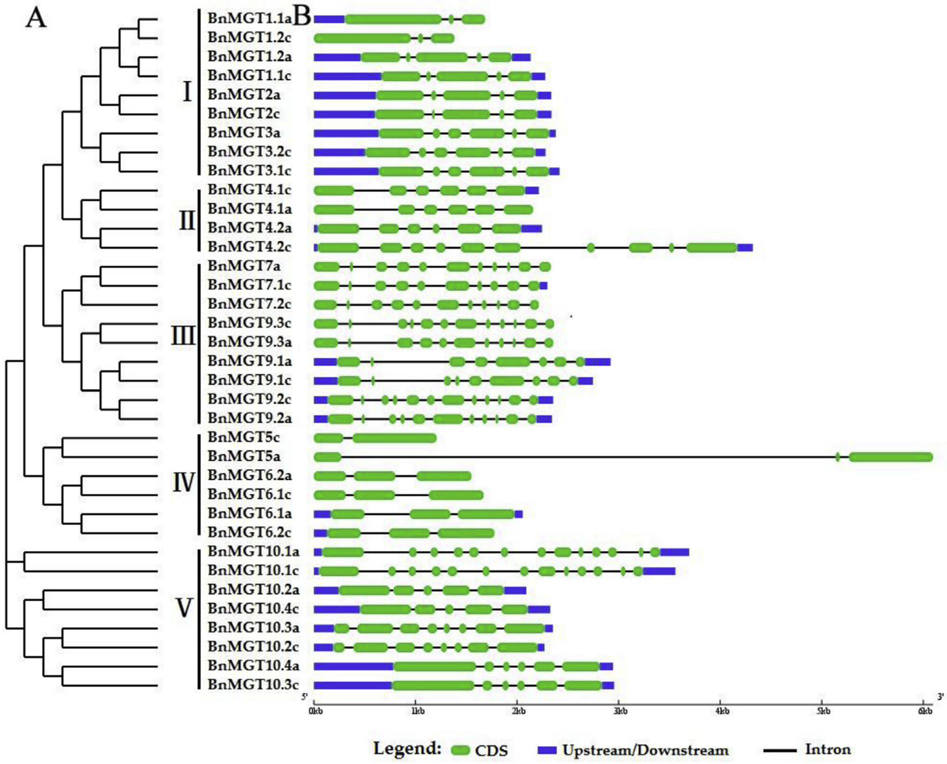


Fig. 3. Phylogenetic relationships and gene structures of the *BnMGT* genes. (A) The phylogenetic tree was generated in the MEGA 7.0 software by using the Neighbor–Joining method. (B) Structures analysis of *BnMGT* genes. Exons and introns were marked with green boxes and black lines, respectively. The scale bar was drawed at bottom.

genome C of *B. napus,* respectively (Table 1; Fig. 2). These gene numbers are different from the number of genes found in the *B. rapa* and *B. oleracea* genomes, indicating that the *MRS2/MGT*-type gene family in *B. napus* underwent expansion and evolution when compared with its ancestor species, *B. rapa and B. oleracea*.

The duplication patterns of the 36 BnMGT genes were also considered. Among the *BnMGT* genes, only *BnMGT6.1a* and *BnMGT9.1a* displayed tandem repeats which belonging to the same tandem gene cluster (Fig. 2). In addition, 27 of the 36 BnMGT genes were segmentally duplicated genes (Fig. S2). These genes were mapped on 16 different chromosomes (Table 1; Fig. 2). Thus, segmental duplications played a more important role in the expansion of the *BnMGT* gene family than did tandem duplications.

Chromosomal location and gene duplication events of *B. napus* suggest that, after the generation of *B. napus* from a cross between *B. rapa* and *B. oleracea* and under different environmental pressures, new family members were generated in the *BnMGT* gene family of *B. napus*. This lead to variations in gene numbers among *B. napus*, *B. rapa*, and *B. oleracea* (Supplementary Tables S1 and S2).

To further investigate the gene structure, the coding sequences and genomic sequences of the *BnMGT* genes were aligned in ClustalW 2.0 and the conserved domains logo was generated by the Weblogo online program. The number of introns varied from 2 to 12, with groups III and V having the largest numbers of introns. *BnMGT* genes from the same group exhibited a similar exon-intron structures (Fig. 3B). The analysis of conserved domains are important for investigating the functions of the BnMGT family. Using MEME online tool identified 10 motifs among the BnMGT proteins in *B. napus* (Fig. 4), the all motifs were annotated by SMART program (http://smart.embl-heidelberg.de/). Motif 2 contains a Cir_N domain (N-terminal domain of CBF1 interacting co-repressor CIR) and a SAP domain (DNA-binding motif predicted to be involved in chromosomal organization), motif 5 contains a CorA domain. The CorA domain has a highly charged N-terminal domain and two transmembrane domains on its C-terminal region (Fig. 5). The first transmembrane domain contains a conserved GMN motif which are essential for functional Mg^{2+} transport (Szegedy and Maguire, 1999). The BnMGT proteins in the same group showed parallel motif components. The BnMGT proteins were aligned in ClustalW 2.0 and the conserved domains logo was generated by the Weblogo online program. The results showed that all the BnMGT proteins possessed the CorA domain (Figs. 4 and 5).

