The functional analysis of ABCG transporters in the adaptation of pigeon pea (*Cajanus cajan*) to abiotic stresses

Hanghang Li [Equal first author, 1], **Zhihua Song** [Equal first author, 1], **Lili Niu** [2], **Biying Dong** [1], **Hongyan Cao** [1], **Tengyue Liu** [1], **Tingting Du** [1], **Wanlong Yang** [1], **Rohul Amin** [1], **Litao Wang** [1], **Qing Yang** [1, 2], **Dong Meng** [Corresp., 1, 2], **Yujie Fu** [Corresp. 1, 2, 3]

[1] The College of Forestry, Beijing Forestry University, Bei Jing, People's Republic of China

[2] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Bei Jing, People's Republic of China

[3] Key Laboratory of Forestry Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, People's Republic of China

Corresponding Authors: Dong Meng, Yujie Fu
Email address: mengdongjlf@163.com, yujie_fu@163.com

ATP-binding cassette (ABC) transporters are a class of proteins that widely present in living organisms and mediate transmembrane transport of substances by hydrolyzing ATP, play a vital role in the physiological process of growth and development in plants. The ABCG is the largest subclass of ABC transporters family, which has the most complex functions in plant's response to abiotic stress. This study focused on the effect of ABCG transporters in pigeon pea adaptation to the adverse environments (such as drought, salt, temperature, etc.). Based on this, the functional analysis of ABCG transporters in pigeon pea to abiotic stresses was conducted. In this study, a total of 51 *ABCG* genes (*CcABCGs*) were identified, further, their phylogenetic analysis and physicochemical properties of the encoded proteins, subcellular localization prediction, and conserved domains were performed. Expression analysis showed that *ABCG* genes have different expression profiles under tissues and abiotic stresses. Results showed that *CcABCG28* was up-regulated at low temperature, however, *CcABCG7* was up-regulated by drought and aluminum stress. Initial results revealed that ABCG transporters is more effective in the abiotic stresses resistance of pigeon peas, which broaden its research direction in abiotic stress resistance.

The functional analysis of ABCG transporters in the adaptation of pigeon pea (*Cajanus cajan*) to abiotic stresses

Hanghang Li[1, *], Zhihua Song[1, *], Lili Niu[2], Biying Dong[1], Hongyan Cao[1], Tengyue Liu[1], Tingting Du[1], Wanlong Yang[1], Rohul Amin[1], Litao Wang[1], Qing Yang[1,2], Dong Meng[1, 2, #], Yujie Fu[1, 2, 3, #]

[1] The College of Forestry, Beijing Forestry University, Beijing, People's Republic of China

[2] Beijing Advanced Innovation Center for Tree Breeding by Molecular Design, Beijing, People's Republic of China

[3] Key Laboratory of Forestry Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin, People's Republic of China

[*] These authors contributed equally to this work.

Corresponding Author:

Dong Meng[1,2], Yujie Fu[1,2,3]

The College of Forestry, Beijing Forestry University, Beijing 100083, People's Republic of China

Email address: mengdongjlf@163.com (D.M.); yujie_fu@163.com (Y.F.)

Abstract

ATP-binding cassette (ABC) transporters are a class of proteins that widely present in living organisms and mediate transmembrane transport of substances by hydrolyzing ATP, play a vital role in the physiological process of growth and development in plants. The ABCG is the largest subclass of ABC transporters family, which has the most complex functions in plant's response to

abiotic stress. This study focused on the effect of ABCG transporters in pigeon pea adaptation to the adverse environments (such as drought, salt, temperature, etc.). Based on this, the functional analysis of ABCG transporters in pigeon pea to abiotic stresses was conducted. In this study, a total of 51 *ABCG* genes (*CcABCGs*) were identified, further, their phylogenetic analysis and physicochemical properties of the encoded proteins, subcellular localization prediction, and conserved domains were performed. Expression analysis showed that *ABCG* genes have different expression profiles under tissues and abiotic stresses. Results showed that *CcABCG28* was up-regulated at low temperature, however, *CcABCG7* was up-regulated by drought and aluminum stress. Initial results revealed that ABCG transporters is more effective in the abiotic stresses resistance of pigeon peas, which broaden its research direction in abiotic stress resistance.

Keywords: Pigeon pea (*Cajanus Cajan*); ABCG transporters; Abiotic stresses; Gene expression

Introduction

ABC transporters, also known as ATP-binding cassette transporters, are a part of the largest and oldest known protein families and are widely found in the eukaryotic and prokaryotic organisms (Martinoia et al., 2002; Mosser et al., 1993). ABC transporters play an extremely important role in the growth and development of plants by participating in the detoxification of exogenous toxins for the response to abiotic stress, transporting the molecular of metabolites such as intercellular peptides, sugars, lipids, alkaloids, and inorganic ions and other metabolic substances (Mendez & Salas, 2001; Morris & Zhang, 2006; Mourez et al., 1997). *AtABCB1* (also known as *AtMDR1*) identified in *Arabidopsis* was the first reported in plants in 1992 (Dudler & Hertig, 1992). In the plant genomes, ABC transporters are divided into 8 subfamilies (ABCA-ABCG and ABCI) according to the HUGO (Human Genome Organization) scheme. Then, 129, 128, 261 ABC transporters have been identified in Arabidopsis (*Arabidopsis thaliana*), rice (*Oryza sativa*) and soybean (*Glycine max*) genomes, respectively (Mishra et al., 2019; Schulz & Kolukisaoglu, 2006; Sanchez-Fernandez et al., 2001).

The ATP-binding cassette subfamily G is the largest subfamily of ABC transporters family. It is found that ABCG protein is widely distributed in plants and plays an important role in many fundamental physiological processes (Kretzschmar et al., 2011). A typical structural feature of ABCG transporters has a highly conservative amino acid of 1-2 nucleotide-binding domains (NBDs) and a highly hydrophobic transmembrane domain of 1-2 trans-membrane domains (TMDs) (Verrier et al., 2008). ABCG subfamily is divided into WBCs (white-brown complex) and PDRs (pleiotropic drug resistance). WBCs are semi-molecular ABCG transporter, contain one NBD domain, and one TMD domain, while PDRs belong to the full-molecule ABCG transporter, include two NBD domains and two TMD domains (Jasinski et al., 2009). The NBD

domain consists of three highly conserved regions of approximately 200 amino acids and include Walker A box [GX_4GK (ST)], Walker B box [(RK) X_3GX_3L (hydrophobic)$_3$], and Walker C between them with approximately 120 amino acids in length(J. E. Walker, 1982). The TMD domain contains 4~6 a-helices, which constitute the channel for the transport of substrate molecules for transmembrane transport (Hyde et al., 1990; Schneider & Hunke, 1998). Therefore, it is generally believed that the mechanism of ABCG transporters is that the substrate recognition site on the TMD domain recognize and bind the transport substrates located near the cell membrane, while NBD domain on the cell membrane hydrolyzes ATP to provide energy for substrate transport and cause conformational changes in the membrane structure (Davidson & Maloney, 2007). *SpTUR2*, the first full-molecule *ABCG* transporter gene in plants, have been identified in the perennial aquatic plant *Spirodella polyrhiza* in 2002 (van den Brule et al., 2002). So far, more than 40 ABCG transporters have been identified in the *Arabidopsis*, rice and soybean genomes, respectively. ABCG transporters are involved with many physiological activities of the plant. For example, *OsABCG31* identified in rice could decrease the evaporation of plant leaves, which is predicted to relate to the drought stress response of rice (Chen et al., 2011). *Arabidopsis AtPDR8* is involved with Na^+ excretion, increasing the plant's tolerance to salt and drought stress (Kim et al., 2007). It was also found that *AtPDR36* in *Arabidopsis thaliana* responded to the toxic effects of heavy metal lead and participated in the stomatal self-regulation of leaves (Kim et al., 2010).

Cajanus cajan (L.) Millsp, as known as pigeon pea, is a diploid plant (2n=22) with a genome size of approximately 858 Mbp and grows in tropical and subtropical regions, which has formed a stable regulatory system to adapt to the adverse environment of high temperature, high salt and drought (Singh et al., 2013; Varshney et al., 2011; Wu et al., 2011; Yadu et al., 2018). Meanwhile, chilling is an important environmental factor in the practice of pigeon pea migration to the north. Besides, pigeon pea has widely grown in acidic soil (pH5-7), and its good aluminum resistance has bought it much attention. Pigeon pea produces numerous secondary metabolites that play an important role in the adaptation of the plant to the adverse environment (H Shepherd, 1986). Pigeon pea possesses medicinal properties and widely used in chemical industry (Ogoda et al., 2002; Pandey & Pandey, 1991).

However, the current research work focused on the trans-membrane transport of secondary metabolite, antibiotics, heavy metal ions by ABCG transport protein hydrolysis of ATP (Yazaki, 2006; Badri et al., 2008; Le Hir et al., 2013; Fourcroy et al., 2014). The inhibition of ABCG transporters decreases plant flavonoid content (Morris & Zhang, 2006; Imai et al., 2004). It was found that plants are transporting some hormones (such as ABA) which increases the survival of plants in a drought and other adversity environments (Kuromori et

al., 2010; Kang et al., 2010). It reminds us that ABCG transporters can positively affect plant adversity by transporting certain substances. To reveal the important role of ABCG transporters in resisting environmental stress in pigeon peas, identification, phylogenetic analysis, and expression analysis, etc. of the pigeon pea ABCG gene family were performed in our study. A total of 51 ABCG transporters were identified and further analyzed. We have found that some cis-acting elements related to stress-responsive existed in several genes identified above, suggesting that ABCG transporter had a related regulatory effect in pigeon pea. To explore the expression profile of ABCG gene in pigeon pea, we analyzed the expression of ABCG gene under different organs and different stress treatments and observed the tissue specificity of ABCG gene and stress expression response. Our findings will lay the foundation for exploring the mechanism of ABCG transporters in resistance in several abiotic stresses of pigeon pea.

Materials and Methods

Identification of ABCG transporters in pigeon pea genome

The whole 129 *Arabidopsis* ABC protein sequences were downloaded in the Phytozome v12.1 database (https://phytozome.jgi.doe.gov/pz/portal.html). All of these *Arabidopsis* ABCs as queries were used to identify the ABC transporters in *Cajanus cajan* (L.) Millsp (taxid: 3821) database using BLASTP search in NCBI (National Center for Biotechnology Information) with an initial cut-off e-value of 1.0 e^{-10} and max target sequence of 500. Also, the HMM (Hidden Markov Model) profiles of ABC transporters (such as PF00005, PF00664, and PF10614) were downloaded from the Pfam database (http://pfam.xfam.org/search#tabview=tab1) and used HMMER search server against the pigeon pea proteome with an E-value setting of 1.0 e^{-5} (https://www.ebi.ac.uk/Tools/hmmer/) (Potter et al., 2018). Then, the resulting protein sequences were further identified whether it contained a conserved domain of ABC based on CD-search (Conserved Domain search) on the NCBI website with a threshold e-value of 1.0 e^{-5}. Through the above methods, members of the ABC transporters were identified.

Then, multiple-sequence alignments of ABC transporters identified above and several ABC proteins of *Arabidopsis thaliana*, rice, and soybean were performed using clustalW with default settings. The phylogenetic tree of the ABC transporters was constructed using the neighbor-joining (NJ) method with the 1000 replications of bootstrap and p-distance of a model in MEGA6.0 software (https://www.megasoftware.net/) (Tamura et al., 2013). The phylogenetic tree was visualized with iTOL website (http://itol.embl.de/help.cgi) (Sugiyama et al., 2007).

Finally, ABCG transporters were identified through the phylogenetic analysis of the ABC family of pigeon pea.

Phylogenetic tree construction and chromosome localization

The chromosomal information of *ABCG* genes was obtained through the NCBI website (https://www.ncbi.nlm.nih.gov/) and was systematically named based on the number and relative position of the *ABCG* genes in the pigeon pea chromosome. The phylogenetic tree of pigeon pea ABCG transporters was constructed in MEGA6.0 as described above.

Based on the chromosomal location information of the ABCG transporters, the chromosomal location of the *CcABCGs* was mapped using the MapChart software (https://www.wur.nl/en/show/Mapchart.html) (Voorrips, 2002).

Protein property prediction, subcellular localization

The exon information of *CcABCGs* was collected on the NCBI website. The number of amino acid sequences, relative molecular weight, and the isoelectric point (pI) of CcABCGs was predicted in the ExPASy ProtParam server database (http://expasy.org/). Subcellular localization prediction of ABCG proteins was performed by the Cell-PLoc 2.0 database (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) (Chou & Shen, 2008).

