Molecular Plant Breeding 2022, Vol.13, No.31, 1-15
http://genbreedpublisher.com/index.php/mpb

Research Article Open Access

Identification and Bioinformatics Analysis of ABC Transporter Gene Family in Hydrangea under Aluminum Stress

Chen Haixia, Wang Xuan, Xu Lu
Horticulture College of Hunan Agricultural University, Hunan Mid-subtropical Quality Plant Breeding and Utilization Engineering Technology Research Center, Changsha, 410128, China
Corresponding author email: chenhaixia@hunau.edu.cn
Molecular Plant Breeding, 2022, Vol.13, No.31 doi: 10.5376/mpb.2022.13.0031
Received: 15 Dec., 2022
Accepted: 22 Dec., 2022
Published: 30 Dec., 2022

Preferred citation for this article:
Chen H.X., Wang X., and Xu L., 2022, Identification and bioinformatics analysis of ABC transporter gene family in hydrangea under aluminum stress, Molecular Plant Breeding, 13(31): 1-15 (doi: 10.5376/mpb.2022.13.0031)

Abstract ABC transporters is a type of transmembrane transporters widely found in prokaryotes and eukaryotes. They are involved in plant signal transduction, secondary metabolite transport and abiotic stress response. According to the transcriptome data of Hydrangea, this study was proposed analyze the members of ABC transporter gene family, physical and chemical properties, gene structure, phylogeny and gene expression patterns under aluminum stress using bioinformatics method. The results showed that there were 48 members of ABC transporter gene family in Hydrangea, which belonged to 7 subfamilies respectively, among which the C subfamily had the most members; the subcellular localization results showed that 50% of the family members were located on the plasma membrane; the physical and chemical properties and domain analysis showed that most of the ABC transporters were hydrophobic proteins, and different subfamilies had specific conserved motifs, but motif1 and motif2 were all present in hydrangea ABC transporter; A phylogenetic tree was constructed with 129 ABC transporters of *Arabidopsis thaliana*, and 48 ABC transporters from Hydrangea were clustered into 7 subfamilies of Arabidopsis thaliana respectively. The expression profile analysis showed that the *HmABCA1, HmABCC1, HmABCC6, HmABCC14* and *HmABCD1* gene were upregulated in the root and *HmABCG1* gene were up-regulated in leaves after aluminum stress treatment. The results provide a reference for further study on aluminum tolerance and expression regulation of ABC transporter genes in hydrangea.
Keywords Hydrangea macrophylla; Aluminum tolerance; ABC transporter genes; Bioinformatics

ABC transporters (ATP-binding cassette transporters) is a type of transmembrane transporters widely found in prokaryotes and eukaryotes, which could transport substrates, including sugar, amino acids, proteins, metal ions and other biological molecules, across the membrane with the help of the energy generated by ATP hydrolysis. They are involved in a variety of biological processes, such as plant hormone transport, signal transduction, cell detoxification, virus defense and antigen transmission (Wilkens, 2015; Aryal et al., 2016). ABC transporter family has a conservative structure. The typical structure is composed of two nucleotide binding domains (NBD) and two transmembrane domains (TMD). NBD domain is relatively conservative, which performs ATP hydrolase function and provides energy for transmembrane transport. TMD is a transmembrane channel, and its structure and sequence have substrate specificity (Lane et al., 2016).

In the early 1990s, the first ABC transporter AtABCB1 was first found in *Arabidopsis thaliana*, and then ABC transporters were identified from more than 20 plants such as rice (*Oryza sativa*), tobacco (*Nicotiana tabacum*) and maize (*Zea mays*). At the same time, the mechanisms of ABC transporters' transmembrane transport, absorption and transport of nutrient ions, and resistance to abiotic stress were studied. ABC transporters can improve the resistance of crops to abiotic stresses such as high temperature, drought, salinity and heavy metals. ABC transporters could improve the resistance of crops to abiotic stresses such as high temperature, drought, salinity and heavy metals. Studies have shown that the ABCB transporter AtABCB27/AtTAP2, located in the vacuolar membrane and vascular system of Arabidopsis root tips, transports aluminum into vacuoles in the form of chelating peptide conjugates to isolate, thereby improving plant aluminum tolerance (Larsen et al., 2007). *OsSTAR1* and *OsSTAR2* identified from *indica* rice are ABC transporter genes in response to aluminum stress,

which transport UDP-glucose to limit aluminum accumulation and reduce aluminum toxicity. Another semi-molecular transporter OsALS1 is in vacuolar membrane and encodes aluminum transporter (Huang et al., 2012). Subsequently, homologous genes were identified in maize (*Zea mays*), sweet sorghum (*Sorghum bicolor*), alfalfa (*Medicago sativa*) and other plants, all of which belong to ABC transporters related to aluminum toxicity stress (Song et al., 2013; Liang, 2018).

Hydrangea is an aluminum enriched plant. Aluminum treatment for a few months can make the accumulation of aluminum in leaves reach 5 mg/g dry weight, and the aluminum content in leaf cells is 15.7 mmol/kg fresh weight. These aluminums are absorbed by roots and transported to plants to chelate with citric acid in a molar ratio of 1:1 to synthesize a non-toxic citric acid aluminum complex. Therefore, it is considered that there should be a system for aluminum ion absorption, transport and storage in hydrangea. In order to clarify the correlation between blue hydrangea coloration and aluminum ion transport, two aluminum ion transporter genes *HmPALT1* and *HmVALT* located on the plasma membrane and vacuolar membrane respectively were isolated and identified from the blue hydrangea sepals, both of which belong to the anion permease family (Negishi et al., 2012). The amino acid permeability enzyme family gene *HmPALT2* cloned from blue sepals is located in the plasma membrane and expressed in all tissues of plants. It participates in the transportation of many ions and can also transport aluminum ions from extracellular to cytosol (Negishi et al., 2013). Therefore, the molecular mechanism of response to aluminum stress and enrichment in hydrangea plants has always been a research hotspot. Transcriptome sequencing analysis showed that the genes encoding ABC transporters were up-regulated in roots and leaves of Hydrangea after aluminum stress, but no systematic study of ABC transporter gene family was carried out, and its gene function was unclear (Chen et al., 2015). According to the transcriptome data of Hydrangea, ABC transporters were selected as keywords in the gene function annotation library in this study, and the obtained ABC transporters were identified, and bioinformatics analyzed to explore the relationship between ABC transporter family and aluminum resistance, in order to provide a theoretical basis for the function mining of ABC transporter gene family.

1 Results and Analysis

1.1 Identification and physical and chemical properties analysis of ABC transporter in hydrangea

The 95 714 gene clusters in the transcriptome of hydrangea were searched in the three databases of GO, NR and KEGG with ABC transporters as the keywords. A total of 208 gene clusters were annotated, which belong to seven ABC transporter subfamilies, namely ABCA, ABCB, ABCC, ABCD, ABCE, ABCF and ABCG. SMART was used to predict the domain of 208 ABC transporters, and the conserved NBDs domain was selected as the final screening index. A total of 48 ABC transporters in hydrangea with NBDs domain were screened (Table 1).