3.3. Phylogenetic analysis of the MRS2/MGT genes among B. napus, Arabidopsis and rice

To understand the evolutionary relationships and functions of the BnMGT proteins in *B. napus*, we carried out the phylogenetic analysis of the *BnMGT* genes in *B. napus.* We used MEGA7.0 to produce a phylogenetic tree with all BnMGT protein sequences. The 36 BnMGT proteins in *B. napus* were resolved into five distinguishing groups (group I-V; Fig. 3A; Supplementary Fig. S1).

To investigate the functions of the BnMGT proteins, we performed comparative analysis of the evolutionary relationships among *B. napus*, *Arabidopsis* and rice, we constructed an integrated phylogenetic tree using BnMGTs, AtMGTs, and OsMRS2 proteins with the neighbor-joining method. The phylogenetic tree also distinctly displayed five diverse clades (I to V), which comprise the 55 MRS2/MGT proteins from *B. napus*, *Arabidopsis*, and rice, and each clade contained representative MRS2/MGT proteins from the three plant species (Supplementary Fig. S1; Fig. S3). Group I has 14 members and was the largest group, accounting for 39% of the total MRS2/MGTs, and clade II constituted the smallest group, containing only eight members and accounting for 22% of the total MRS2/MGTs. As shown in the phylogenetic tree, the genes in group V were more divergent compared with those in other groups. *BnMGT* genes showed a higher level of similarity to their orthologues from *Arabidopsis* than to the rice genes in the same group. These results indicate a diversification of MRS2/MGT genes between monocot and dicot plants and confirmed the presence of genome variation and obvious genome expansion in *B. napus*, which as an allotetraploid plant.