To verify the above predictions of subcellular localization, two-week-old seedlings of pigeon pea were used as cDNA template to amplify the full-length coding region of *CcABCG7* gene. The primers were: F: 5′-ATGGTGATGATATGGGAAAATGTTAC-3′ and R: 5′-TTATATTGGAAGGTTTGGGGACA-3′. The recovered PCR production was ligated to the T vector pMD19-T (TaKaRa, Japan). Subsequently, it was cloned into the eGFP-pROKII vector by double digestion with KpnI/XbaI to construct the CcABCG7-eGFP-pROKII expression vector and transformed into agrobacterium strain GV3101(Shanghai Weidi Biotechnology, China). One-month-old tobacco seedlings were injected into the Agrobacterium liquid on the back of the tobacco leaves with a disposable syringe and placed in a dark place for 3 days for observation. Approximately 0.5 cm^2 of the material was taken around the injection site, observed, and photographed under a Leica SP8 laser confocal microscope (Leica, Germany).

Analysis of motifs and conserved domain

To examine the characteristics and properties of the pigeon pea ABCG subfamily protein domain, the conserved motif of CcABCGs was analyzed on the MEME software (http://meme-suite.org/tools/meme) with following parameters: the number of motifs was set 10 and the optimum motif width was set between 20 and 200, the other parameters were the defaults(Bailey

& Elkan, 1994). Then InterPro program (http://www.ebi.ac.uk/interpro/scan.html) was performed to annotate all 10 motifs.

The conserved domain of CcABCGs was performed in HMMER servers (https://www.ebi.ac.uk/Tools/hmmer/) and visualized using TBtools software (Chen, 2018). At the same time, representative WBC and PDR transporter amino acid sequences were selected as target sequences used to select the template with higher homology and better coverage Swiss-model server with automatic mode(https://swissmodel.expasy.org/interactive). The model results were evaluated using SAVES server(https://servicesn.mbi.ucla.edu/SAVES/), and the homology model of CcABCG proteins was visualized by the PyMol software.

Gene structure and cis-elements analysis of CcABCGs

Based on the evolution analysis of *CcABCGs*, the intron/exon structures of *ABCG* genes were analyzed by the gene annotation file (GFF) of C.cajan_V1.0, and visualized using TBtools software(Chen, 2018), Then the 2000bp upstream region of all *CcABCGs* identified was extracted from pigeon pea genome to identify the stress-related or other functions cis-acting regulatory elements of promoter sequences using TBtools software. All promoter sequences were analyzed on the PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Rombauts et al., 1999).

Go annotation and function prediction

GO (Gene Ontology) integrates and unifies the description and standards of gene products in the current database, and provides the most comprehensive description of gene functions and gene products. In this study, the ABCG protein sequences in pigeon pea were used for blast alignment in the Swiss-prot database (https://www.uniprot.org/blast/), and then the alignment results were annotated and classified of the pigeon pea *ABCG* gene(Ashburner et al., 2000; Consortium, 2019; Ehlert et al., 2006; Yang et al., 2016).

Plant materials and treatments

On the one hand, the seeds of pigeon pea (ICPL87119) were sown in mixed soil of nutrient soil, vermiculite and perlite (1:1:1) in the greenhouse with 10-14h/day of average annual lighting conditions, 18~28°C in temperature and 45-70% of the relative humidity at Beijing Forestry University in China. After three months, the roots, stems, leaves, and flowers from pigeon pea trees were selected and stored at -80°C to extract RNA and further analyze tissue-specific expression.

On the other hand, the pigeon pea seeds were surface-sterilized with 75% Ethanol for 30 seconds, then soaked in sodium hypochlorite solution for 6 minutes, and finally washed 5 times with sterile water for 30 seconds each time. These sterilized seeds were grown in Murashige and Skoog (MS) medium at pH=5.8 in growth chambers at 24±2°C with a photoperiod of 16h light/ 8h dark, 400 μM m^{-2} s^{-1} light intensity. To analyze the expression of *CcABCG* genes in various abiotic stresses, the 14-days old pigeon pea seedlings were chosen to different treatments: for heat and cold stress, the seedlings were cultured at 4°C and 42°C in incubators, respectively; for salt stress and metal stress, the seedlings were grown in solid MS medium with 200 nmol/L NaCl and 100 μmo/L $AlCl_3$, respectively; for drought stress, seedlings of pigeon pea were treated with 250 nmol/L mannitol in solid MS medium. Roots and leaves of these seedlings were sampled at 0h, 6h, 12h after various treatments. Three biological replicates of each sample were immediately frozen in liquid nitrogen and stored at -80°C until expression analysis of variously abiotic stresses.

RNA isolation and quantitative real-time PCR analysis

Based on the above analysis of cis-elements in the promoter region, 10 genes containing elements that responded to adversity were selected for tissue specificity analysis and abiotic stress analysis. The total RNA was isolated using CTAB method. And then, the first-strand cDNA was synthesized from 1μg of total RNA using a PrimeScript RT kit (Takara) according to the manufacturer's manual. The quality and concentration of cDNA were assessed using a Nano Photometer N50 (Implen GmbH, Munich, Germany). Expression analysis of ABCG transporter under different tissues and abiotic stress was performed using qRT-PCR. Real-time RT-PCR analysis was performed on a CFX connect (Bio-Rad, California, USA) using the SYBR Green PCR Master Mix (TaKaRa, Tokyo, Japan) with *CcActin* as a reference gene. The primers of genes selected were synthesized on Sangon Biotech website (http://www.sangon.com/newPrimerDesign) and were listed on Table S1. All the analysis were performed with three biological replicates in this study, and qRT-PCR date was analyzed using IBM SPSS 22 software (IBM Corporation, USA).

Results

Identification of ABCG transporter genes in pigeon pea genome

To identify all members of the pigeon pea ABCG transporters, 129 Arabidopsis ABC proteins were downloaded and used as queries to search in pigeon pea genome database using BLASTP server. A total of 222 pigeon pea ABC proteins were identified after removing the redundancy. Then, the HMMER search was performed with Hidden Markov Model (HMM) of

ABC transporters (such as PF00005, PF00664, and PF10614) to screen for ABC transporters identified by BLASTP. Further, 155 pigeon pea ABC proteins were eventually confirmed that have the NBD/TMD domains (Table S2). To explore the phylogenetic evolutionary relationship between pigeon pea and other species ABC proteins such as Arabidopsis, rice, and soybean, a Neighbor-Joining (NJ) tree was constructed (Fig. S1). Phylogenetic relationship indicated that the pigeon pea *ABC* gene family was classified into 8 different groups (ABCA-ABCI), and ABCG subfamily, contained 51 members, was the largest group of pigeon pea ABC transporters.

Phylogenetic tree construction and chromosome localization

All of the ABCG transporters identified above were named *CcABCG1-CcABCG51* based on chromosomal location. A Neighbor-Joining (NJ) tree was constructed to analyze the phylogenetic relationship of ABCG transporters (Fig. 1). Phylogenetic analysis indicated that the ABCG transporters were further divided into WBC and PDR, of which WBC contained 33 ABCG transporters and PDR contained 18 transporters.

Chromosome localization of ABCG transporters in pigeon pea genome was performed, which indicated all identified *CcABCGs* were diversely distributed on pigeon pea chromosomes except chromosome 7. Chromosome 1 and 8 both contained one ABCG transporter, which had the least ABCG transporter in all the chromosomes of pigeon. Chromosome 2, 9,11 included 5,5,8 transporters, respectively. Besides, 17 genes, distributed on unplaced scaffolds, were mapped onto a single chromosome called the ChrUn (Fig. 2). Two pairs of genes (*CcABCG8/9, CcABCG32/33*) closely linked on the chromosome and belonged to the paralogous genes. Then, the *ABCG* gene localization was annotated using NCBI website and added the information in Table 1.

Protein property prediction, subcellular localization of CcABCGs

All of 51 CcABCGs identified were predicted their protein property, such as amino acid lengths, relative molecular weight, and isoelectric points (pIs) (Table 1). Results indicated that the lengths of all identified pigeon ABCG proteins ranged from 609 aa (CcABCG34) to 1500 aa (CcABCG29) amino acid; similarly, relative molecular mass varied from 68159.95 Kd (CcABCG34) to 170370.83 Kd (CcABCG29), and the theoretical isoelectric points ranged from 6.75 (CcABCG29) to 9.47(CcABCG4). The exons number of *ABCG* genes changed from 1 to 26.

To determine the active site of CcABCGs, subcellular localization of CcABCGs was performed. The prediction of subcellular localization indicated that all CcABCGs were localized in the cell membrane. Also, 3 ABCG transporters (CcABCG26, 28, 29) were found to be localized in chloroplast, not just on cell membranes (Table 1). To verify the above prediction, the

subcellular localization of ABCG transporter was verified by transiently expressing *CcABCG7-eGFP* in tobacco leaves. The results showed that the green fluorescent signal of CcABCG7-eGFP-pROKⅡ was mainly detected in the cell membrane (Fig. S2).

Analysis of motifs and conserved domain of CcABCGs

To identify the structural and functional of ABCGs in pigeon pea, motif analysis was performed based on the phylogenetic analysis (Fig. 3A). In our study, a total of 10 conserved motifs were predicted, and the width of those motifs ranged from 32 to 123 amino acids, the number of motifs in each amino acid varied from 2 to 10. Results showed that the number of motifs in the WBC was significantly less than the number of motifs detected in the PDR. Further, all of 10 motifs were annotated using the InterPro program to identify information of motif structure. Annotation analysis demonstrated that Motif 3,7,9,10 were annotated nothing; Motif 6 and Motif 8 were annotated ABC_2_trans structure (IPR013525); Motif 1,2,4,5 were annotated P_loop_NTPase structure (IPR027417); Motif 1,4,5 were annotated ABC_transporter_like structure (IPR003439). Results indicated that motifs of the same annotation were relatively conservative in structure, such as Motif 1,4,5 (Table S3).

The conserved domain of CcABCGs was performed to explore ABCG transporter's domain function using HMMER servers (Fig. 3B). In the HMME model, "ABC2_membrane" indicates the TMD domain, and "ABC_tran" indicates the NBD domain. The results showed that the ABCG transporters were different from other members of the ABC transporters family with an inverted "TMD-NBD" domain arrangement pattern. All ABCG transporters contained the NBD domain, but several ABCG transporters didn't contain the TMD domain. WBC subgroup contained one NBD domain with a length changed from 97 to 153 and one or no TMD domain with a length of about 200, while PDR subgroup contained two NBD domain with amino acid length of 138 to 200 and an approximately 200-length TMD domain.

CcABCG35 was selected for homology modeling to explore the molecular functions of CcABCGs. The best template of CcABCG35 was 6hij.1.A (Seq Identity:33.05%, GMQE:0.58, Coverage:0.85) using the Swiss-model program. According to the PROCHECK evaluation results, Ramachandran Plot results in the model evaluation shown that in each ABCG model,> 90% of the amino acid residues were distributed in the allowed region, indicating that the quality of the CcABCG protein model obtained by homology modeling is reliable (Fig. S3A). Model of CcABCG35 shown that Walker A and Walker B were located in the NBD domain, while the TMD domain contained 6 alpha helices (Fig. S3B).

Gene structure and cis-elements analysis of CcABCGs

Gene structure was analyzed based on the phylogenetic relationship of the ABCG gene family to better understand their structural evolution (Fig. 4A). The highly conserved exon sequence is an essential sequence for the ABCG transporter to perform gene functions, and the differences in introns may be different regulatory mechanisms for the existence of genes. The results indicated that all ABCG transporters in pigeon pea contained different numbers of exons and introns, but the significant difference was that the number of exons in *CcPDRs* was significantly higher than that in *CcWBCs*. The exons number of *CcPDRs* ranged from 5 to 25, while the exons number of *CcWBCs* varied from 1 to 14. Moreover, there were large differences in the number of introns. The average number of introns in *CcPDRs* was about 20, while those in *CcWBCs* was about 5.