The online software ProtParam was used to predict the physical and chemical properties of the protein encoded by the ABC transporter gene family in hydrangea (Table 1). The length of 48 ABC transporters in hydrangea is ranged from 195 to 1 895 aa, and the theoretical value of molecular weight is 21413.55~104980.71. The theoretical isoelectric point (pI) is 5.18~9.49, of which 28 are acidic proteins with pI less than 7 and the remaining 20 are basic proteins. Instability coefficient analysis showed that 38 proteins are stable proteins with instability coefficient less than 40, and the remaining 10 are unstable proteins. The aliphatic index is 80.4~111.16, with an average value of 96.33, of which 19 have an aliphatic index higher than 100. A relatively high aliphatic index could maintain good stability of protein and help to play a normal function in different environments. The grand average of hydropathicity is -0.625~0.287, of which 30 are negative, indicating that most of them are hydrophobic proteins, and the hydrophobic degree is different.

Table 1 The ABC transporter protein family members and physico-chemical analysis in Hydrangea

Gene	Transcriptome code	NBD domain (aa)	Number of Amino acids (aa)	Molecular weight (ku)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity	Subcellular localization
HmABCA1	c200548.graph_c1	595~774	774	84 773.43	5.75	37.89	102.83	0.129	Plasma membrane
HmABCA2	c210858.graph_c0	555~379	965	107 091.19	6.54	41.09	89.35	0.066	Plasma membrane
HmABCA3	c211005.graph_c0	589~771 1 497~1 684	1 895	210 905.64	7.33	36.75	99.14	0.121	Plasma membrane
HmABCB1	c162347.graph_c1	416~610	635	68 490.99	9.00	28.99	104.30	0.123	Plasma membrane
HmABCB2	c196483.graph_c0	212~398	463	49762.13	9.07	33.23	92.76	-0.088	Mitochondrion
HmABCB3	c221727.graph_c0	110~296	383	40962.45	5.57	46.04	90.44	-0.095	Chloroplast
HmABCC1	c156671.graph_c0	342~521 949~1136	1156	127494.92	6.00	39.57	106.31	0.133	Plasma membrane
HmABCC2	c158016.graph_c0	671~852 1 293~1 478	1509	169341.89	8.64	35.36	105.11	0.161	Plasma membrane
HmABCC3	c163586.graph_c0	642~825 1 266~1 459	1625	182946.00	7.94	43.69	105.87	0.078	Plasma membrane
HmABCC4	c176480.graph_c0	549~742	775	86007.39	5.58	36.45	102.53	0.113	Plasma membrane
HmABCC5	c185684.graph_c0	652~825 1 260~1 452	1480	165230.03	8.51	38.38	104.22	0.180	Plasma membrane
HmABCC6	c194263.graph_c0	38~163 587~779	797	88147.70	6.37	37.12	99.11	0.132	Plasma membrane
HmABCC7	c199439.graph_c0	245~428 912~1 098	1131	124928.99	6.90	39.02	100.08	-0.028	Plasma membrane
HmABCC8	c201746.graph_c0	607~781 1 220~1 410	1431	159549.09	6.38	43.29	107.38	0.279	Plasma membrane
HmABCC9	c206954.graph_c0	71~261 737~923	952	104331.25	6.28	39.93	101.36	-0.028	Mitochondrion
HmABCC10	c209063.graph_c0	665~807	807	90561.23	8.98	29.85	110.36	0.287	Plasma membrane
HmABCC11	c209888.graph_c0	101~274 699~895	943	104980.71	5.94	35.19	95.96	-0046	Plasma membrane
HmABCC12	c210623.graph_c0	658~831	1105	124382.84	8.86	36.53	111.16	0.270	Plasma membrane
HmABCC13	c210746.graph_c1	288~478	1160	129578.10	7.55	33.29	94.38	-0.019	Plasma membrane

Continuing Table 1

Gene	Transcriptome code	NBD domain (aa)	Number of Amino acids (aa)	Molecular weight (ku)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity	Subcellular localization
HmABCC14	c211816.graph_c0	422~601 1 022~1 214	1232	136954.82	8.24	41.23	100.99	0.143	Plasma membrane
HmABCC15	c211939.graph_c0	612~786 1 224~1 415	1453	163353.08	8.66	37.59	107.54	0.146	Plasma membrane
HmABCC16	c211964.graph_c0	636~809 1 256~1 441	1480	164840.23	6.23	37.08	105.45	0.186	Plasma membrane
HmABCC17	c212580.graph_c0	655~828 1 284~1 476	1509	168549.07	7.16	40.71	105.06	0.104	Plasma membrane
HmABCD1	c145066.graph_c1	187~365	447	50591.64	5.67	41.37	86.82	-0.452	Chloroplast
HmABCD2	c181610.graph_c0	489~680	710	81049.43	8.89	39.08	97.73	-0.167	Chloroplast
HmABCD3	c185390.graph_c0	110~302	422	46660.23	5.26	38.56	80.40	-0.501	Chloroplast
HmABCD4	c190425.graph_c0	473~657	661	75060.47	9.49	35.88	100.14	-0.089	Chloroplast
HmABCD5	c201208.graph_c0	99~291	352	39600.73	5.18	32.30	86.68	-0.500	Chloroplast
HmABCD6	c207731.graph_c5	121~370	380	42868.70	5.35	44.70	90.32	-0.269	Chloroplast
HmABCD7	c210535.graph_c1	471~650 1 126~1 328	1338	149587.46	9.21	41.02	102.88	-0.053	Plasma membrane
HmABCE1	c143732.graph_c0	19~187	248	27992.16	9.11	29.09	87.22	-0.334	Plasma membrane
HmABCE2	c180499.graph_c1	101~292 369~497	497	55436.49	8.09	29.75	98.83	-0.137	Cytoplasm
HmABCE3	c197794.graph_c0	206~375	457	51409.11	6.31	35.03	90.22	-0.295	Chloroplast
HmABCE4	c207506.graph_c0	102~297 374~543	607	68563.02	8.08	34.88	88.93	-0.312	Nucleus
HmABCF1	c120618.graph_c0	1~170	195	21413.55	9.18	33.39	91.49	-0.202	Chloroplast
HmABCF2	c173288.graph_c1	58~232	252	27894.75	6.03	35.11	93.97	-0.206	Chloroplast
HmABCF3	c177935.graph_c1	53~195	201	21912.13	5.48	52.05	98.46	-0.069	Plasma membrane
HmABCF4	c183630.graph_c0	251~381	388	43383.37	6.20	39.10	88.04	-0.321	Nucleus
HmABCF5	c192449.graph_c0	58~236	271	30341.90	6.80	30.80	93.99	-0.284	Chloroplast
HmABCF6	c202770.graph_c1	227~510	557	61901.62	6.82	33.74	80.70	-0.452	Chloroplast
HmABCF7	c203124.graph_c0	195~397 527~703	728	81305.86	5.85	36.92	83.17	-0.625	Chloroplast