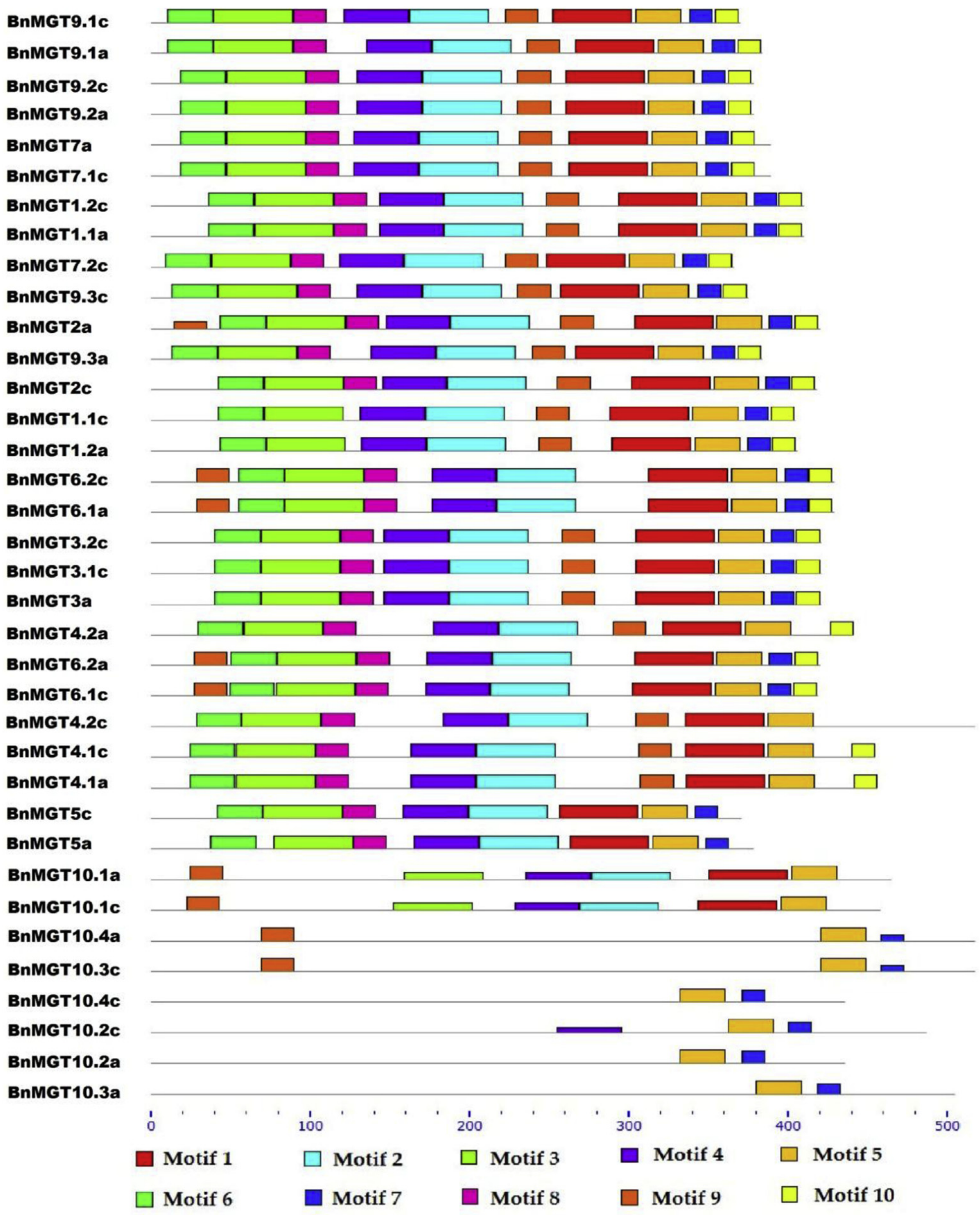


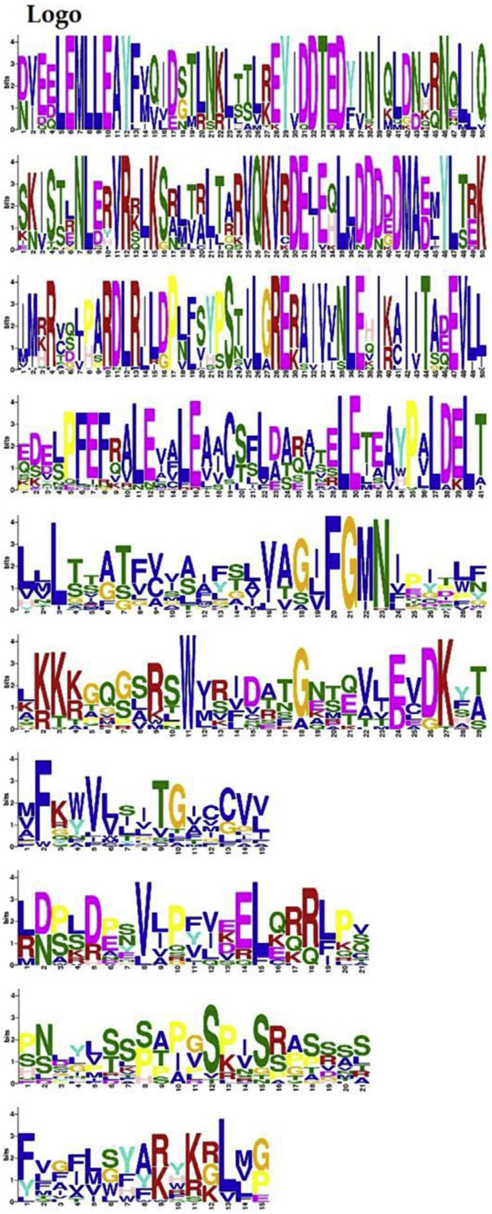
Fig. 4. Conserved motif structure of BnMGT proteins. Ten motifs were identified by MEME online tool. Different color blocks represent different motifs. The motif 5 indicates the CorA domain.

3.4. Functional complementation analyses of BnMGT genes in Salmonella typhimurium

To identify whether *BnMGT* genes encode Mg^{2+} transporters, we performed the functional complementation analysis of the *BnMGT* genes in *S. typhimurium*. We chose eight *BnMGT* genes and transformed them into the *S. typhimurium* mutant strain MM281. This strainis lacking the Mg^{2+} transport systems CorA, MgtA and MgtB, and it only grows well on the medium containing 10 mM Mg^{2+} (Maguire, 1992). The MM281 mutant transformed with vector as a negative control, and AtMGT1 which is a high affinity magnesium transporter as a positive control (Li et al., 2001). The eight BnMGTs were BnMGT1.1a, BnMGT2c, BnMGT3a, BnMGT4.1c, BnMGT6.1a, BnMGT7a, BnMGT9.1a and BnMGT10.1a. The complementation test results showed that the negative control (MM281 and MM281 transformed with pTrc99A vector) did not grow on the medium containing less than 10 mM Mg^{2+}, The MM281 cells transformed with AtMGT1 and BnMGT1.1a, BnMGT2c, BnMGT3a and BnMGT10.1a grew well on the medium containing 0.01 mM Mg^{2+}, and those transformed with BnMGT4.1c, BnMGT7a and BnMGT9.1a grew successfully on the medium containing 2 mM Mg^{2+} (Fig. 6). The growth rates analysis in liquid medium were also consistent with the complementation results on agar plates (Fig. 7). These results suggest that seven of these eight *BnMGT* genes were capable of mediating Mg^{2+} uptake, but their affinities to Mg^{2+} differed from high in *BnMGT1.1a*, *BnMGT2c*, *BnMGT3a*, and *BnMGT10.1a* to low in *BnMGT4.1c*, *BnMGT7a* and *BnMGT9.1a* (Fig. 6). Although BnMGT6.1a is a putative Mg^{2+} transporter based on the gene structure, the MM281 complementation showed that BnMGT6.1a is similar to the control. It will be necessary to determine whether this transporter has Mg^{2+} transport ability in plant.

3.5. Expression profiles analyses of BnMGT genes

The expression profiles of genes are usually related to their molecular function. In order to better investigate the physiological functions of the *BnMGT* genes, we analyzed the expression patterns of the *BnMGT*



Sequence	Name
BVEELEMLLEAYFVQIDSTLNKLTTLREYIDDTEDYINIQLDNVRNQLIQ	Motif 1
SKISTLNLERVRKLKSRLTALTARVQKVRDEJEQLLDDDDDMAEMYLTRK	Motif 2
IMRRVQJPARDLRJLDPLFSYPSTILGRERAIVVNLEHIKAIITADEVLL	Motif 3
ZDELPFEFRALEVALEAACSFLDARATELETEAYPALDELT	Motif 4
LLLTTATFVVAIFSLVAGIFGMNIPITLF	Motif 5
LKKKGQGSRSWVRIDATGNTEVJEVDKYT	Motif 6
MFKWVLIITGICCVV	Motif 7
LBPLDPNVJPFVEELQRRLPS	Motif 8
PNLYLSSPAPGSPISRASSSS	Motif 9
FVGFLSYAKYKRLMG	Motif 10

Fig. 5. Sequence logo of conserved domains found in BnMGT family. Sequence logo was created with Weblogo online program. The *X-axis* shows the conserved sequences of the domains. The coservation of residues are indicated by the height of the letters. The *Y-axis* represents the conservation of the amino acid.