To explore the expression elements of the promoter region, a region of 2000 bp upstream of the promoter was selected for cis-acting element analysis of the pigeon pea ABCG gene family (Fig. 4B). Three cis-acting elements were screened in our study, which focused on hormone-responsive elements, light-responsive elements, and stress-responsive elements. It was found that the number of light-responsive elements in the promoter region of the pigeon pea ABCG transporter was relatively large. Hormonal response elements had a large number of copies in the promoter region of the ABCG transporter, including auxin response elements (AuxRR-core, TGA-element, etc.), gibberellin response elements, etc. Compared with hormone response elements, there were fewer stress response elements, and some genes didn't contain stress response elements (*CcABCG14*, *CcABCG10*, etc.). At the same time, drought-related cis-acting elements were identified in *CcABCG7*, *CcABCG24*. Low-temperature stress-related cis-acting elements (LTR) were also identified in *CcABCG28*, in which their elements were highly conserved with short sequences composed of CCGAAA.

Go annotation and function prediction

Since the gene term of pigeon pea was not be annotated, the blast alignment method was used to perform go annotation in the Swiss-Prot database. The *ABCG* gene in pigeon pea was annotated and divided into three categories: molecular function, biological process, and cell component. The annotation results showed that all 50 genes were annotated into three major categories, but CcABCG48 were not blast anything UniProtKB ID, and all usable data were addressed in Raw Data. S5. Among the major categories of biological processes, the gene frequency with higher prominent is the transmembrane transport (GO:0055085), and 45 genes were found annotated in transmembrane transport. Furthermore, CcABCG28 and CcABCG29 were blasted in AB36G_ARATH(UniProtKB ID), and annotated 42 Go: annotations, which contained "response to salt stress"(GO:0009651) and "root development"(GO:0048364), etc. In the broad category of molecular functions, 50 *ABCG* genes are annotated to the molecular

functions related to ATPase activity and ATP binding; while in the major category of cellular components, most of the *ABCG* genes are annotated on the membrane (Fig. 5).

Expression analysis of the pigeon pea ABCG gene family in different organs

To explore the expression pattern of the ABCG gene in different organs during the growth and development of pigeon, organs (roots, stems, leaves, and flowers) of the one-year-old pigeon pea were collected to extract RNA and used it for qRT-PCR for analyzing the expression of the ABCG genes. Tissue-specific expression analysis found that the expression of ABCG transporters in different tissues was not significantly different. However, the relatively obvious difference was that the expression level of the ABCG transporter gene in flowers was significantly lower than that in roots, stems, and leaves. The expression of the gene *CcABCG24* was significantly higher than that of any other gene (Fig. 6).

Expression analysis of the pigeon pea ABCG transporters under different abiotic stresses

To explore the expression level of the pigeon pea *ABCG* gene family under different abiotic stresses, two-week-old pigeon pea seedlings were selected and transplanted to the culture environment of 4°C, 42°C, 200 nmol/L NaCl, 100 μmol/L $AlCl_3$ and 250 nmol/L mannitol in solid MS medium, respectively. Among them, *CcABCG5,7,14,19,21* belong to the WBC subgroup, and *CcABCG10,24,28,29,32* belong to the PDR subfamily. It was found that the expression difference of 10 genes in roots was significantly higher than that in leaves (Fig. 7).

In the roots (Fig. 7A, C, E, G, I), the two major subfamilies of ABCG transporters had significant differences in expression under 4°C stress and NaCl stress. The *PDR* family genes were significantly up-regulated under both stresses, and *CcABCG24* was up-regulated 40-fold after 12 h under 4°C treatment. The *CcABCG28* increased by 65 times. Under the initial NaCl treatment, *CcABCG24* increased by 10 times, but it was worth noting that with the further increase of the treatment time, the expression of *CcABCG24* decreased to below the initial level at 12h. Further, we found that the relative expression changes of WBC subgroups under drought and aluminum stress treatments were opposite differences, as shown in Fig. 7E, G. Expression level of *CcABCG5* increased under drought stress at 6h, while wasn't change significantly under aluminum stress. Similarly, *CcABCG19* also exhibited the same pattern of opposite expression in the treatments of mannitol stress and $AlCl_3$ stress. Under high-temperature stress, we observed that the expression of *CcABCG7* was down-regulated to a certain extent, but the expression of *CcABCG7* in leaves was up-regulated.

The expression differences in leaves were relatively gentle (Fig. 7B, D, F, H, J). It was worth noting that *CcABCG28* also was a certain up-regulation under 4°C treatment. However, *CcABCG7* was significantly up-regulated after 6h of drought stress. In the same situation, *CcABCG7* was up-regulated 11.3 times after 12h of aluminum stress treatment. However, under sodium stress, *CcABCG7, 19, 21* were down-regulated. In conclusion, under different stress conditions, the ABCG transporter has different expressions in response to different environmental stresses.

Discussion

ABC transporters are distributed in animals and plants, but ABC transporters in plants are characterized by a large number and complex function, such as 129,127 ABC transporters were identified in Arabidopsis and rice. As shown in Fig. S1, a total of 155 ABC transporters were identified in the pigeon pea genome, which was divided into 8 subgroups of ABCA-ABCI. Among them, ABCE and ABCF subfamily had no transport function because of their proteins were localized to the endoplasmic reticulum without a transporter region. ABCB subfamily might be involved in the transport of auxins, secondary metabolites, heavy metal salts (Kang et al., 2011; Verrier et al., 2008). ABCC subfamily might be involved in plant chlorophyll transport, cell detoxification, and other functions (Hashimoto & Yamada, 2003). ABCG transporters contained 51 members, was the largest group of ABC transporters in the pigeon pea, which was larger than reported for *Arabidopsis* (44) and rice (50), but smaller than the 116 members of ABCG reported for soybean (Mishra et al., 2019; Jasinski et al., 2003).

As shown in Fig. 1, the ABCG subfamily could be further divided into a full-molecular transporter PDRs and a semi-molecular transporter WBCs, in which PDRs contained 18 ABCG transporters and WBCs included 33 ABCG transporters. The conserved domain of the ABCG transporter is composed of NBD and TMD, and the ABCG transporter is different from the conserved domain arrangement of other ABC transporter subfamilies. The conserved domain composition of the ABCG transporter is a trans- "TMD-NBD" structure (Fig. 3)(van den Brule & Smart, 2002). The arrangement of ABC domains and their transmembrane domains are highly conserved, while the number of transmembrane helixes and their arrangements is not necessarily conserved, which determines their functional differences (Andolfo et al., 2015; Goodman et al., 2004; Locher, 2004). ABCG transporters were involved in many physiological activities of plants, transport small molecular compounds, secondary metabolites, and played an important role in disease resistance, hormonal regulation, and adaptation to changes in the external environment (Alejandro et al., 2012; Bird et al., 2007; Lee et al., 2010). Arabidopsis cutin and wax secretion required *AtWBC11* participation (Panikashvili et al., 2007). Rice *OsPDR9* could be

induced by methyl jasmonate, and *AtPDR8* could be induced by salicylic acid (Kim et al., 2007; Kuromori et al., 2010; Moons, 2008). When plants are stressed by external environmental factors, the signal receptors on the plant cell first sense the external stress signal and generate a second messenger transmitted inside the cell, such as Ca^{2+} ions, reactive oxygen molecules, ABA, etc., and then mediates the phosphorylation of downstream proteins to activates downstream transcription factors, which can specifically activate target genes related to stress response, and thus resist cell damage from factors such as drought and temperature changes (Mittler et al., 2012; Farooq et al., 2009) As shown in Fig. 4B and Fig. 5, there were found that many *ABCG* genes were annotated to hormone transport and regulation related functions. *AtABCG22* was induced by drought stress, possibly by affecting stomata and increasing transpiration (Kuromori et al., 2011). In our study, *CcABCG28*(PDR) might be involved in the response of pigeon pea to abiotic stresses such as chilling, salt; while *CcABCG7*(WBC) tended to respond to drought and aluminum stress as shown in Fig. 7.

The analysis of the location of the ABCG family in the pigeon pea chromosome found that most of the *ABCG* gene was located on chromosome 11 as shown in Fig. 2. The major part of most plant genomes consisted of different repeating DNA elements. These sequence elements are essential for the large-scale organization and evolution of the plant genome (Kubis et al., 1998). Our results showed that there were two pairs of paralogous genes (*CcABCG8/9, CcABCG32/33*) closely linked to chromosome 3 and chromosome 11, and the tandem replication leaded to the expansion of these two genes. Introns are important components in the genome of eukaryotes. The typical 5'-GT ··· AG-3'of intron is an important marker of gene splicing and an important feature of introns in eukaryotic mRNA sequences(Rose et al., 2016; Mukherjee et al., 2018). Further, the exon/intron analysis of ABCG transporter was performed to learn the stress regulation of the splicing process in Fig. 4A. Our results indicated that PDR contained more intron structures than WBC, speculating PDR had more variable splices and functions, and might been more changes in response to stress. Furthermore, introns may also affect gene expression, and more introns may have a stronger regulatory effect (Rose et al., 2016; Mukherjee et al., 2018). But the argument that having more exon make them prone to more regulation is theoretical and no evidence was provided in support of that statement. PDR/WBC indeed have a different regulatory effect more depends on deeper molecular mechanisms. The cis-acting elements are involved in the binding of transcription factors (TF) and regulating the expression of the gene (Toledo et al., 2011). Our study found that there were many low temperature-related elements in the promoter region of the pigeon pea, which might be induced by chilling induction in pigeon pea (Fig. 4B). It was worth noting that the promoter region of the pigeon pea ABCG transporter had a large number of hormone-regulated expression elements (abscisic acid responsiveness,

MeJA responsiveness), which also demonstrated the important role of ABCG transporters in the regulation of plant hormones(Kuromori et al., 2010b; Wu et al., 2007). Kuromori et al. (Kuromori et al., 2011) found that *AtABCG25* is mainly expressed in vascular tissues and can transport ABA out of cells.

ABC transporters are essential for plant development and play a role in the processes of gametogenesis, seed development, seed germination, organ formation, and secondary growth (Thanh, etc, 2018). Our study performed the expression analysis of pigeon pea in different organs and under different abiotic stresses (Fig. 6 and Fig. 7). The expression levels of all genes in flowers were not obvious than in other tissues, while *CcABCG24* was highly expressed in other tissues. *CcABCG24* was expressed in the stems of pigeon peas significantly higher than other tissues, showing that ABCG transporters active regions as trans-membrane proteins. *AtABCG25* is mainly expressed in vascular tissues and can transport ABA to the outside of cells (Kuromori et al., 2011). It is demonstrated that it is closely related to the formation of plant vascular bundles. Analysis of the expression levels of the pigeon seedlings under different stresses shown that the expression of most genes in the roots was significantly higher than that in the leaves. Interestingly, *CcABCG28*, regardless of any tissue (root or leaf), the expression level is up-regulated, especially in the leaves, the up-regulation of this expression is significantly higher than any other gene under low-temperature stress. At present, the regulation mechanism of *CcABCG28* homologous gene in other species is still unclear, but this gene certainly plays an important role in the growth and development stage of pigeon pea. In our study, we also found that *CcABCG24* was highly expressed under NaCl treatment, and its homologous gene *AtPDR12* was of great significance for Pb(II) resistance in Arabidopsis (Lee et al., 2005). However, it was proved that *CcABCG24* played an important role in response to salt stress in this experiment. In response to drought and salt stress in plants, the cuticle lipid coding gene ABCG transporter gene will be significantly up-regulated, indicating that the ABCG transporter has an important role in adapting plants to drought and saline-alkali environments (Luo et al., 2007; Panikashvili et al., 2007b). The expression level of *CcABCG7* was up-regulated in roots under drought and aluminum stress, which proved its important role in stress tolerance of pigeon pea.

Conclusions

In the present study, a total of 51 ABCG transporters were identified and divided into two subgroups, WBC and PDR. Further, analysis of protein structure, gene structure cis-elements on the pigeon pea ABCG transporters were performed. The highly conserved NBD domain determines the important function of the ABCG transporter. *CcABCG28* was significantly up-regulated under low temperature stress, while *CcABCG7* responded to drought stress. In

conclusion, the results will initially reveal the role of ABCG transporters in abiotic stress resistance and broaden research direction in abiotic stress resistance of pigeon peas.

Acknowledgments

The authors gratefully acknowledge the support by The College of Forestry, Beijing Forestry University. and Beijing Advanced Innovation Center for Tree Breeding by Molecular Design.