Continuing Table 1

Gene	Transcriptome code	NBD domain (aa)	Number of Amino acids (aa)	Molecular weight (ku)	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity	Subcellular localization
HmABCF8	c203715.graph_c0	320~487	578	63657.96	5.85	34.35	87.23	-0.174	Cytoplasm
HmABCF9	c204186.graph_c0	97~299 409~576	599	57010.75	6.03	35.15	89.45	-0.321	Chloroplast
HmABCF10	c205016.graph_c0	74~268 383~550	571	65497.26	5.77	39.96	93.43	-0.393	Chloroplast
HmABCF11	c208754.graph_c0	106~309	614	68354.81	6.54	31.41	87.39	-0.387	Chloroplast
HmABCF12	c210038.graph_c0	471~638 714~991	1063	118017.55	6.08	33.75	88.69	-0.354	Cytoplasm
HmABCF13	c211855.graph_c0	485~650 727~1 009	1068	116716.78	6.21	35.29	94.22	-0.211	Chloroplast
HmABCG1	c187439.graph_c0	99~289	661	73932.17	9.08	39.41	101.74	0.101	Plasma membrane

Based on the subcellular localization analysis of ABC transporter gene family in hydrangea, it was found that it was distributed in plasma membrane, chloroplast, mitochondria, cytoplasm and nucleus. The three ABCA subfamily genes were located on the plasma membrane; Three ABCB subfamily genes were located in plasma membrane, mitochondria and chloroplast, respectively; Most of the 17 ABCC subfamily genes were located in the plasma membrane, and only one was located in the mitochondria; Only one of the ABCD subfamily genes was located in the plasma membrane and the other six were located in the chloroplast; The four genes of ABCE subfamily were located in plasma membrane, cytoplasm, chloroplast and nucleus, respectively; 13 ABCF subfamily genes were distributed in plasma membrane, cytoplasm, chloroplast and nucleus; One ABCG subfamily gene was located in the plasma membrane. Therefore, 50% of the ABC transporter genes of hydrangea are located in the plasma membrane, and 94.1% of the ABCC subfamily genes are located in the plasma membrane, which is common with the known plant ABC transporters, indicating that the ABC transporter family has a certain degree of conservation in function and plays a role in transmembrane material transport and information transmission.

1.2 Phylogenetic analysis of ABC transporters in Hydrangea

According to the sequence similarity as the classification basis of gene subfamilies, the phylogenetic tree was constructed by using ClustalW software for the 48 ABC transporter sequences in Hydrangea (Figure 1). The members of the ABC transporter family in Hydrangea could be divided into 7 subfamilies, of which the ABCC subfamily contained up to 17 protein members, followed by the ABCF subfamily with 13 protein members. ABCC subfamily proteins are all molecule ABC transporters, which are involved in many physiological processes in plants, including maintaining intracellular balance, metal detoxification and transporting glutathione conjugates (Wilkens, 2015). 17 and 15 ABCC transporter members have been identified in rice and Arabidopsis genomes, respectively. Therefore, it is speculated that the ABCC transporter in Hydrangea under aluminum stress may participate in the transmembrane transport of aluminum ions. The ABCB and ABCC transporter subfamilies in Hydrangea are in the same branch, and it is speculated that the two subfamily genes have homology. The proteins in the ABCF subfamily belong to soluble ABC proteins, with two nucleic acid domains and no transmembrane domain. It was found that the *ABCF3* gene in *Arabidopsis thaliana* plays an important role in the response to cadmium stress (Li, 2018). Therefore, it is speculated that the ABCF subfamily genes in Hydrangea may also be involved in the process of responding to aluminum stress.

In order to further study the evolutionary relationship of the members of the ABC transporter family in Hydrangea, a phylogenetic tree was constructed by using the 129 ABC protein sequences published in Arabidopsis and 48 protein sequences in Hydrangea (Figure 2). Referring to the Sanchez-Fernandez et al. (2001), 129 ABC transporters in Arabidopsis were classified into 7 subfamilies, and the 48 ABC transporters in Hydrangea were also clustered into 7 subfamilies with the help of PLA (Proximity Ligation Assay). Therefore, according to the position of phylogenetic tree, the function of ABC transporter in Hydrangea could be preliminarily identified.

1.3 Analysis of ABC transporter domains and conserved motifs in Hydrangea

CD-search was used to analyze the ABC protein domains of Hydrangea, and it was shown that each protein has one or two highly conserved nucleotide domains, and some subfamilies contain several transmembrane domains (Figure 3; Table 1). There are 24 ABC transporters with two nucleotide domains, of which 14 belong to the ABCC subfamily, because the C subfamily has only one type of whole molecule protein. ABCE subfamily and ABCF subfamily proteins do not have transmembrane domains and do not function as transporters, but participate in ribosome biosynthesis and translation regulation.

MEME online software was used to predict the conserved motifs of 48 ABC transporters in Hydrangea, and it was found that the number of conserved motifs of different proteins was different, and the types were different (Figure 4). There were 50 motif conserved motifs in the ABC transporters of Hydrangea, of which motif1 and motif2 existed in all ABC transporters. Motif3 and motif13 appeared 28 times and motif4 appeared 27 times, all of which were distributed in each subfamily. ABCB and ABCC subfamilies shared motifs of motif5~12, 16, 19, 23, 25, 27, 29, 30, 36, 39, 41, 44, 47, 49, 50, so it was speculated that the two subfamilies have homology. Motif18, 20, 31,

33, 35, 37, 40, 42, 45, 46, 48 were distributed in the ABCF subfamily. Motif21, 28, 34, 38 were distributed in the ABCD subfamily. Motif22, 24, 26, 32, 43 were distributed in the ABCE subfamily. Conserved motifs clustered in the same subfamily are similar in composition, indicating that the evolutionary relationship between members is close. There are differences in conserved motifs among different subfamilies. The higher the frequency of motif, the higher the degree of conservation.