genes in 12 different tissues and organs based on the RNA-seq data from the *B. napus* 'Zhongshuang 11' (BioProject ID PRJNA394926) (Fig. 8, Supplementary Table S5). According to the results, the genes belong to the *BnMGT2*, *BnMGT4*, *BnMGT6*, and *BnMGT10* subgroups had a higher expression in most organs when compared to other *BnMGT* genes. Among the 36 BnMGT genes, the expression of *BnMGT2a* was higher in the wilting pistil compared with the other genes. The transcripts of the *BnMGT5* subgroup genes were very weak in the 12 different tissues and organs. Among the *BnMGT9* subgroup genes, *BnMGT9.1a* was similarly specifically expressed in the stamen. Several genes shared similar expression patterns, which mirrored the results of the phylogenetic analysis (Supplementary Fig. S3). These results indicate that the expression of *BnMGT* genes was tissue-specific and varied in different tissues.

The expression patterns of the eight *BnMGTs* during the flowering period based on the real-time quantitative PCR (qRT-PCR) results (Fig. 9) were mainly consistent with the results of the transcriptome data analysis. The *BnMGT* transcripts were detected in roots, stems, leaves, flowers, capsules, and young embryos, and they showed distinct expression patterns. Among them, *BnMGT1.1a* was mainly expressed in the capsules and young embryos, and no expression of *BnMGT1.1a* was observed in the roots, stems, and leaves; whereas the expression of *BnMGT2c*, *BnMGT3a, BnMGT4.1c*, *BnMGT6.1a*, *BnMGT7a*, *BnMGT9.1a*, and *BnMGT10.1a* was detected in all organs. For *BnMGT7a*, there was no expression observed in the stems and leaves according to the transcriptome data, and expression was very low in the qRT-PCR results (Figs. 8 and 9). These results were consistent with the analysis of transcriptome data. In contrast, the transcripts of *BnMGT3.1c* and *BnMGT7.2c* were not detected in any tested tissues according to the transcriptome data, indicating that the two genes may be expressed at specific developmental stages in other tissues not tested here.

Except for the expression pattern of *BnMGTs*, the subcellular localization of MGT transporters also shows significant information about comprehending their in vivo functions. By means of the WoLF PSORT program, there are 13–14 of the 36 BnMGT proteins were presumed to be plasma membrane localized and 13–14 of the 36 BnMGT proteins were predicted to be chloroplasts localized, the rest of BnMGT proteins

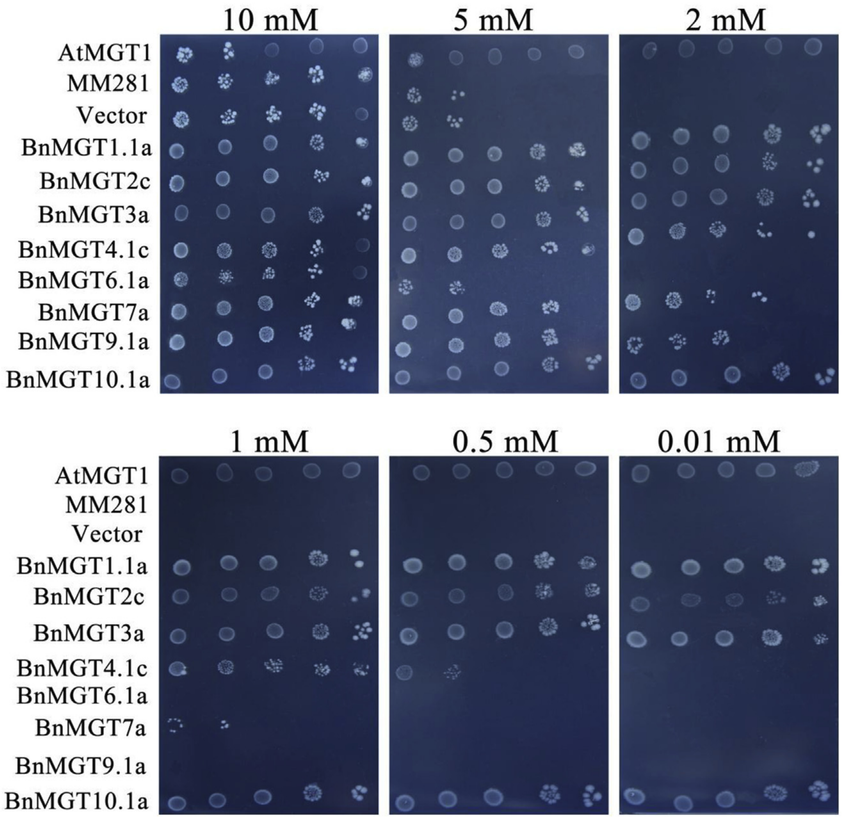


Fig. 6. Complementation of the MM281 mutant by *BnMGT* genes. Solid medium $MgSO_4$ concentrations from 0.01 to 10 mM. From left to right is a 10-fold dilution series of bacterial cultures. The MM281 mutant transformed with the AtMGT1 as a positive control, the MM281 mutant and MM281 mutant transformed with the pTrc99A vector as negative controls.