References

Alejandro, S., Lee, Y., Tohge, T., Sudre, D., Osorio, S., Park, J., Bovet, L., Lee, Y., Geldner, N., Fernie, A.R., and Martinoia, E. 2012. AtABCG29 is a monolignol transporter involved in lignin biosynthesis. *CURRENT BIOLOGY* 22:1207-1212. 10.1016/j.cub.2012.04.064

Andolfo, G., Ruocco, M., Di Donato, A., Frusciante, L., Lorito, M., Scala, F., and Ercolano, M.R. 2015. Genetic variability and evolutionary diversification of membrane ABC transporters in plants. *BMC PLANT BIOLOGY* 15:51. 10.1186/s12870-014-0323-2

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., and Sherlock, G. 2000. Gene Ontology: tool for the unification of biology. *NATURE GENETICS* 25:25-29. 10.1038/75556

Badri, D.V., Loyola-Vargas, V.M., Broeckling, C.D., De-la-Peña, C., Jasinski, M., Santelia, D., Martinoia, E., Sumner, L.W., Banta, L.M., Stermitz, F., and Vivanco, J.M. 2008. Altered Profile of Secondary Metabolites in the Root Exudates of Arabidopsis ATP-Binding Cassette Transporter Mutants. *PLANT PHYSIOLOGY* 146:762-771. 10.1104/pp.107.109587

Badri, D.V., Loyola-Vargas, V.M., Du, J., Stermitz, F.R., Broeckling, C.D., Iglesias-Andreu, L., and Vivanco, J.M. 2008. Transcriptome analysis of Arabidopsis roots treated with signaling compounds: a focus on signal transduction, metabolic regulation and secretion. *NEW PHYTOLOGIST* 179:209-223. 10.1111/j.1469-8137.2008.02458.x

Bailey, T.L., and Elkan, C. 1994. Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proceedings. International Conference on Intelligent Systems for Molecular Biology* 2:28-36.

Bird, D., Beisson, F., Brigham, A., Shin, J., Greer, S., Jetter, R., Kunst, L., Wu, X., Yephremov, A., and Samuels, L. 2007. Characterization of Arabidopsis ABCG11/WBC11, an ATP binding cassette (ABC) transporter that is required for cuticular lipid secretion. *PLANT JOURNAL* 52:485-498. 10.1111/j.1365-313X.2007.03252.x

Chen. 2018. TBtools, a Toolkit for Biologists integrating various biological data handling tools with a user-friendly interface. 10.1101/289660

Chen, G., Komatsuda, T., Ma, J.F., Nawrath, C., Pourkheirandish, M., Tagiri, A., Hu, Y.G., Sameri, M., Li, X., Zhao, X., Liu, Y., Li, C., Ma, X., Wang, A., Nair, S., Wang, N., Miyao, A., Sakuma, S., Yamaji, N., Zheng, X., and Nevo, E. 2011. An ATP-binding cassette subfamily G full transporter is essential for the retention of leaf water in both wild barley and rice. *Proc Natl Acad Sci U S A* 108:12354-12359. 10.1073/pnas.1108444108

Chou, K., and Shen, H. 2008. Cell-PLoc: a package of Web servers for predicting subcellular localization of proteins in various organisms. *Nature Protocols* 3:153-162. 10.1038/nprot.2007.494

Consortium, T.G.O. 2019. The Gene Ontology Resource: 20 years and still GOing strong. *NUCLEIC ACIDS RESEARCH* 47:D330-D338. 10.1093/nar/gky1055

Davidson, A.L., and Maloney, P.C. 2007. ABC transporters: how small machines do a big job. *TRENDS IN MICROBIOLOGY* 15:448-455. 10.1016/j.tim.2007.09.005

Dudler, R., and Hertig, C. 1992. Structure of an mdr-like gene from Arabidopsis thaliana. Evolutionary implications. *JOURNAL OF BIOLOGICAL CHEMISTRY* 267:5882-5888.

Ehlert, A., Weltmeier, F., Wang, X., Mayer, C.S., Smeekens, S., Vicente-Carbajosa, J., and Dröge-Laser, W. 2006. Two-hybrid protein-protein interaction analysis in Arabidopsis protoplasts: establishment of a heterodimerization map of group C and group S bZIP transcription factors. *The Plant Journal* 46:890-900. 10.1111/j.1365-313X.2006.02731.x

Etc., T.H.T.D. 2018. Functions of ABC transporters in plant growth and development Thanh Ha Thi Do1, Enrico Martinoia2 and Youngsook Lee1. *PLANT BIOLOGY*.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., and Basra, S.M.A. 2009. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development* 29:185-212. 10.1051/agro:2008021

Fourcroy, P., Siso-Terraza, P., Sudre, D., Saviron, M., Reyt, G., Gaymard, F., Abadia, A., Abadia, J., Alvarez-Fernandez, A., and Briat, J.F. 2014. Involvement of the ABCG37 transporter in secretion of scopoletin and derivatives by Arabidopsis roots in response to iron deficiency. *NEW PHYTOLOGIST* 201:155-167. 10.1111/nph.12471

Goodman, C.D., Casati, P., and Walbot, V. 2004. A multidrug resistance-associated protein involved in anthocyanin transport in Zea mays. *PLANT CELL* 16:1812-1826. 10.1105/tpc.022574

H Shepherd, R.B. 1986. Moisture-dependent Physical Properties of Pigeon Pea. *Journal of Agricultural Engineering Research*.

Hashimoto, T., and Yamada, Y. 2003. New genes in alkaloid metabolism and transport. *Curr Opin Biotechnol* 14:163-168.

Hyde, S.C., Emsley, P., Hartshorn, M.J., Mimmack, M.M., Gileadi, U., Pearce, S.R., Gallagher, M.P., Gill, D.R., Hubbard, R.E., and Higgins, C.F. 1990. Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *NATURE* 346:362-365. 10.1038/346362a0

Imai, Y., Tsukahara, S., Asada, S., and Sugimoto, Y. 2004. Phytoestrogens/flavonoids reverse breast cancer resistance protein/ABCG2-mediated multidrug resistance. *CANCER RESEARCH* 64:4346-4352. 10.1158/0008-5472.CAN-04-0078

J. E. Walker, M.S.M.J. 1982. Distantly related sequences in the alpha-and beta-subunits of ATP synthase, myosin, kinases and ther ATP-requiring enzymes Distantly related sequences in the alpha - and beta - subunits of ATP synthase, myosin, kinases and other ATP - requiring enzymes and a common nucleotide binding fold. *EMBO J (1982)1:945-951*.

Jasinski, M., Banasiak, J., Radom, M., Kalitkiewicz, A., and Figlerowicz, M. 2009. Full-size ABC transporters from the ABCG subfamily in medicago truncatula. *Mol Plant Microbe Interact* 22:921-931. 10.1094/MPMI-22-8-0921

Jasinski, M., Ducos, E., Martinoia, E., and Boutry, M. 2003. The ATP-binding cassette transporters: structure, function, and gene family comparison between rice and Arabidopsis. *PLANT PHYSIOLOGY* 131:1169-1177. 10.1104/pp.102.014720

Kang, J., Hwang, J., Lee, M., Kim, Y., Assmann, S.M., Martinoia, E., and Lee, Y. 2010. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proceedings of the National Academy of Sciences* 107:2355-2360. 10.1073/pnas.0909222107

Kang, J., Park, J., Choi, H., Burla, B., Kretzschmar, T., Lee, Y., and Martinoia, E. 2011. Plant ABC Transporters. *Arabidopsis Book* 9:e153. 10.1199/tab.0153

Kim, D.Y., Bovet, L., Maeshima, M., Martinoia, E., and Lee, Y. 2007. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. *PLANT JOURNAL* 50:207-218. 10.1111/j.1365-313X.2007.03044.x

Kim, D.Y., Jin, J.Y., Alejandro, S., Martinoia, E., and Lee, Y. 2010. Overexpression of AtABCG36 improves drought and salt stress resistance in Arabidopsis. *Physiol Plant* 139:170-180. 10.1111/j.1399-3054.2010.01353.x

Kretzschmar, T., Burla, B., Lee, Y., Martinoia, E., and Nagy, R. 2011. Functions of ABC transporters in plants. *Essays in Biochemistry* 50:145-160. 10.1042/bse0500145

KUBIS, S., SCHMIDT, T., and HESLOP-HARRISON, J.S.P. 1998. Repetitive DNA Elements as

a Major Component of Plant Genomes. *ANNALS OF BOTANY* 82:45-55. https://doi.org/10.1006/anbo.1998.0779

Kuromori, T., Miyaji, T., Yabuuchi, H., Shimizu, H., Sugimoto, E., Kamiya, A., Moriyama, Y., and Shinozaki, K. 2010. ABC transporter AtABCG25 is involved in abscisic acid transport and responses. *Proceedings of the National Academy of Sciences* 107:2361-2366. 10.1073/pnas.0912516107

Kuromori, T., Sugimoto, E., and Shinozaki, K. 2011. Arabidopsis mutants of AtABCG22, an ABC transporter gene, increase water transpiration and drought susceptibility. *PLANT JOURNAL* 67:885-894. 10.1111/j.1365-313X.2011.04641.x

Le Hir, R., Sorin, C., Chakraborti, D., Moritz, T., Schaller, H., Tellier, F., Robert, S., Morin, H., Bako, L., and Bellini, C. 2013. ABCG9, ABCG11 and ABCG14 ABC transporters are required for vascular development in Arabidopsis. *The Plant Journal* 76:811-824. 10.1111/tpj.12334

Lee, I., Ambaru, B., Thakkar, P., Marcotte, E.M., and Rhee, S.Y. 2010. Rational association of genes with traits using a genome-scale gene network for Arabidopsis thaliana. *NATURE BIOTECHNOLOGY* 28:149-156. 10.1038/nbt.1603

Lee, M., Lee, K., Lee, J., Noh, E.W., and Lee, Y. 2005. AtPDR12 Contributes to Lead Resistance in Arabidopsis. *PLANT PHYSIOLOGY* 138:827-836. 10.1104/pp.104.058107

Letunic, I., and Bork, P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *NUCLEIC ACIDS RESEARCH* 47:W256-W259. 10.1093/nar/gkz239

Locher. 2004. Structure and mechanism of ABC transporters Kaspar P Locher. *CURRENT OPINION IN STRUCTURAL BIOLOGY*.

Luo, B., Xue, X., Hu, W., Wang, L., and Chen, X. 2007. An ABC Transporter Gene of Arabidopsis thaliana, AtWBC11, is Involved in Cuticle Development and Prevention of Organ Fusion. *PLANT AND CELL PHYSIOLOGY* 48:1790-1802. 10.1093/pcp/pcm152

Martinoia, E., Klein, M., Geisler, M., Bovet, L., Forestier, C., Kolukisaoglu, U., Muller-Rober, B., and Schulz, B. 2002. Multifunctionality of plant ABC transporters--more than just detoxifiers. *PLANTA* 214:345-355.

Mendez, C., and Salas, J.A. 2001. The role of ABC transporters in antibiotic-producing organisms: drug secretion and resistance mechanisms. *RESEARCH IN MICROBIOLOGY* 152:341-350.

Mishra, A.K., Choi, J., Rabbee, M.F., and Baek, K.H. 2019. In Silico Genome-Wide Analysis of the ATP-Binding Cassette Transporter Gene Family in Soybean (Glycine max L.) and Their Expression Profiling. *Biomed Research International* 2019:8150523. 10.1155/2019/8150523

Mittler, R., Finka, A., and Goloubinoff, P. 2012. How do plants feel the heat? *TRENDS IN BIOCHEMICAL SCIENCES* 37:118-125. 10.1016/j.tibs.2011.11.007

Moons, A. 2008. Transcriptional profiling of the PDR gene family in rice roots in response to plant growth regulators, redox perturbations and weak organic acid stresses. *PLANTA* 229:53-71. 10.1007/s00425-008-0810-5

Morris, M.E., and Zhang, S. 2006. Flavonoid-drug interactions: effects of flavonoids on ABC transporters. *LIFE SCIENCES* 78:2116-2130. 10.1016/j.lfs.2005.12.003

Mosser, J., Douar, A.M., Sarde, C.O., Kioschis, P., Feil, R., Moser, H., Poustka, A.M., Mandel, J.L., and Aubourg, P. 1993. Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *NATURE* 361:726-730. 10.1038/361726a0

Mourez, M., Hofnung, M., and Dassa, E. 1997. Subunit interactions in ABC transporters: a conserved sequence in hydrophobic membrane proteins of periplasmic permeases defines an important site of interaction with the ATPase subunits. *EMBO JOURNAL* 16:3066-3077. 10.1093/emboj/16.11.3066

Mukherjee, D., Saha, D., Acharya, D., Mukherjee, A., Chakraborty, S., and Ghosh, T.C. 2018. The role of introns in the conservation of the metabolic genes of Arabidopsis thaliana. *GENOMICS* 110:310-317. 10.1016/j.ygeno.2017.12.003

Ogoda, O.J., Akubue, P.I., and Okide, G.B. 2002. The kinetics of reversal of pre-sickled erythrocytes by the aqueous extract of Cajanus cajan seeds. *PHYTOTHERAPY RESEARCH* 16:748-750. 10.1002/ptr.1026

Pandey, P.C., and Pandey, V. 1991. Urease purification from the seeds of Cajanus cajan and its application in a biosensor construction. *Appl Biochem Biotechnol* 31:247-251.