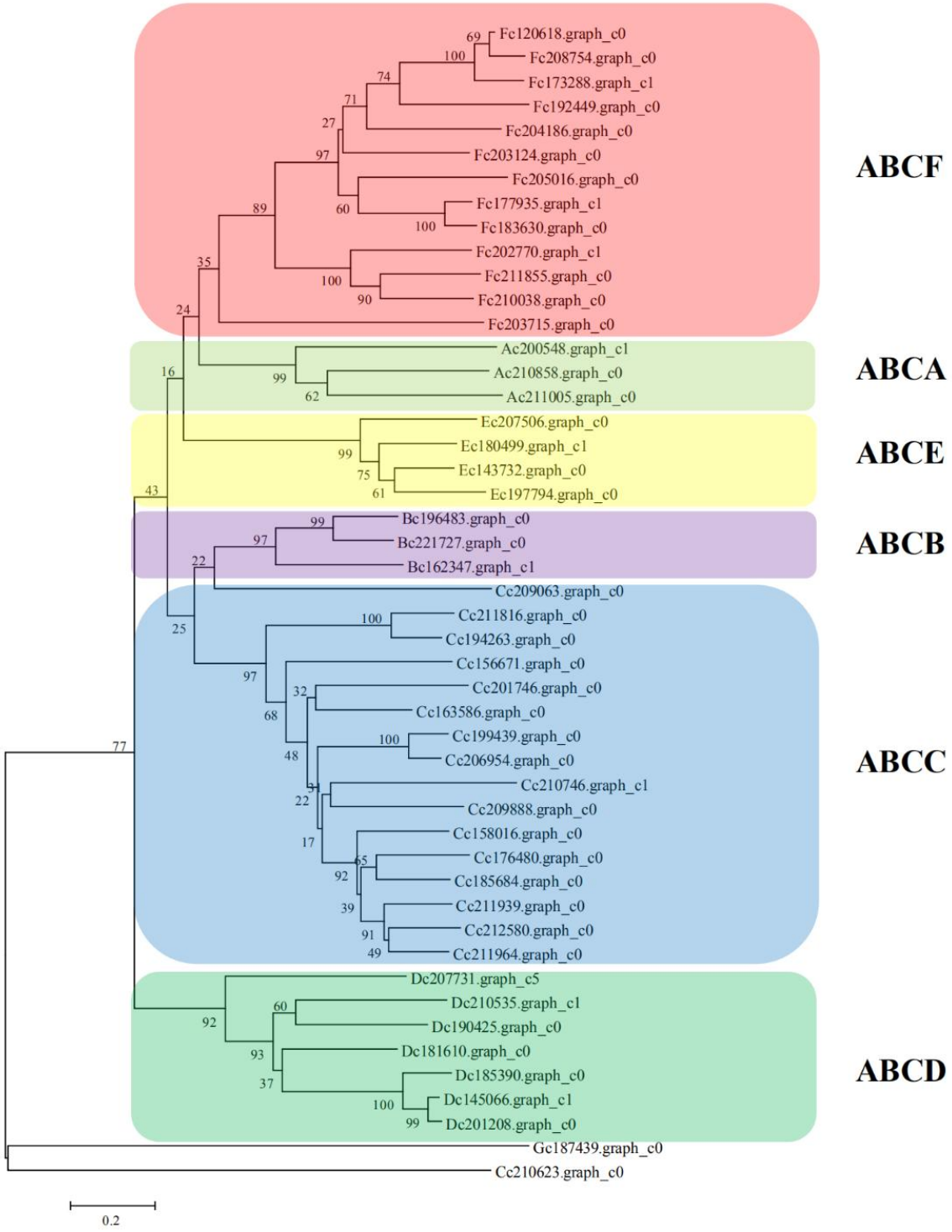

Figure 1 Evolution tree of ABC transporter in Hydrangea

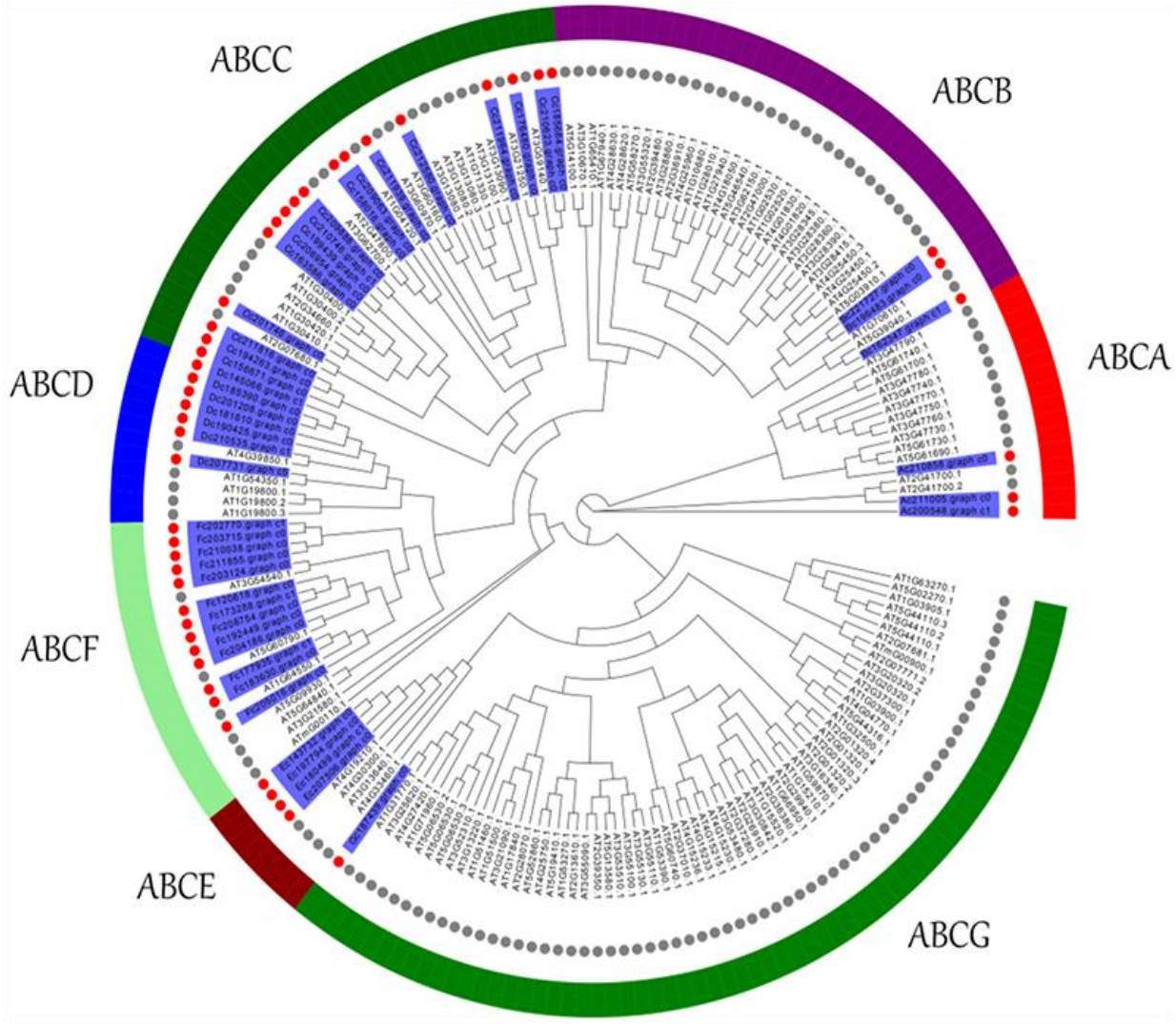

Figure 2 Phylogenetic tree of ABC transporter proteins from Hydrangea and *Arabidopsis thaliana*
Note: Shade blue: ABC transporters in Hydrangea

1.4 Analysis of differential expression pattern

In order to analyze the expression pattern of ABC transporter gene, the transcriptome data of roots and leaves in Hydrangea under aluminum stress for 4 h were screened and the expression results of 48 ABC transporter genes were analyzed, with the no aluminum stress treatment as control. The heat map of the expression patterns of 48 ABC transporter genes in the roots and leaves was drawn with the help of pheatmap software (Figure 5). The results showed that all transporter genes were expressed in the roots of Hydrangea, but 38 genes were slightly expressed or not expressed in the leaves. Compared with the Hydrangea without aluminum stress treatment, 12 genes were up-regulated in the root and only 1 gene was up-regulated in the leaf after aluminum stress. Among them, there were 6 genes with significant differences in expression (Table 2), which were *HmABCA1*, *HmABCC1*, *HmABCC6*, *HmABCC14* and *HmABCD1* expressed in the root and *HmABCG1* expressed in the leaf. Therefore, it is believed that the ABC transporter gene in the root of Hydrangea is involved in the absorption, transport and storage of aluminum ions in response to aluminum stress.