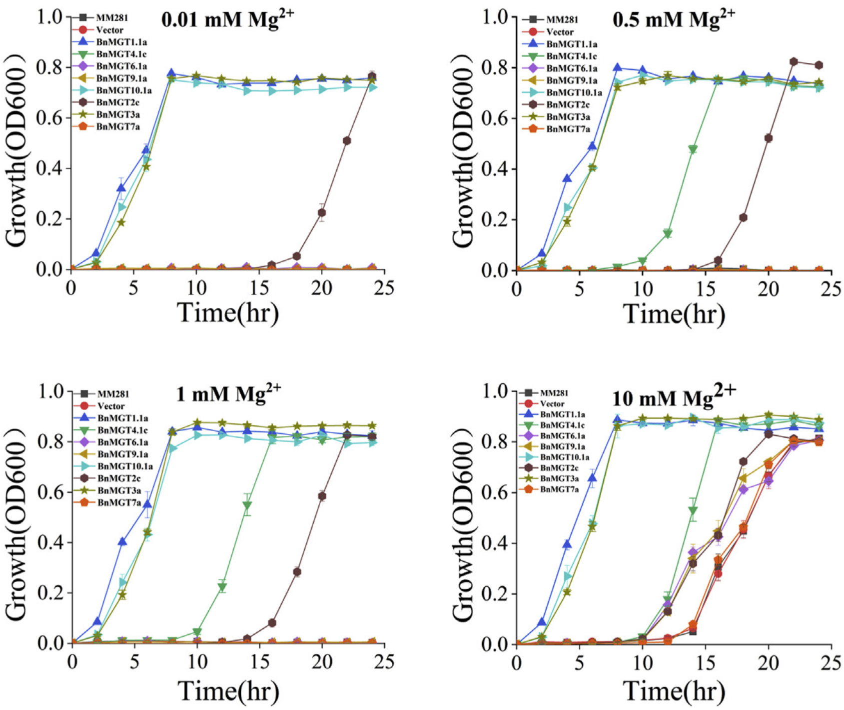


Fig. 7. Growth curve of the MM281 strains of *BnMGT* genes in liquid medium. Liquid medium $MgSO_4$ concentrations from 0.01 to 10 mM. The bacteria concentration in liquid culture medium (OD_{600}) was detected every 2 h for 24 h. The MM281 strain transformed with the pTrc99A vector as negative control. Three independent experiments were preformed, and date were shown as means ± SE.

may be localized to the nucleus, Golgi, and the endoplasmic reticulum (ER) (Table 1).

4. Discussion

Despite the importance of Mg^{2+} on plant growth and development, the molecular mechanism of plant cells regulating Mg^{2+} homeostasis remains poorly understood. The CorA-like Mg^{2+} transporters have been identified in *Arabidopsis* and rice by various in vivo and in vitro tests. The main functions of *CorA*-like genes in *Arabidopsis* and rice have been related to Mg^{2+} transport, whereas their function in *B. napus*, one of the important oil crop, has remained undocumented. In our study, we identified and characterized 36 MGT gene family members in *B. napus*. Of those, 17 BnMGT members and 19 BnMGT members were derived