Panikashvili, D., Savaldi-Goldstein, S., Mandel, T., Yifhar, T., Franke, R.B., Höfer, R., Schreiber, L., Chory, J., and Aharoni, A. 2007a. The Arabidopsis DESPERADO/AtWBC11 Transporter Is Required for Cutin and Wax Secretion. *PLANT PHYSIOLOGY* 145:1345-1360. 10.1104/pp.107.105676

Potter, S.C., Luciani, A., Eddy, S.R., Park, Y., Lopez, R., and Finn, R.D. 2018. HMMER web server: 2018 update. *NUCLEIC ACIDS RESEARCH* 46:W200-W204. 10.1093/nar/gky448

Rombauts, S., Dehais, P., Van Montagu, M., and Rouze, P. 1999. PlantCARE, a plant cis-acting regulatory element database. *NUCLEIC ACIDS RESEARCH* 27:295-296. 10.1093/nar/27.1.295

Rose, A.B., Carter, A., Korf, I., and Kojima, N. 2016. Intron sequences that stimulate gene expression in Arabidopsis. *PLANT MOLECULAR BIOLOGY* 92:337-346. 10.1007/s11103-016-0516-1

Sanchez-Fernandez, R., Davies, T.G., Coleman, J.O., and Rea, P.A. 2001. The Arabidopsis thaliana ABC protein superfamily, a complete inventory. *JOURNAL OF BIOLOGICAL CHEMISTRY* 276:30231-30244. 10.1074/jbc.M103104200

Schneider, E., and Hunke, S. 1998. ATP-binding-cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS MICROBIOLOGY REVIEWS* 22:1-20. 10.1111/j.1574-6976.1998.tb00358.x

Schulz, B., and Kolukisaoglu, H.U. 2006. Genomics of plant ABC transporters: the alphabet of photosynthetic life forms or just holes in membranes? *FEBS LETTERS* 580:1010-1016. 10.1016/j.febslet.2006.01.002

Singh, A.K., Rai, V.P., Chand, R., Singh, R.P., and Singh, M.N. 2013. Genetic diversity studies and identification of SSR markers associated with Fusarium wilt (Fusariumudum) resistance in cultivated pigeonpea (Cajanus cajan). *JOURNAL OF GENETICS* 92:273-280.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *MOLECULAR BIOLOGY AND EVOLUTION* 30:2725-2729. 10.1093/molbev/mst197

Toledo, M.A., Schneider, D.R., Azzoni, A.R., Favaro, M.T., Pelloso, A.C., Santos, C.A., Saraiva, A.M., and Souza, A.P. 2011. Characterization of an oxidative stress response regulator, homologous to Escherichia coli OxyR, from the phytopathogen Xylella fastidiosa. *Protein Expr Purif* 75:204-210. 10.1016/j.pep.2010.10.004

van den Brule, S., Muller, A., Fleming, A.J., and Smart, C.C. 2002. The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. *PLANT JOURNAL* 30:649-662.

van den Brule, S., and Smart, C.C. 2002. The plant PDR family of ABC transporters. *PLANTA* 216:95-106. 10.1007/s00425-002-0889-z

Varshney, R.K., Chen, W., Li, Y., Bharti, A.K., Saxena, R.K., Schlueter, J.A., Donoghue, M.T., Azam, S., Fan, G., Whaley, A.M., Farmer, A.D., Sheridan, J., Iwata, A., Tuteja, R., Penmetsa, R.V., Wu, W., Upadhyaya, H.D., Yang, S.P., Shah, T., Saxena, K.B., Michael, T., McCombie, W.R., Yang, B., Zhang, G., Yang, H., Wang, J., Spillane, C., Cook, D.R., May, G.D., Xu, X., and Jackson, S.A. 2011. Draft genome sequence of pigeonpea (Cajanus cajan), an orphan legume crop of resource-poor farmers. *NATURE BIOTECHNOLOGY* 30:83-89. 10.1038/nbt.2022

Verrier, P.J., Bird, D., Burla, B., Dassa, E., Forestier, C., Geisler, M., Klein, M., Kolukisaoglu, U., Lee, Y., Martinoia, E., Murphy, A., Rea, P.A., Samuels, L., Schulz, B., Spalding, E.J., Yazaki, K., and Theodoulou, F.L. 2008. Plant ABC proteins--a unified nomenclature and updated inventory. *TRENDS IN PLANT SCIENCE* 13:151-159. 10.1016/j.tplants.2008.02.001

654 Voorrips, R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs.
655 *JOURNAL OF HEREDITY* 93:77-78. 10.1093/jhered/93.1.77

656 Wu, G., Lewis, D.R., and Spalding, E.P. 2007. Mutations inArabidopsis Multidrug Resistance-
657 Like ABC Transporters Separate the Roles of Acropetal and Basipetal Auxin Transport in Lateral
658 Root Development. *The Plant Cell* 19:1826-1837. 10.1105/tpc.106.048777

659 Wu, N., Kong, Y., Fu, Y., Zu, Y., Yang, Z., Yang, M., Peng, X., and Efferth, T. 2011. In vitro
660 antioxidant properties, DNA damage protective activity, and xanthine oxidase inhibitory effect of
661 cajaninstilbene acid, a stilbene compound derived from pigeon pea [Cajanus cajan (L.) Millsp.]
662 leaves. *J Agric Food Chem* 59:437-443. 10.1021/jf103970b

663 Yadu, B., Chandrakar, V., Korram, J., Satnami, M.L., Kumar, M., and S, K. 2018. Silver
664 nanoparticle modulates gene expressions, glyoxalase system and oxidative stress markers in
665 fluoride stressed Cajanus cajan L. *JOURNAL OF HAZARDOUS MATERIALS* 353:44-52.
666 10.1016/j.jhazmat.2018.03.061

667 Yang, X., Coulombe-Huntington, J., Kang, S., Sheynkman, G.M., Hao, T., Richardson, A., Sun,
668 S., Yang, F., Shen, Y.A., Murray, R.R., Spirohn, K., Begg, B.E., Duran-Frigola, M., MacWilliams,
669 A., Pevzner, S.J., Zhong, Q., Trigg, S.A., Tam, S., Ghamsari, L., Sahni, N., Yi, S., Rodriguez, M.D.,
670 Balcha, D., Tan, G., Costanzo, M., Andrews, B., Boone, C., Zhou, X.J., Salehi-Ashtiani, K.,
671 Charloteaux, B., Chen, A.A., Calderwood, M.A., Aloy, P., Roth, F.P., Hill, D.E., Iakoucheva, L.M.,
672 Xia, Y., and Vidal, M. 2016. Widespread Expansion of Protein Interaction Capabilities by
673 Alternative Splicing. *CELL* 164:805-817. 10.1016/j.cell.2016.01.029

674 Yazaki, K. 2006. ABC transporters involved in the transport of plant secondary metabolites. *FEBS*
675 *LETTERS* 580:1183-1191. 10.1016/j.febslet.2005.12.009

676

Figure 1

Phylogenetic analysis of the ABCG transporters among pigeon pea.

Using the MEGA6.0 program, the NJ (Neighbor-Joining, NJ) tree was constructed using the amino acid sequence of the pigeon pea ABCG transporters. The numbers beside the branches represent bootstrap values based on 1000 replications. The outer side of the phylogenetic tree is a branch labeled with two subgroups of the pigeon pea ABCG transporters, and shown in different colors. WBC: white-brown complex; PDR: pleiotropic drug resistance.

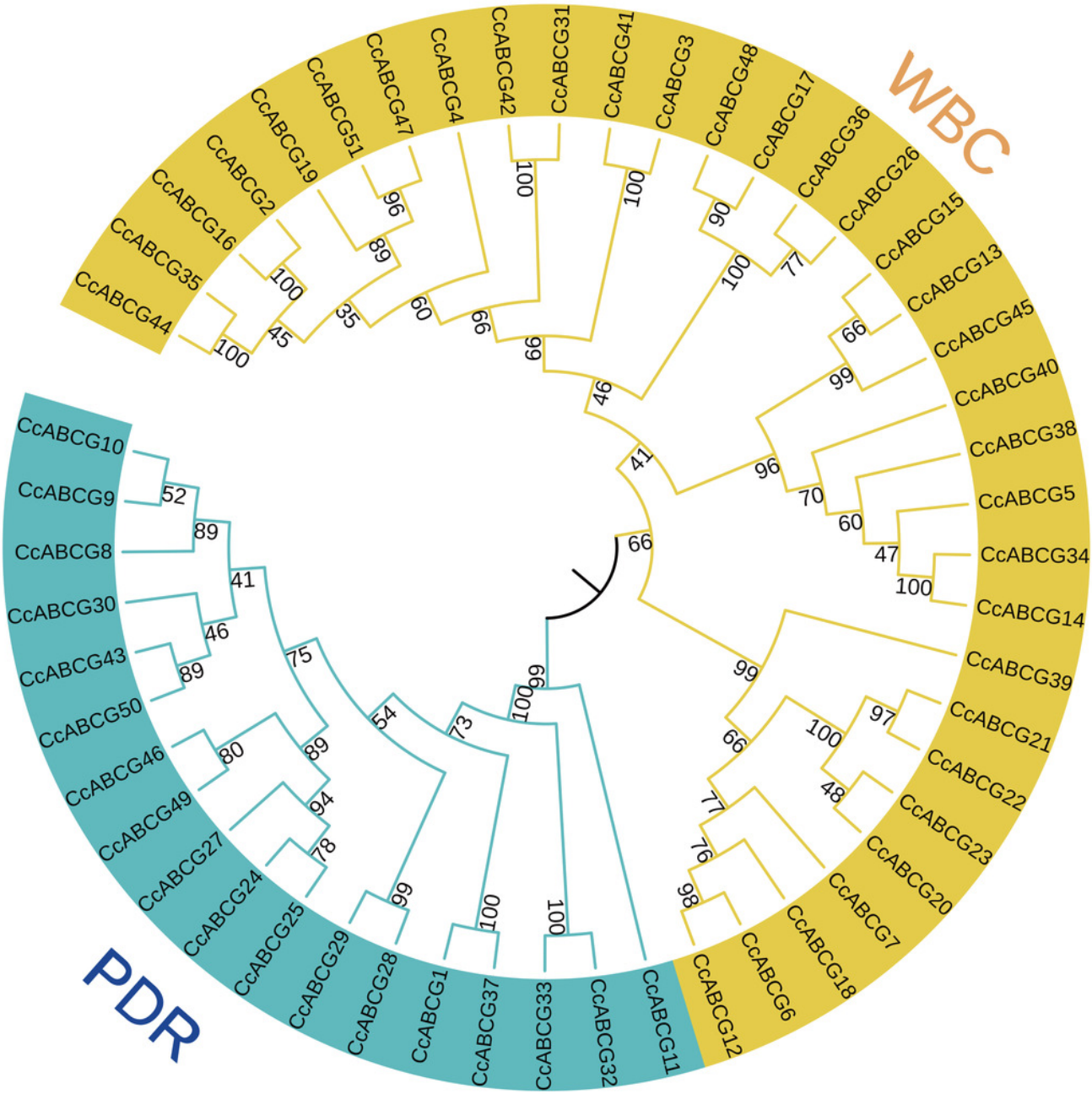

WBC
PDR
CcABCG10
CcABCG9
CcABCG8
CcABCG30
CcABCG43
CcABCG50
CcABCG46
CcABCG49
CcABCG27
CcABCG24
CcABCG25
CcABCG29
CcABCG28
CcABCG1
CcABCG37
CcABCG33
CcABCG32
CcABCG11
CcABCG12
CcABCG6
CcABCG18
CcABCG7
CcABCG20
CcABCG23
CcABCG22
CcABCG21
CcABCG39
CcABCG14
CcABCG34
CcABCG5
CcABCG38
CcABCG40
CcABCG45
CcABCG13
CcABCG15
CcABCG26
CcABCG36
CcABCG17
CcABCG48
CcABCG3
CcABCG41
CcABCG31
CcABCG42
CcABCG4
CcABCG47
CcABCG51
CcABCG19
CcABCG2
CcABCG16
CcABCG35
CcABCG44

Figure 2

The chromosomal location of the pigeon pea *ABCG* genes.