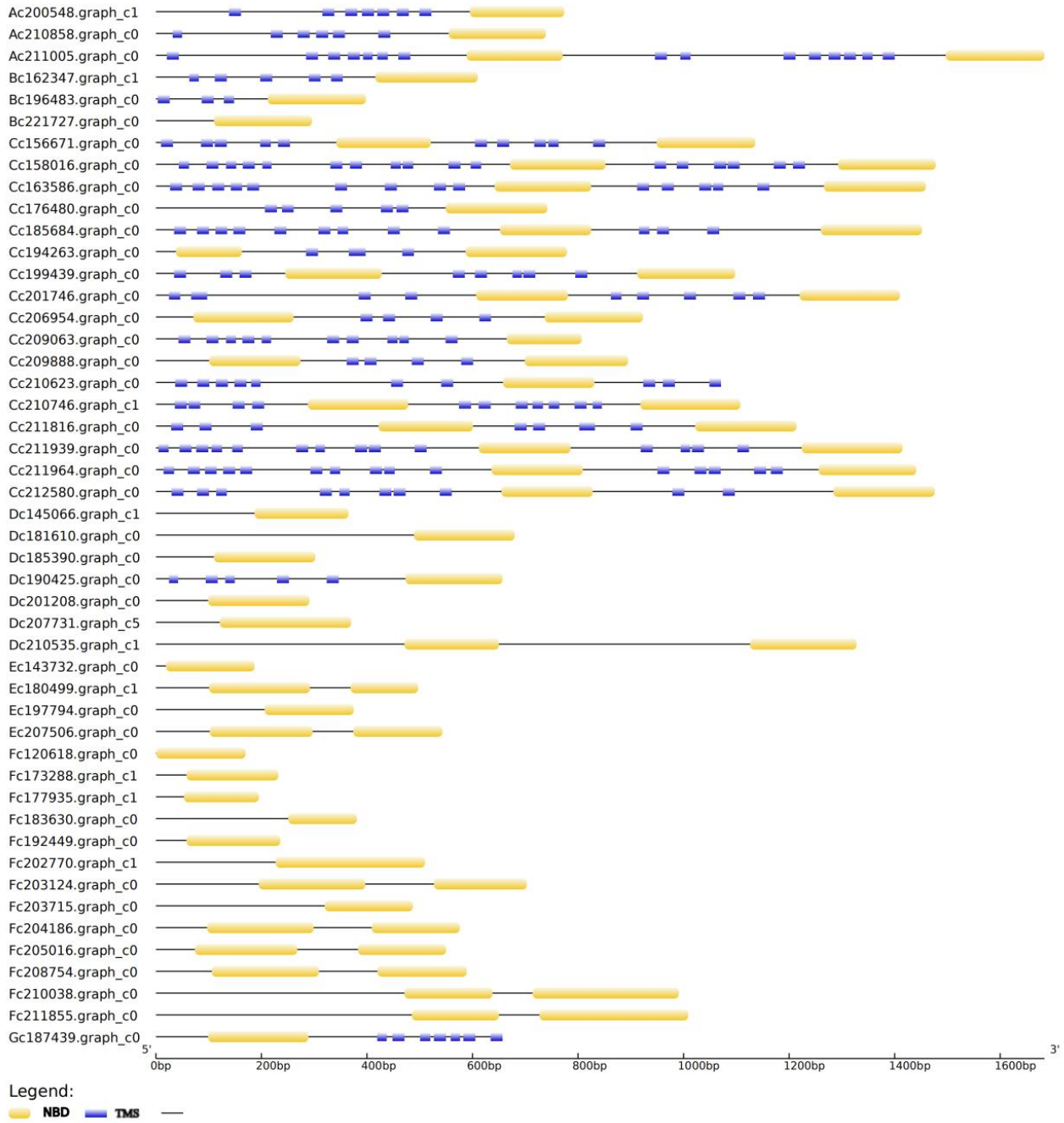

Figure 3 Domain distribution of ABC transporter family in Hydrangea

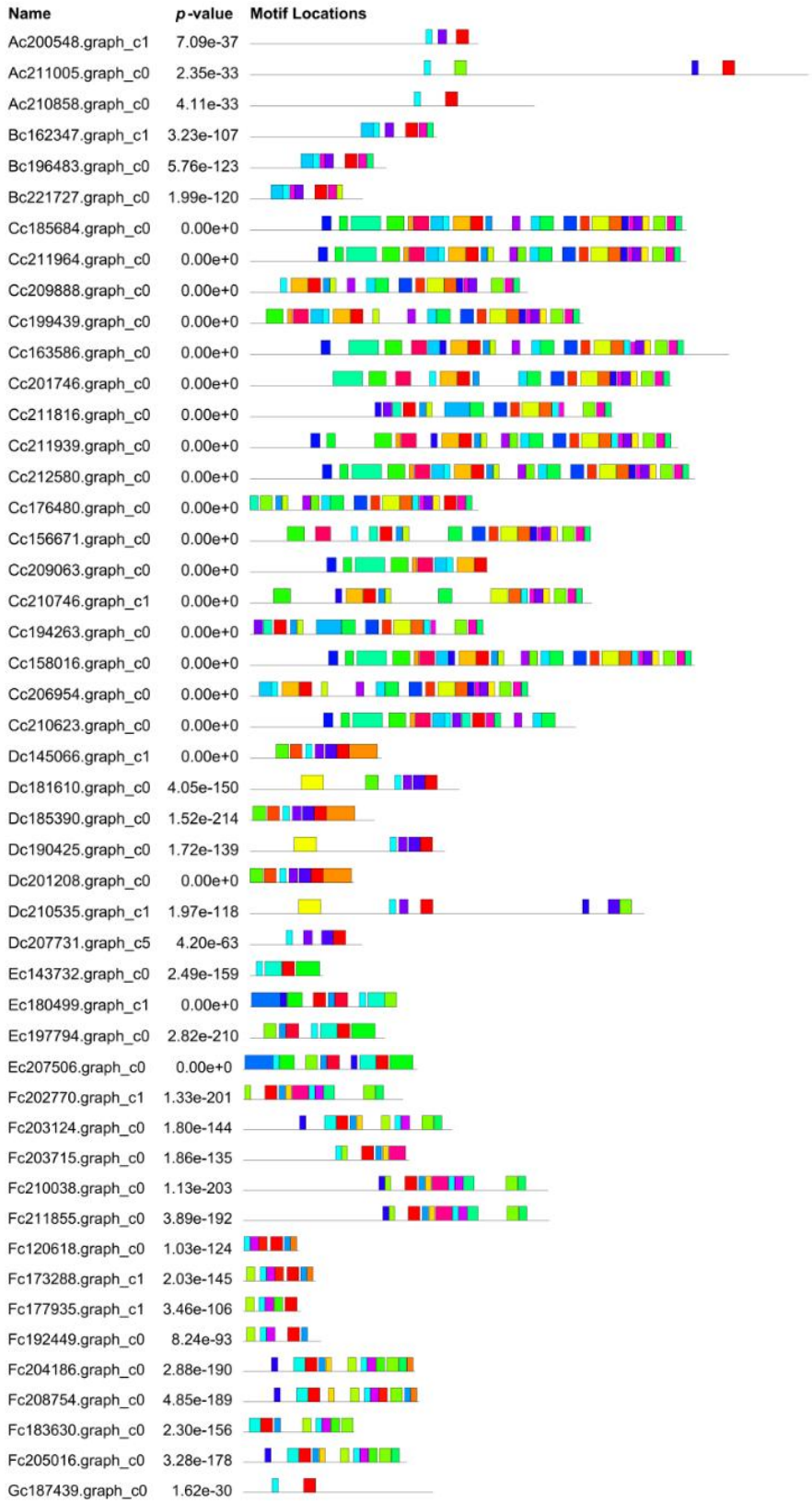

Figure 4 Distribution of conserved motifs of ABC transporter family in Hydrangea
Note: Different color squares represent different conservative motifs

Table 2 Differential expression of ABC transporter gene in hydrangea under aluminum stress

Gene	Transcriptome code	Differentially expressed site	log2FC	Express trends
HmABCA1	Ac200548.graph_c1	Root	3.377822103	Up-regulation
HmABCC1	Cc211816.graph_c0	Root	6.439104379	Up-regulation
HmABCC6	Cc156671.graph_c0	Root	5.820422296	Up-regulation
HmABCC14	Cc194263.graph_c0	Root	6.454978981	Up-regulation
HmABCD1	Dc145066.graph_c1	Root	3.89568012	Up-regulation
HmABCG1	Gc187439.graph_c0	Leaf	1.993847525	Up-regulation

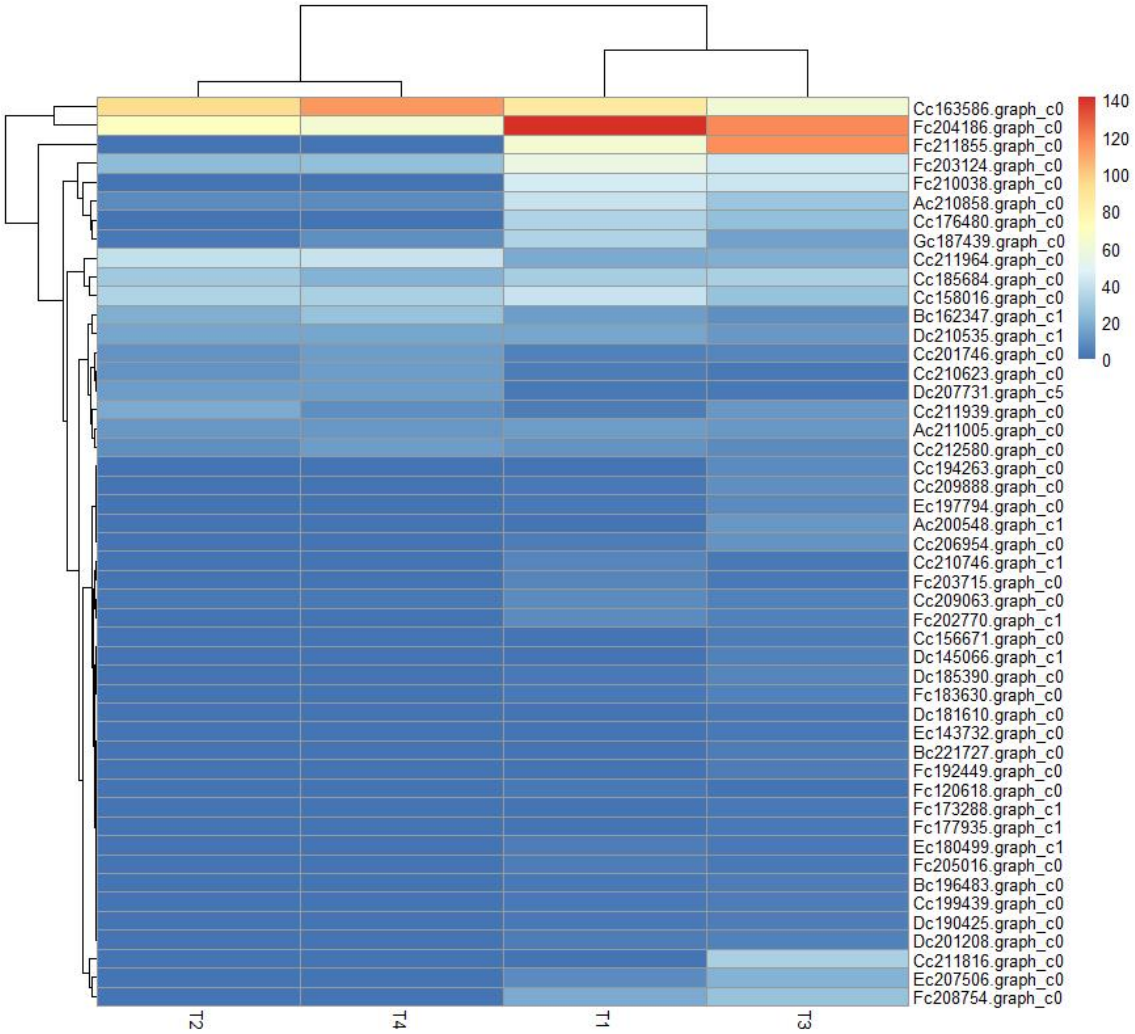

Figure 5 The heat map of ABC transporter gene expression under Al stress in Hydrangea

Note: T1: -Al treatment in root; T2: -Al treatment in leaf; T3: +Al treatment in root; T4: +Al treatment in leaf

2 Discussion

This study systematically analyzed the response of 48 ABC transporter genes in Hydrangea to aluminum stress. The results showed that the expression of different genes was different in the root and leaf after aluminum stress treatment, and the root was the main part of Hydrangea in response to aluminum stress. Based on the phylogenetic tree analysis, 48 proteins were clustered into 7 subfamilies, and the clustering results were basically consistent with the ABC transporters of Arabidopsis, indicating that the ABC transporters in Hydrangea and Arabidopsis have high homology, which provides a reference for the functional analysis of the ABC transporters of Hydrangea.

Through the analysis of the evolutionary relationship of the ABC transporter family between Hydrangea and Arabidopsis, it was found that *HmABCA3* (c211005.graph_c0) and Arabidopsis *AtABCA1*/*AtAOH1* (AT2G41700)

clustered in one branch; *HmABCA2* (c210858.graph_c0) was closely related to *AtABCA9*/*AtATH15*, which encoded an ABC transporter located in the endoplasmic reticulum (Kim et al., 2013; Wang et al., 2017). There are few studies on the function of subfamily A transporters in plants. According to homology analysis, they have the function of lipid transport in plants.