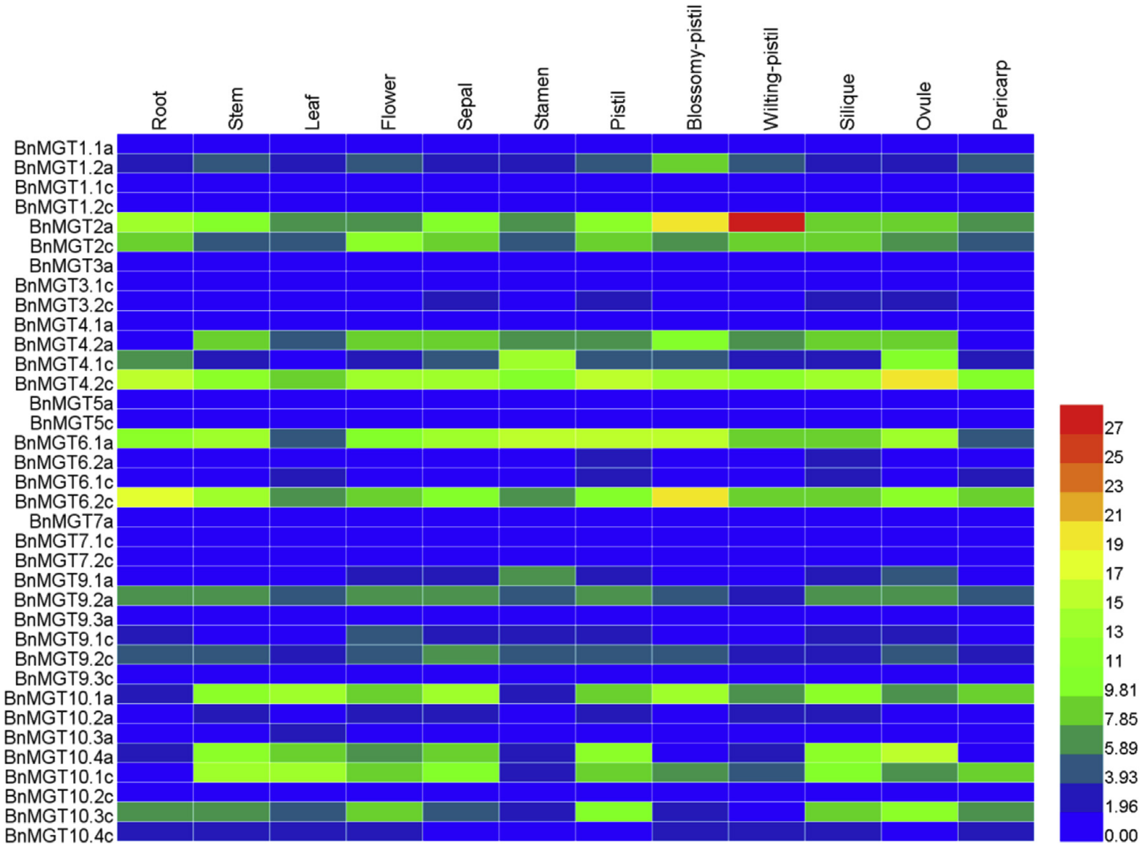


Fig. 8. Expression patterns of *BnMGT* family genes in twelve tissues. The expression datas were gained from the RNA-seq data and calculated by fragments per kilo base of exon model per million (FPKM) values. The label above the heatmap represents the different tissues of B. napus ZS11, the left side of the heatmap represents different *BnMGT* genes. The bar in the right side indicates the scale plate of FPKM values. The heatmap was constructed with Heatmap Illustrator v1.0 software (http://hemi.biocuckoo.org/down.php).

from the A genome and C genome of *B. napus*, respectively. We also identified 14 MGT proteins in *B. rapa* and 11 MGT proteins in *B. oleracea* (Supplementary Table S1; Supplementary Table S2). The *MGTs* members of *B. napus* have underwent expansion and variation when compared with the *MGTs* members of its ancestor species *B. rapa* and *B. oleracea*. And the *BnMGTs* members showed a greater degree of expansion compared with the *MGT* members in *Arabidopsis* and rice because of the triploid nature of the genome.

The CorA-like Mg^{2+} transporters were identificated with two TM regions and a conserved GMN motif. In this study, all BnMGTs possess two conserved TM regions at the C-terminus, similar to the MRS2/MGT family members in *Arabidopsis* and rice. In *Arabidopsis*, all the AtMRS2/AtMGT family members contain the conserved GMN motif, whereas in rice, it is modified in some OsMRS2 transporters. For example, the GMN motif of the OsMRS2-4 and OsMRS2-5 is altered to AMN, and is changed to GIN in OsMRS2-8 (Saito et al., 2013). Furthermore, the analysis of all the BnMGTs protein sequences showed that the complete GMN motif was also observed in 31 BnMGTs, but it was altered to GMR in BnMGT7c and GIN in BnMGT10e, BnMGT10f, BnMGT10g, and BnMGT10h (Fig. 1). These changes may be the result of gene variation during evolution and genome expansion under environmental selection pressure.

The *B. napus MGT* family was greatly expanded, having 36 family members compared with 10 members in *Arabidopsis* and 9 members in rice. The 36 BnMGT genes were classified into five groups by the phylogenetic relationships of the *MRS2/MGT* genes from *Arabidopsis* and rice. The 3-fold greater amount of *MGT* genes in *B. napus* compared with that in *Arabidopsis* indicates that the expansion of *B. napus MGT* genes occurred after the genome tripling events of *B. rapa* and *B. oleracea*.

In *Arabidopsis*, the *AtMGT* family members are expressed in all kinds of plant tissues and the proteins are localized to the plasma membrane and other specific organelle membranes, indicating their roles in Mg^{2+} uptake, translocation, and redistribution into organelles. *MGT1* is plasma membrane localized, and it have expression in the root hair, elongation zones, and vascular tissues, and in trichomes of adult leaves, suggesting its function in Mg^{2+} uptake by the root or transport between the organs (Li et al., 2001; Gebert et al., 2009). *MGT2* and *MGT3* are tonoplast localized and may be related to Mg^{2+} homeostasis (Conn et al., 2011; Gebert et al., 2009). *MGT5* is mitochondria localized and has an important role in pollen development (Li et al., 2008). *MGT6* is plasma membrane localized and is required for growth under low-Mg^{2+} conditions (Mao et al., 2014). *MGT7* is endomembrane localized and may be involved in the Mg^{2+} homeostasis (Gebert et al., 2009). *MGT9* is important for male fertility (Chen et al., 2009). *MGT10* is chloroplast envelope membrane system localized and is expressed in the rosette leaves and vascular tissues of cauline leaves, suggesting a role in Mg^{2+} translocation (Drummond et al., 2006). Previous studies indicated these *AtMGTs* showed different Mg^{2+} transport ability.

The *BnMGT* transcripts were detected in roots, stems, leaves, flowers, capsules, and young embryos. Among them, *BnMGT1.1a* was mainly expressed in the capsules and young embryos, the expression of *BnMGT2c*, *BnMGT3a*, *BnMGT4.2a*, *BnMGT4.1c*, *BnMGT9.1a*, *BnMGT9.3a*, and *BnMGT10.1a* was detected in all organs. *BnMGT4.1c and BnMGT9.1a* were mainly expressed in the flowers and young embryos, *BnMGT2c, BnMGT6.1a and BnMGT10.1a*were mainly expressed in the stems and leaves. The 36 BnMGT proteins were predicted to be localized to the plasma membrane, chloroplasts or other cellular compartments. The above results indicated that most of the *BnMGT* genes possess Mg^{2+} transport ability and the Mg^{2+} transport ability of