The pigeon pea *ABCG* genes were named *CcABCG1-CcABCG51* based on chromosomal location information. All of *ABCG* genes were location in all pigeon pea chromosomal except of chromosomal 7. ChrUn contained 17 members, which were mapped based on the length of those unplaced scaffold sequences with 17 genes, respectively.

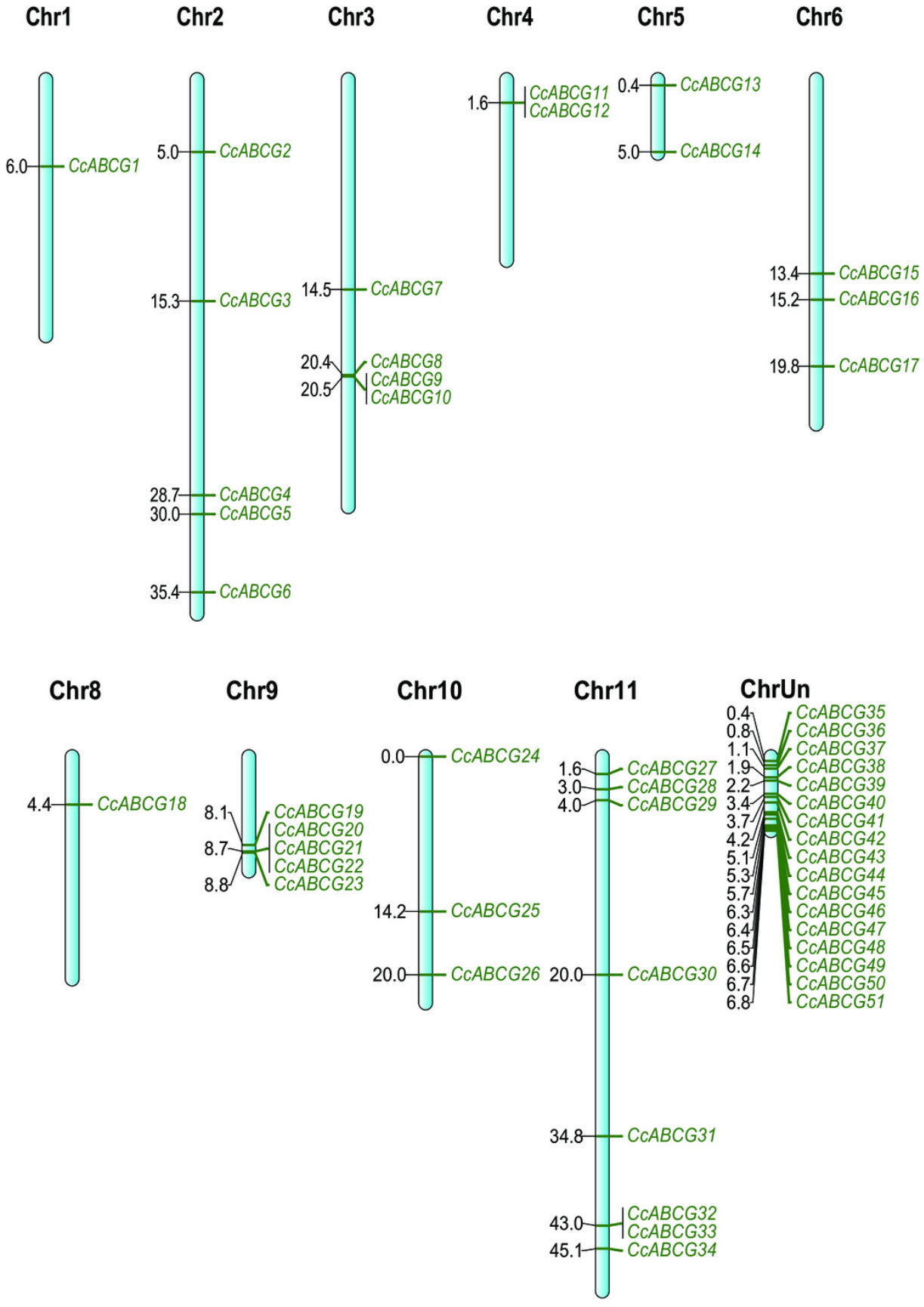
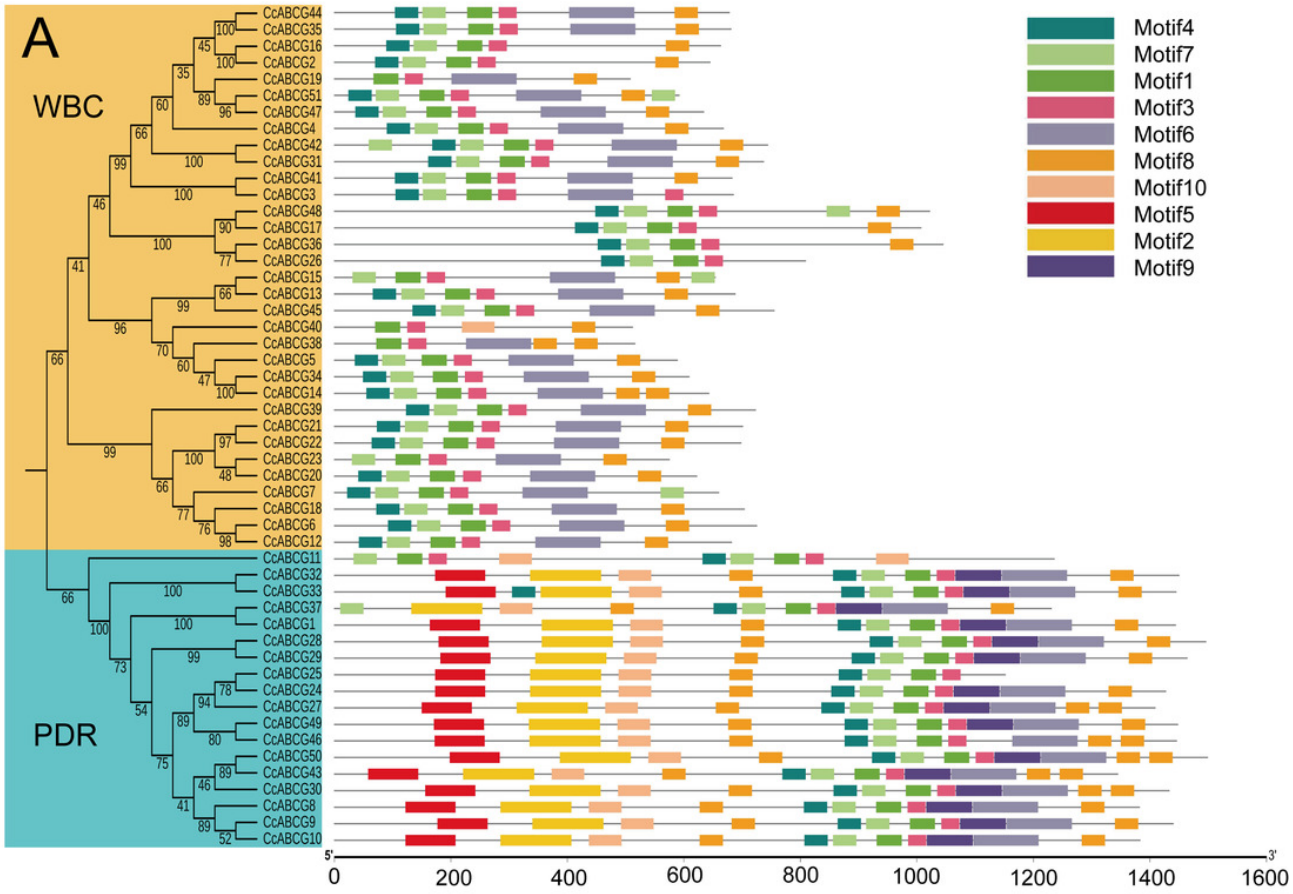

Chr1
6.0 CcABCG1
Chr2
5.0 CcABCG2
15.3 CcABCG3
28.7 CcABCG4
30.0 CcABCG5
35.4 CcABCG6
Chr3
14.5 CcABCG7
20.4 CcABCG8
20.5 CcABCG9
CcABCG10
Chr4
1.6 CcABCG11
CcABCG12
Chr5
0.4 CcABCG13
5.0 CcABCG14
Chr6
13.4 CcABCG15
15.2 CcABCG16
19.8 CcABCG17
Chr8
4.4 CcABCG18
Chr9
8.1 CcABCG19
CcABCG20
8.7 CcABCG21
CcABCG22
8.8 CcABCG23
Chr10
0.0 CcABCG24
14.2 CcABCG25
20.0 CcABCG26
Chr11
1.6 CcABCG27
3.0 CcABCG28
4.0 CcABCG29
20.0 CcABCG30
34.8 CcABCG31
43.0 CcABCG32
CcABCG33
45.1 CcABCG34
ChrUn
0.4 CcABCG35
0.8 CcABCG36
1.1 CcABCG37
1.9 CcABCG38
2.2 CcABCG39
3.4 CcABCG40
3.7 CcABCG41
4.2 CcABCG42
5.1 CcABCG43
5.3 CcABCG44
5.7 CcABCG45
6.3 CcABCG46
6.4 CcABCG47
6.5 CcABCG48
6.6 CcABCG49
6.7 CcABCG50
6.8 CcABCG51

Figure 3

Motifs and conserved domain of CcABCGs.

(A) The 10 conserved motif of CcABCGs on the MEME software. Motif annotation is shown as color legends. Annotates of motif were listed on the Supplemental Table S3. (B) Conserved domain of CcABCGs. Blue indicates the NBD (nucleotide binding domain), and orange indicates the another conserved domain TMD (trans-membrane domains).


A
WBC
PDR
CcABCG44
CcABCG35
CcABCG16
CcABCG2
CcABCG19
CcABCG51
CcABCG47
CcABCG4
CcABCG42
CcABCG31
CcABCG41
CcABCG3
CcABCG48
CcABCG17
CcABCG36
CcABCG26
CcABCG15
CcABCG13
CcABCG45
CcABCG40
CcABCG38
CcABCG5
CcABCG34
CcABCG14
CcABCG39
CcABCG21
CcABCG22
CcABCG23
CcABCG20
CcABCG7
CcABCG18
CcABCG6
CcABCG12
CcABCG11
CcABCG32
CcABCG33
CcABCG37
CcABCG1
CcABCG28
CcABCG29
CcABCG25
CcABCG24
CcABCG27
CcABCG49
CcABCG46
CcABCG50
CcABCG43
CcABCG30
CcABCG8
CcABCG9
CcABCG10
Motif4
Motif7
Motif1
Motif3
Motif6
Motif8
Motif10
Motif5
Motif2
Motif9
5'
3'
0
200
400
600
800
1000
1200
1400
1600