Subfamily B proteins in plants have diverse functions and are mainly involved in the transport of auxin, secondary metabolites and heterologous substances, including multidrug resistance related protein (MDR), ABC transporter of the mitochondria (ATM), heavy metal tolerance protein (HMT) and transporter associated with antigen processing (TAP). *ABCB27*/*AtTAP2* (AT5G39040), which is located in the same branch as *HmABCB1* (c162347.graph_c1) of Hydrangea, encodes an ABCB transporter located in the vacuole. The gene product may play an important role in the intracellular transport of aluminum chelates and is an important factor in the aluminum sequestration mechanism (Larsen et al., 2007). After aluminum stress, *HmABCB1* in Hydrangea was up-regulated in the root, but the differential expression was not significant, which may be related to the concentration and time of aluminum stress treatment.

The original member of subfamily C was the human multidrug resistance related protein HsMRP1, and MRPs were subsequently found in plants. MRP is not only involved in intracellular detoxification and oxidative stress resistance, but also mediates the transport of glutathione (GSH) conjugates. ABC transporters on the vacuolar membrane are mainly members of PRP subfamily, which are mainly involved in the transport of toxic substances and realize their isolation by vacuoles, thus playing a detoxification role. *HmABCC4*, *HmABCC5*, *HmABCC12* (c176480.graph_c0, c185684.graph_c0, c210623.graph_c0) in Hydrangea and *ABCC8*/*ATMRP6*, *ABCC10*/*ATMRP14* (AT3G59140.1, AT3G21250.1) in Arabidopsis are clustered in the same branch. At present, it has been reported that ATMRP14 is a heterologous transmembrane transporter, and ATMRP6 has an important role in detoxifying cadmium (Gaillard et al., 2008). *ABCC5*/*AtMRP5* (AT1G04120.1), which is clustered with the *HmABCC15* (c211939.graph_c0) in Hydrangea, participates in the regulation of ion channels in abscisic acid and calcium ion signal transduction pathways in guard cells, helping plants resist drought. The expression of *ABCC13*/*AtMRP11* (AT2G07680.1), which is similar to the relationship between *HmABCC8* (c201746.graph_c0) in Hydrangea, is induced by gibberellin and inhibited by naphthalene acetic acid, abscisic acid and zeatin. In the presence of cadmium or hydrogen peroxide, the differential expression of *ABCC13* gene in the root system of wheat seedlings plays an important role in alleviating the oxidative stress caused by heavy metals (Bhati et al., 2015). *HmABCC1* (c156671.graph_c0), *HmABCC6* (c194263.graph_c0) and *HmABCC14* (c211816.graph_c0) clustered into independent branches in the evolutionary tree, and their expression levels were significantly increased after aluminum stress. It is speculated that they participated in the response of Hydrangea to aluminum stress, and the mechanism of their response to aluminum stress needs further study.

The ABCD subfamily belongs to PMP subfamily, also known as peroxisome transmembrane transport proteins. There are only two kinds of AtPMP1 and AtPMP2 in Arabidopsis. The six genes in ABCD subfamily in Hydrangea are clustered in the same group with ABCD1 (AT4G39850.1). It is reported that ABCD1 is involved in the physiological process of glucose regulated meristem maintenance in Arabidopsis roots (Huang et al., 2019). In the process of aluminum stress, a series of complex oxidative stress reactions occur after the roots are damaged. Therefore, the function of *HmABCD1* (c145066.graph_c1) gene, which is significantly upregulated in the roots of Hydrangea, deserves further study.

The ABCG subfamily is a transporter of the NBD-TMD reverse sequence domain type, including two types of PDR and WBC. It has been reported that *AcABCG1* gene in *Axonopus compressus* is localized on the cytoplasmic membrane in response to aluminum stress (Li et al., 2019). *HmABCG1* (c187439.graph_c0) in Hydrangea and *AtABCG14* (AT1G31770.1) in Arabidopsis clustered on the same branch. AtABCG14 is a cytokinin transporter, which can not only regulate the growth and development of Arabidopsis root, but also mediate cytokinin transport to regulate the drought resistance response (Ko et al., 2013; Zhang et al., 2014). Therefore, it can be speculated that the root tip of Hydrangea is damaged after aluminum stress, and the expression of *HmABCG1* gene is up-regulation. In response to aluminum stress, its gene function needs further study.

3 Materials and Methods

3.1 Identification of ABC transporter gene family members in Hydrangea

The data used to identify the members of the ABC transporter gene family in Hydrangea is the transcriptome sequencing data obtained by our research group based on the Illumina high-throughput sequencing platform (Chen et al., 2015). As there is no genomic information of the Hydrangea, according to the NR annotation results, the ABC transporters of the Hydrangea were screened by using "ATP-binding cassette transporters" or "ABC" as the keywords. Then NCBI-blastp program was used to compare the screened sequences with the ABC transporter gene families of 22 species such as Arabidopsis, papaya and grape. Then, the SMART online database was used to analyze the conserved domain of the above genes, and the sequences that did not contain the ABC transporter gene features were removed, so as to obtain the transcript sequence of the ABC transporter gene of Hydrangea. According to the ABC transporter gene naming pattern of *Arabidopsis thaliana*, it was named based on sequence similarity, and the unigenes in the transcriptome database were renumbered accordingly.

ProtParam online software (https://web.expasy.org/protparam/) was used to predict and analyze the molecular weight, isoelectric point, instability index, aliphatic index, hydrophobicity and other physical and chemical properties of the ABC transporter sequence of Hydrangea. Plant-mPloc online software (http://www.csbio.sjtu.edu.cn/bioinf/plant/) was used for subcellular localization prediction of proteins. The protein conserved motif was analyzed by MEME online software (http://meme-suite.org/tools/meme), and the ABC protein domain was predicted by CD-search, and the gene structure map was drawn.

3.2 Construction of the phylogenetic tree of ABC transporter gene family in Hydrangea

Selected the amino acid sequence of the identified ABC family gene of Hydrangea. mafft software was used to conduct multiple sequence alignment of ABC protein. Neighbor joining (NJ) method was used to construct evolutionary tree (MEGA7).

3.3 Comparative study on ABC transporter gene family members between Arabidopsis and Hydrangea

First, download the amino acid sequence of *Arabidopsis thaliana* from the website, and then use Muscle software for global alignment. Use iqtree software to construct the evolutionary tree, with the base substitution model of LG+F+G4. Finally, iTOL software was used to color the evolutionary tree.

3.4 Analysis of expression pattern

In order to construct the expression profile of ABC transporter gene in different tissues of Hydrangea under aluminum stress, analyze the tissue expression specificity of ABC transporter gene, obtain the FPKM value of the gene expressed in roots and leaves without aluminum stress treatment and 4 h stress treatment from the transcriptome database, and finally draw the gene expression heat map with pheatmap software.

Authors' contributions

CHX and WX are the executors of the experimental design and research of this study, completed the data analysis and wrote the first draft of the paper. WX and XL participated in the experimental design and analysis of the experimental results. CHX is the designer and principal of the project, guiding the experimental design, data analysis, writing and revision of the paper. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (31201656) and the Natural Science Foundation of Hunan Province (2017JJ3105).