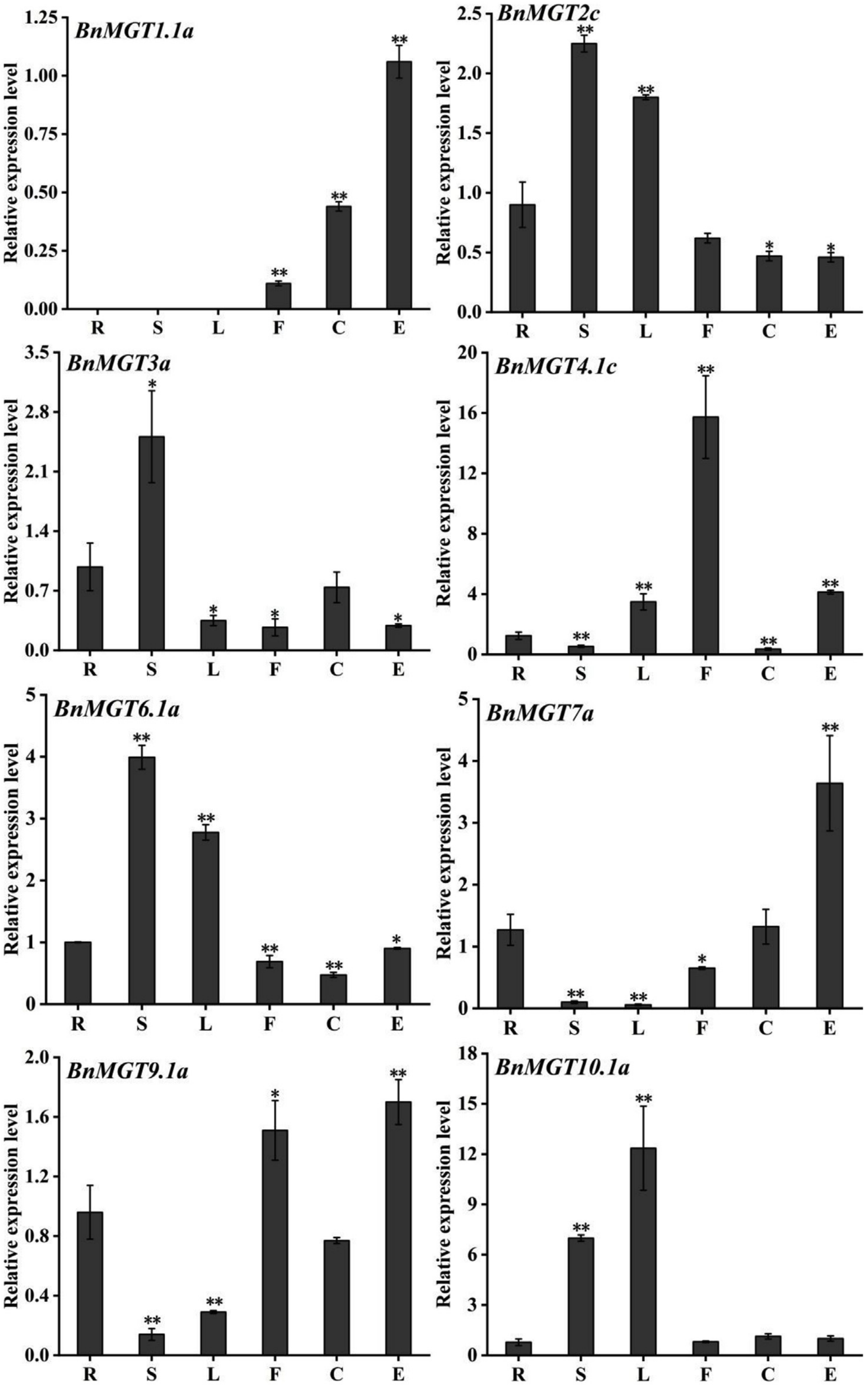


Fig. 9. Relative expression level of *BnMGT* genes in *B. napus* by real-time quantitative PCR (qRT-PCR). R: root; S: stem; L: leaf; F: flower; C: capsule; and E: embryo. Data were normalized to the expression level of the inner control gene *BnUBC21*. * represents the significant level, ** represents the extremely significant level (Student's t-test; *$P < 0.05$, **$P < 0.01$). Data are means of three replicates of one experiment. The experiment was repeated three times with similar results. Error bars represent ± SD.

BnMGT genes will be further proved in plant.

In conclusion, we identified 36 BnMGT genes from *B. napus* and analyzed the specific information about their identification, duplication pattern, conserved motifs, evolutionary relationship, expression profiles, and Mg^{2+} transport ability. Following research using overexpression and RNAi strategies is to reveal the specific function of each *BnMGT* gene are necessary.

Author contributions statement

LZ and DDM designed the experiments. LZ performed the experiments. ANW, XQW, XLP, NW, XC, and YC contributed to the development of new reagents/analytic tools. LZ, SL, LBC, and DM analyzed the data. LZ, SL, and DDM wrote the manuscript.

Declarations of interest

None.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant number NSFC-31500200); and the Excellent Youth Foundation of Hunan Province Educational Committee (grant number 17B165).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.01.017.

References

Chen, J., Li, L., Liu, Z., Yuan, Y., Guo, L., Mao, D., Tian, L., Chen, L., Luan, S., Li, D., 2009. Magnesium transporter AtMGT9 is essential for pollen development in *Arabidopsis*. Cell Res. 19, 887–898.