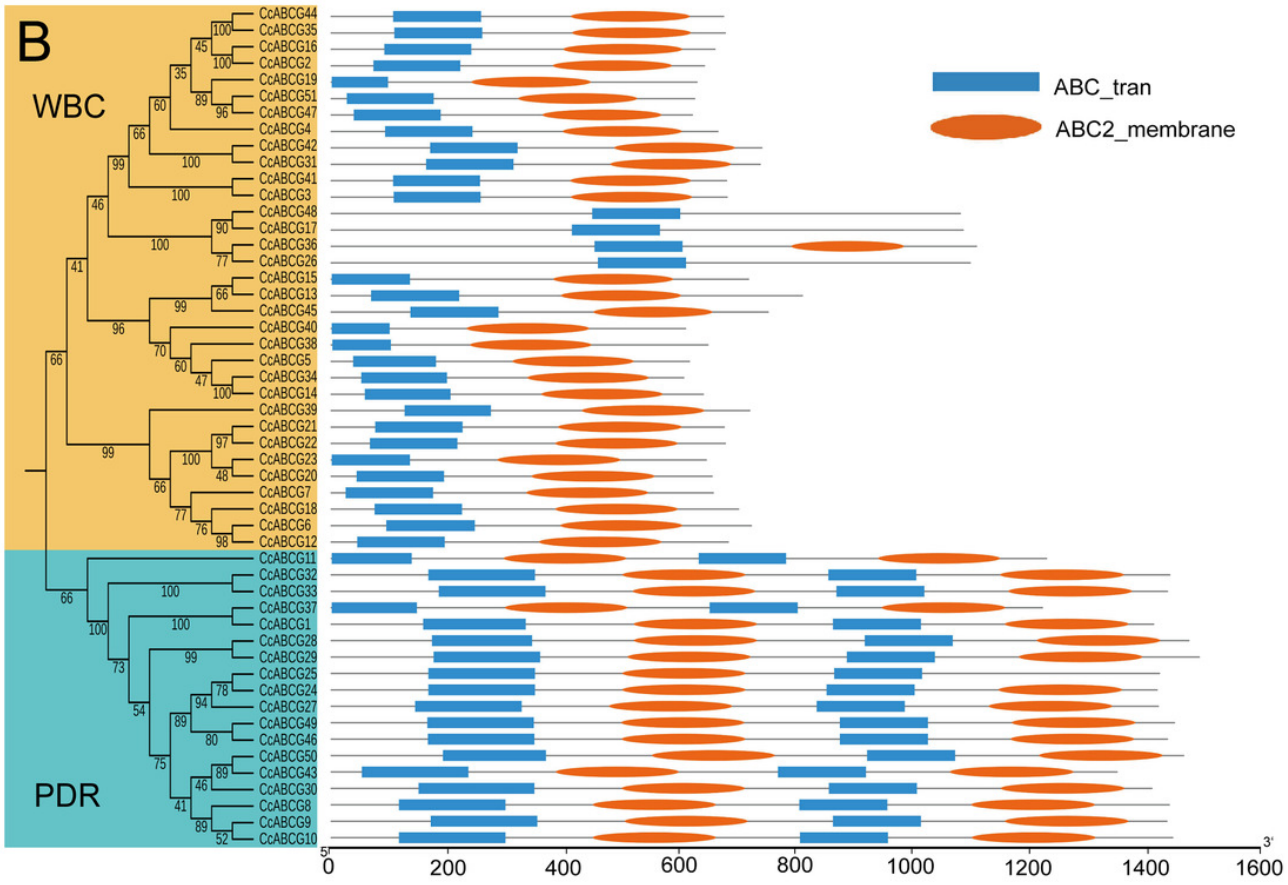

B
WBC
PDR
CcABCG44
CcABCG35
CcABCG16
CcABCG2
CcABCG19
CcABCG51
CcABCG47
CcABCG4
CcABCG42
CcABCG31
CcABCG41
CcABCG3
CcABCG48
CcABCG17
CcABCG36
CcABCG26
CcABCG15
CcABCG13
CcABCG45
CcABCG40
CcABCG38
CcABCG5
CcABCG34
CcABCG14
CcABCG39
CcABCG21
CcABCG22
CcABCG23
CcABCG20
CcABCG7
CcABCG18
CcABCG6
CcABCG12
CcABCG11
CcABCG32
CcABCG33
CcABCG37
CcABCG1
CcABCG28
CcABCG29
CcABCG25
CcABCG24
CcABCG27
CcABCG49
CcABCG46
CcABCG50
CcABCG43
CcABCG30
CcABCG8
CcABCG9
CcABCG10
ABC_tran
ABC2_membrane
5'
3'
0
200
400
600
800
1000
1200
1400
1600

Figure 4

Gene structure and cis-elements analysis of *ABCG* genes.

(A) Intron-exon structures of *CcABCG* genes. Yellow rectangles: coding sequences (CDSs); thin lines: introns; blue rectangles: untranslated regions (UTRs). (B) Putative regulatory cis-elements in the *ABCG* gene promoters. The relative positions of elements are labeled with capital letters in the figure.

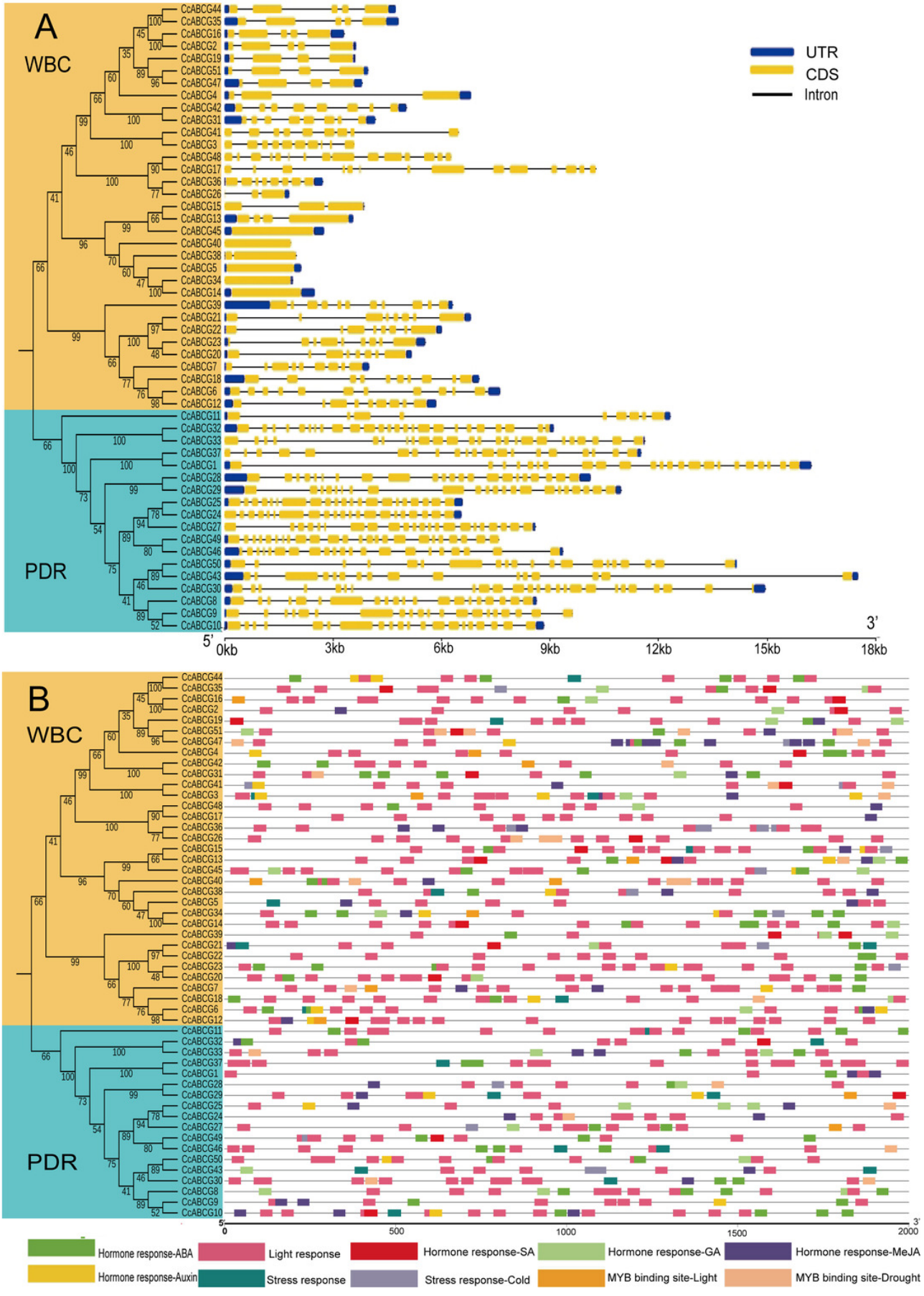

A
WBC
PDR
UTR
CDS
Intron
5'
3'
0kb
3kb
6kb
9kb
12kb
15kb
18kb
B
WBC
PDR
5'
3'
0
500
1000
1500
2000
Hormone response-ABA
Light response
Hormone response-SA
Hormone response-GA
Hormone response-MeJA
Hormone response-Auxin
Stress response
Stress response-Cold
MYB binding site-Light
MYB binding site-Drought

Figure 5

Go annotation of ABCG transporters in pigeon pea

GO annotations of ABCG transporters in pigeon pea were predicted using Swiss-prot database serve. Red means molecular function, yellow means cell component and blue means biological process. Displaying only results for FDR $P < 0.05$