References

Aryal B., Laurent C., and Geisler M., 2016, Correction: learning from each other: ABC transporter regulation by protein phosphorylation in plant and mammalian systems, Biochemical Society Transactions, 44(2): 663-673
https://doi.org/10.1042/BST20150128_2
PMid:27068986

Bhati K.K., Sharma S., Aggarwal S., Kaur M., Shukla V., Kaur J., Mantri S., and Pandey A.K., 2015, Genome-wide identification and expression characterization of ABCC-MRP transporters in hexaploid wheat, Front. Plant Sci., 6: 488
https://doi.org/10.3389/fpls.2015.00488
PMid:26191068 PMCid:PMC4486771

Chen H.X., Lu C.P., Jiang H., and Peng J.H., 2015, Global transcriptome analysis reveals distinct aluminum-tolerance pathways in the al-accumulating species hydrangea *macrophylla* and marker identification, PLoS One, 10(12): e0144927
https://doi.org/10.1371/journal.pone.0144927
PMid:26660093 PMCid:PMC4682798

Gaillard S., Jacquet H., Vavasseur A., Leonhardt N., and Forestier C., 2008, *AtMRP6/AtABCC6*, an ATP-Binding Cassette transporter gene expressed during early steps of seedling development and up-regulated by cadmium in *Arabidopsis thaliana*, BMC Plant Biol., 8: 22
https://doi.org/10.1186/1471-2229-8-22
PMid:18307782 PMCid:PMC2291051

Huang C.F., Yamaji N., Chen Z.C., and Ma J.F., 2012, A tonoplast-localized half-size ABC transporter is required for internal detoxification of aluminum in rice, Plant J., 69: 857-867
https://doi.org/10.1111/j.1365-313X.2011.04837.x
PMid:22035218

Huang L., Yu L.J., Zhang X., Fan B., Wang F.Z., Dai Y.S., Qi H., Xie L.J., ang Xiao S., 2019, Autophagy regulates glucose-mediated root meristem activity by modulating ROS production in *Arabidopsis,* Autophagy, 15(3): 407-422
https://doi.org/10.1080/15548627.2018.1520547
PMid:30208757 PMCid:PMC6351127

Kim S., Yamaoka Y., Ono H., Kim H., Shim D., Maeshima M., Martinoia E., Cahoon E.B., Nishida I., and Lee Y., 2013, AtABCA9 transporter supplies fatty acids for lipid synthesis to the endoplasmic reticulum, Proc. Natl. Acad. Sci. USA, 110(2): 773-778
https://doi.org/10.1073/pnas.1214159110
PMid:23269834 PMCid:PMC3545803

Ko D., Kang J., Kiba T., Park J., Kojima M., Do J., Kim K.Y., Kwon M., Endler A., Song W.Y., Martinoia E., Sakakibara H., and Lee Y., 2014, *Arabidopsis* ABCG14 is essential for the root-to-shoot translocation of cytokinin, Proc. Natl. Acad. Sci. USA, 111(19): 7150-7155
https://doi.org/10.1073/pnas.1321519111
PMid:24778257 PMCid:PMC4024864

Lane T.S., Rempe C.S., Davitt J., Staton M.E., Peng Y.H., Soltis D.E.,Melkonian M., Deyholos M., Leebens-Mack, H J., Chase M., Rothfels C.J., Stevenson D., Graham S.W., Yu J., Liu T., Pires J.C., Edger P.P., Zhang Y., Xie Y.L., Zhu Y., Carpenter E., Wong G.K., and Jr C.N.S., 2016, Diversity of ABC transporter genes across the plant kingdom and their potential utility in biotechnology, BMC Biotechnol., 16: 47
https://doi.org/10.1186/s12896-016-0277-6
PMid:27245738 PMCid:PMC4886425

Larsen P.B., Cancel J., Rounds M., and Ochoa V., 2007, *Arabidopsis ALS1* encodes a root tip and stele localized half type ABC transporter required for root growth in an aluminum toxic environment, Planta, 225(6): 1447-1458
https://doi.org/10.1007/s00425-006-0452-4
PMid:17171374

Li J.F., Han J.R., Jia Y.D., Zhang L.Z., Wang W.Q., Wang Z.Y., and Chen Z.J., 2019, Cloning and expression analysis of *AcABCG*1 in *Axonopus compressus*, Caodi Xuebao (Acta Agrestia Sinica), 27(5): 1147-1153

Li S.2018. The functional study of ABCF subfamily genes in Arabidopsis thaliana. University of Chinese Academy of Sciences, Supervisor: Xu J. pp.24-34

Liang Y.N., 2018, Functional analysis of SbSTAR1 and SbSTAR2 in sweet sorghum (*Sorghum bicolor* L.) under aluminum stress, Thesis for M.S., College of Plant Science, Jilin University, Supervisor: Yang Z.M. pp.36-45

Negishi T., Oshima K., Hattori M., and Yoshida K., 2013, Plasma membrane-localized Al-transporter from blue hydrangea sepals is a member of the anion permease family, Genes to Cells, 18: 341-352
https://doi.org/10.1111/gtc.12041
PMid:23433438

Negishi T., Oshima K., Hattori M., Kanai M., Mano S., Nishimura M., and Yoshida K., 2012, Tonoplast-and plasma membrane-localized aquaporin-family transporters in blue hydrangea sepals of aluminum hyperaccumulating plant, PloS One, 7(8): e43189
https://doi.org/10.1371/journal.pone.0043189
PMid:22952644 PMCid:PMC3430636

Sanchez-Fernandez R., Davies T.G.E., Coleman J.O.D., and Rea P.A., 2001, The *Arabidopsis thaliana* ABC protein superfamily, a complete inventory, Journal of Biological Chemistry, 276(32): 30231-30244
https://doi.org/10.1074/jbc.M103104200
PMid:11346655

Song W.J., Wang P.Q., Zhang B.Y., Yang Q.C., Cai B.J., Lai P., and Kang Y.H., 2013, *MsSTAR2* encodes a half-type ATP-binding cassette transporter protein that is induced by aluminum in *Medicago sativa* L., Nongye Shengwu Jishu Xuebao (Journal of Agricultural Biotechnology), 21(5): 522-529

Wang X.Z., Sun W.M., Ma Y.F., Han E.Q., Han L., Sun L.P., Peng Z.H., and Wang B.J., 2017, Research progress of Arabidopsis ABC transporter, Zhiwu Shengli Xuebao (Plant Physiology Journal), 53(2): 133-144

Wilkens S., 2015, Structure and mechanism of ABC transporters, F1000 Prime Reports, 7(14): 14-18
https://doi.org/10.12703/P7-14
PMid:25750732 PMCid:PMC4338842

Zhang K.W., Novak O., Wei Z.Y., Gou M.Y., Zhang X.B., Yu Y., Yang H.J., Cai Y.H., Strnad M., and Liu C.J., 2014, *Arabidopsis* ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins, Nat. Commun., 5: 3274
https://doi.org/10.1038/ncomms4274
PMid:24513716