Conn, S., Conn, V., Tyerman, S., Kaiser, B., Leigh, R., Gilliham, M., 2011. Magnesium transporters, MGT2/MRS2-1 and MGT3/MRS2-5, are important for magnesium partitioning within *Arabidopsis thaliana* mesophyll vacuoles. New Phytol. 190, 583–594.

Drummond, R., Tutone, A., Li, Y., Gardner, R., 2006. A putative magnesium transporter AtMRS2-11 is localized to the plant chloroplast envelope membrane system. Plant Sci. 170, 78–89.

Eshaghi, S., Niegowski, D., Kohl, A., Molina, D., Lesley, S., Nordlund, P., 2006. Crystal structure of a divalent metal ion transporter CorA at 2.9 angstrom resolution. Science 313, 354–357.

Gebert, M., Meschenmoser, K., Svidová, S., Weghuber, J., Schweyen, R., Eifler, K., Lenz, H., Weyand, K., Knoop, V., 2009. A root-expressed magnesium transporter of the *MRS2/MGT* gene family in *Arabidopsis thaliana* allows for growth in low-Mg^{2+} environments. Plant Cell 21, 4018–4030.

Guo, W., Nazim, H., Liang, Z., Yang, D., 2016. Magnesium deficiency in plants:An urgent problem. Crops J 4, 83–91.

Hortensteiner, S., 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends Plant Sci. 14, 155–162.

Horton, P., Park, K., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C., Nakai, K., 2007. WoLFPSORT: protein localization predictor. Nucleic Acids Res. 35, W585–W587.

Knoop, V., Groth-Malonek, M., Gebert, M., Eifler, K., Weyand, K., 2005. Transport of magnesium and other divalent cations: evolution of the 2-TM-GXN proteins in the MIT superfamily. Mol. Genet. Genom. 274, 205–216.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and clustal X version 2.0. Bioinformatics 23, 2947–2948.

Li, L., Tutone, A., Drummond, R., Gardner, R., Luan, S., 2001. A novel family of magnesium transport genes in *Arabidopsis*. Plant Cell 13, 2761–2775.

Li, L., Sokolov, L., Yang, Y., Li, D., Ting, J., Pandy, G., Luan, S., 2008. A mitochondrial magnesium transporter functions in *Arabidopsis* pollen development. Mol. Plant 1, 675–685.

Lunin, V., Dobrovetsky, E., Khutoreskaya, G., Zhang, R., Joachimiak, A., Doyle, D., Bochkarev, A., Maguire, M., Edwards, A., Koth, C., 2006. Crystal structure of the CorA Mg^{2+} transporter. Nature 440, 833–837.

Maguire, M., 1992. MgtA and MgtB: prokaryotic P-type ATPases that mediate Mg^{2+} influx. J. Bioenerg. Biomembr. 24, 319–328.

Maher, C., Stein, L., Ware, D., 2006. Evolution of *Arabidopsis* microRNA families through duplication events. Genome Res. 16, 510–519.

Mao, D., Tian, L., Li, L., Chen, J., Deng, P., Li, D., Luan, S., 2008. *AtMGT7*: an *Arabidopsis* gene encoding a low-affinity magnesium transporter. J. Integr. Plant Biol. 50, 1530–1538.

Mao, D., Chen, J., Tian, L., Liu, Z., Yang, L., Tang, R., Li, J., Lu, C., Yang, Y., Shi, J., Luan, S., 2014. *Arabidopsis* transporter MGT6 mediates magnesium uptake and is required for growth under magnesium limitation. Plant Cell 26, 2234–2248.

Payandeh, J., Pai, E.F., 2006. A structural basis for Mg^{2+} homeostasis and the CorA translocation cycle. EMBO J. 25, 3762–3773.

Saito, T., Kobayashi, N., Tanoi, K., Iwata, N., Suzuki, H., Iwata, R., Nakanishi, T., 2013. Expression and functional analysis of the CorA-MRS2-ALR-type magnesium transporter family in rice. Plant Cell Physiol. 54, 1673–1683.

Schock, I., Gregan, J., Steinhauser, S., Schweyen, R., Brennicke, A., Knoop, V., 2000. A member of a novel *Arabidopsis thaliana* gene family of candidate Mg^{2+} ion transporters complements a yeast mitochondrial group II intron-splicing mutant. Plant J. 24, 489–501.

Shaul, O., 2002. Magnesium transport and function in plants: the tip of the iceberg. Biometals 15, 307–321.

Smith, R., Banks, J., Snavely, M., Maguire, M., 1993. Sequence and topology of the CorA magnesium transport systems of *Salmonella typhimurium* and *Escherichia coli.* Identification of a new class of transport protein. J. Biol. Chem. 268, 14071–14080.

Sponder, G., Svidová, S., Khan, M.B., Kolisek, M., Schweyen, R.J., Carugo, O., Djinović-Carugo, K., 2013. The GMN motif determines ion selectivity in the yeast magnesium channel Mrs2p. Metallomics 5 (6), 745–752.

Szegedy, M.A., Maguire, M.E., 1999. The CorA Mg^{2+} transport protein of *Salmonella* typhimurium. Mutagenesis of conserved residues in the second membrane domain. J. Biol. Chem. 274 (52), 36973–36979.

Williams, L., Salt, D., 2009. The plant ionome coming into focus. Curr. Opin. Plant Biol. 12, 247–249.

Zhao, H., Ma, H., Yu, L., Wang, X., Zhao, J., 2012. Genome-wide survey and expression analysis of amino acid transporter gene family in rice (*Oryza sativa* L.). PLoS One 7, e49210.