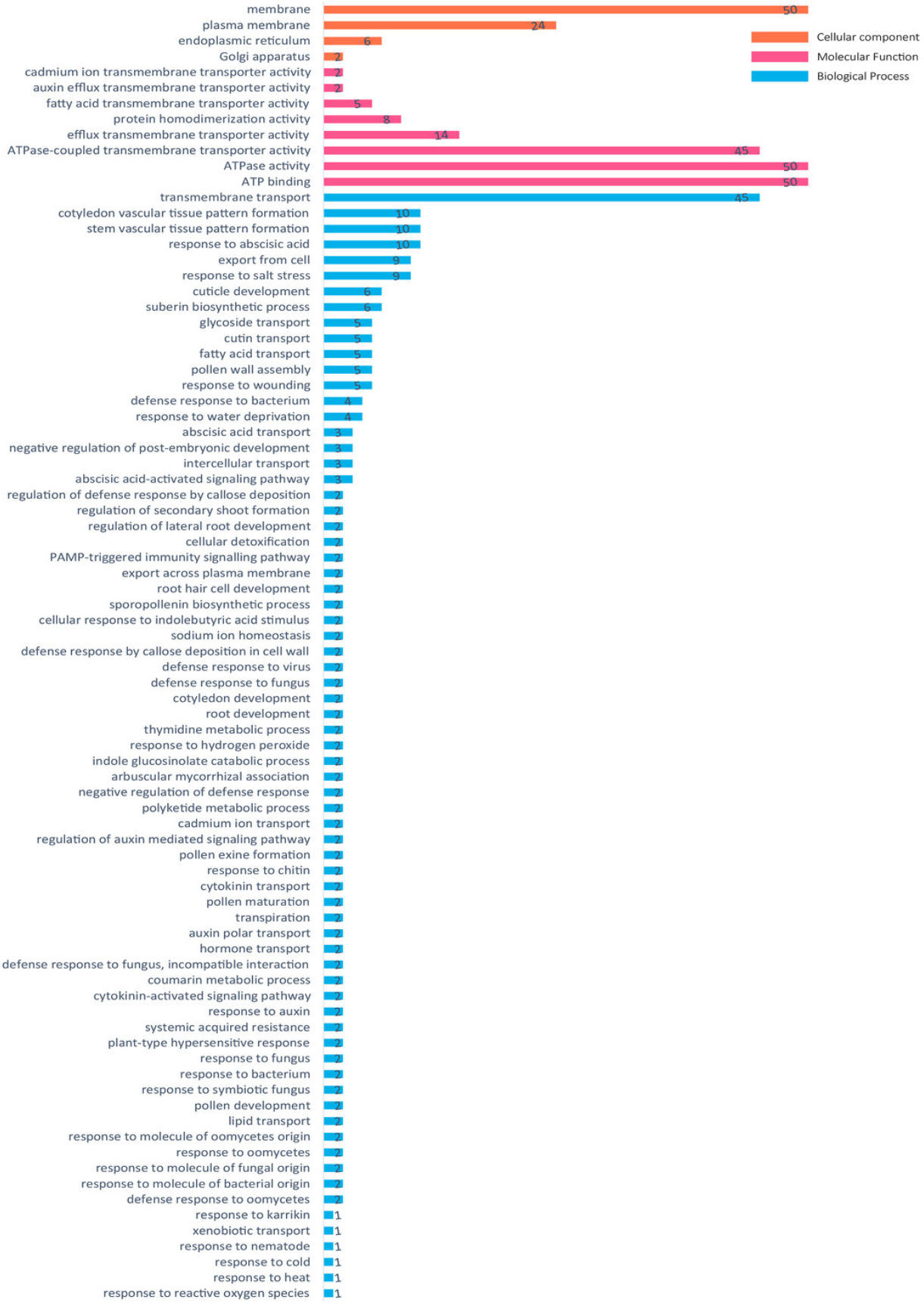

membrane 50
plasma membrane 24
endoplasmic reticulum 6
Golgi apparatus 2
cadmium ion transmembrane transporter activity 2
auxin efflux transmembrane transporter activity 2
fatty acid transmembrane transporter activity 5
protein homodimerization activity 8
efflux transmembrane transporter activity 14
ATPase-coupled transmembrane transporter activity 45
ATPase activity 50
ATP binding 50
transmembrane transport 45
cotyledon vascular tissue pattern formation 10
stem vascular tissue pattern formation 10
response to abscisic acid 10
export from cell 9
response to salt stress 9
cuticle development 6
suberin biosynthetic process 6
glycoside transport 5
cutin transport 5
fatty acid transport 5
pollen wall assembly 5
response to wounding 5
defense response to bacterium 4
response to water deprivation 4
abscisic acid transport 3
negative regulation of post-embryonic development 3
intercellular transport 3
abscisic acid-activated signaling pathway 3
regulation of defense response by callose deposition 2
regulation of secondary shoot formation 2
regulation of lateral root development 2
cellular detoxification 2
PAMP-triggered immunity signalling pathway 2
export across plasma membrane 2
root hair cell development 2
sporopollenin biosynthetic process 2
cellular response to indolebutyric acid stimulus 2
sodium ion homeostasis 2
defense response by callose deposition in cell wall 2
defense response to virus 2
defense response to fungus 2
cotyledon development 2
root development 2
thymidine metabolic process 2
response to hydrogen peroxide 2
indole glucosinolate catabolic process 2
arbuscular mycorrhizal association 2
negative regulation of defense response 2
polyketide metabolic process 2
cadmium ion transport 2
regulation of auxin mediated signaling pathway 2
pollen exine formation 2
response to chitin 2
cytokinin transport 2
pollen maturation 2
transpiration 2
auxin polar transport 2
hormone transport 2
defense response to fungus, incompatible interaction 2
coumarin metabolic process 2
cytokinin-activated signaling pathway 2
response to auxin 2
systemic acquired resistance 2
plant-type hypersensitive response 2
response to fungus 2
response to bacterium 2
response to symbiotic fungus 2
pollen development 2
lipid transport 2
response to molecule of oomycetes origin 2
response to oomycetes 2
response to molecule of fungal origin 2
response to molecule of bacterial origin 2
defense response to oomycetes 2
response to karrikin 1
xenobiotic transport 1
response to nematode 1
response to cold 1
response to heat 1
response to reactive oxygen species 1
Cellular component
Molecular Function
Biological Process

Figure 6

Expression analysis of pigeon pea *ABCG* gene in different organs.

The expression levels of 10 genes in different tissues are shown in the heat map. Three biological replicates per sample. Values represent means ± SEM.

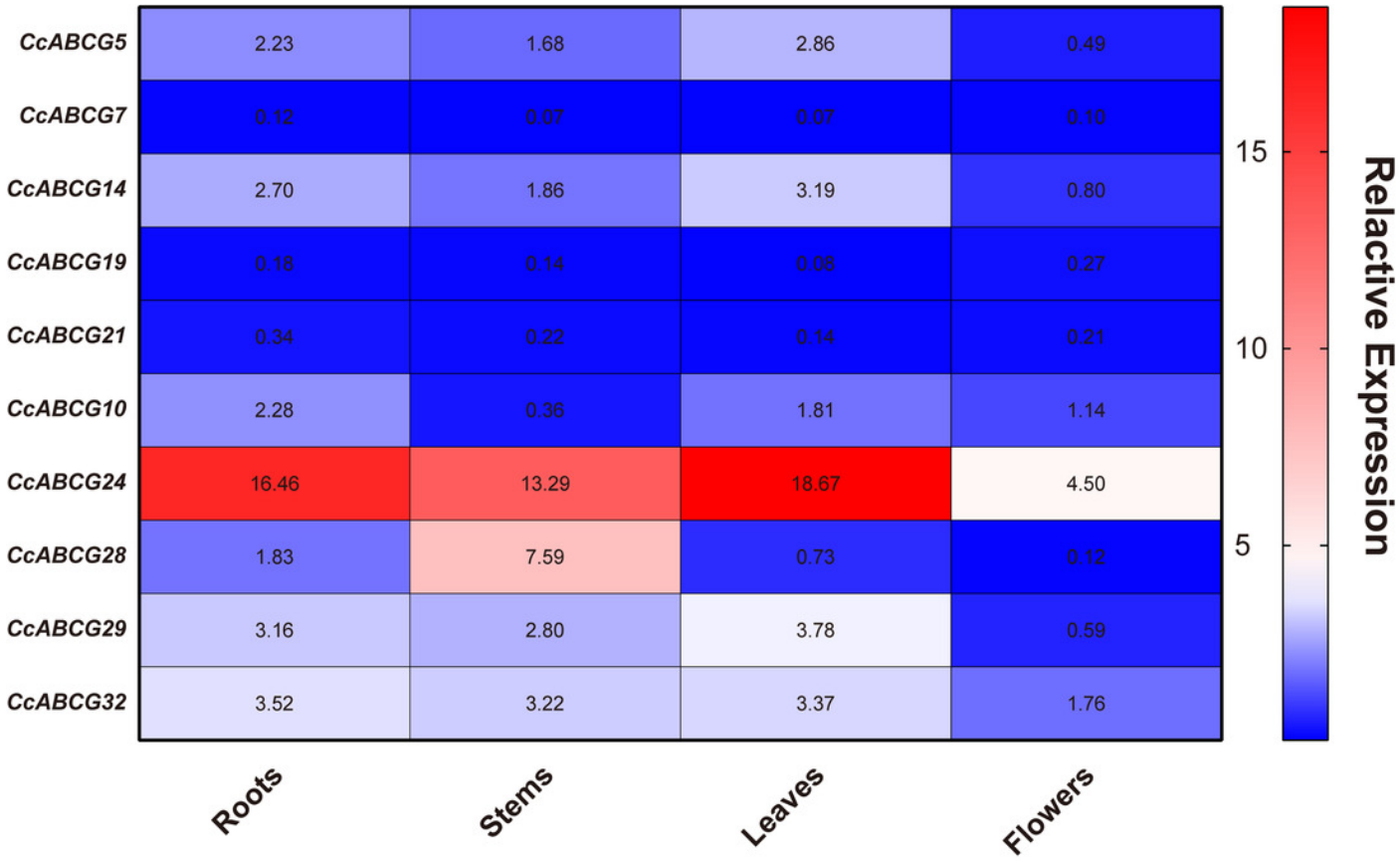


Figure 7

Relative expression analysis of the pigeon pea *ABCG* genes under different abiotic stresses.

Relative expression level of the *ABCG* genes in pigeon pea under different abiotic stresses of 4°C (A, B), 42°C (C, D), 250 nmol/L mannitol (E, F), 100 μmo/L $AlCl_3$ (G, H), 200 nmol/L NaCl (I, J). Expression analysis was performed using a relative quantitative method $^{2\text{-}\triangle\triangle Cq}$, and *CcActin* as internal reference gene. Relative expression level of roots: A,C,E,G,I; relative expression level of leaves: B,D,F,H,J.Three biological replicates of each sample. Values represent means ± SEM. Asterisks indicate significant difference as determined by Dunnett's multiple comparisons test (**, P<0.01). n.s., no significant difference.

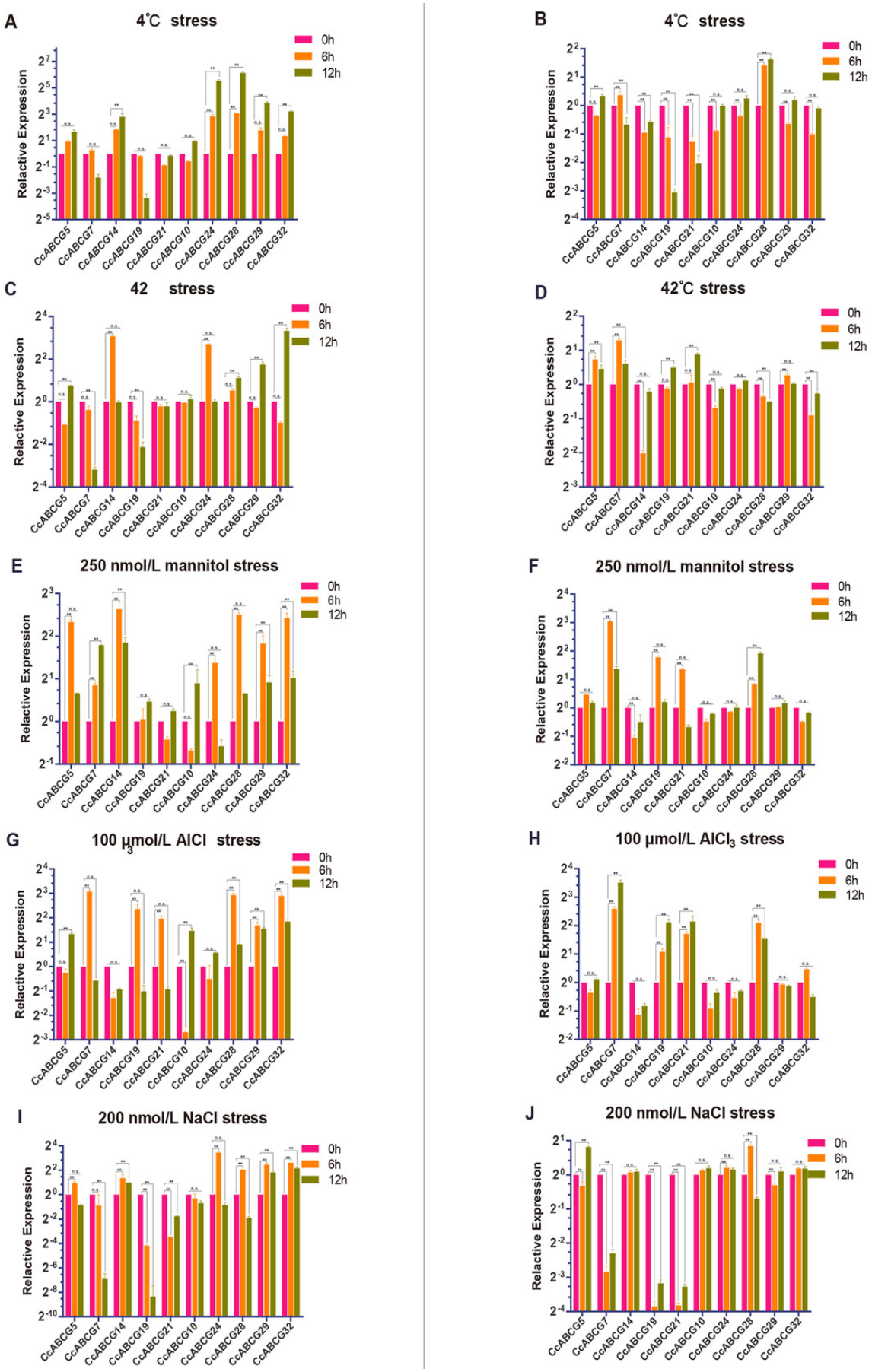

A
4℃ stress
B
4℃ stress
C
42 stress
D
42℃ stress
E
250 nmol/L mannitol stress
F
250 nmol/L mannitol stress
G
100 μmol/L AlCl3 stress
H
100 μmol/L AlCl3 stress
I
200 nmol/L NaCl stress
J
200 nmol/L NaCl stress
Relative Expression
0h
6h
12h
CcABCG5
CcABCG7
CcABCG14
CcABCG19
CcABCG21
CcABCG10
CcABCG24
CcABCG28
CcABCG29
CcABCG32

Table 1(on next page)

Prediction of physicochemical properties and subcellular localization of ABCG transporters.

Table 1.
Prediction of physicochemical properties and subcellular localization of ABCG transporters.

Gene	Accession	Location	Position	Length (aa)	Mw	pI	Chr	Exon	Sub-loc
CcABCG1	KYP76113	LOC109804364	6006382-6022606	1421	159155.33	7.76	1	23	Cell membrane
CcABCG2	KYP72481	LOC109789679	5026814-5030449	645	72010.85	9.08	2	5	Cell membrane
CcABCG3	KYP73490	LOC109810734	15320209-15323794	684	77318.74	8.11	2	10	Cell membrane
CcABCG4	KYP74721	LOC109795335	28723419-28730237	668	73512.6	9.47	2	3	Cell membrane
CcABCG5	KYP74838	LOC109806747	29968224-29970344	619	69012.83	9.4	2	1	Cell membrane
CcABCG6	KYP75362	LOC109815813	35437420-35445042	726	79570.36	8.27	2	12	Cell membrane
CcABCG7	KYP70503	LOC109797228	14492247-14496242	660	73883.51	8.81	3	9	Cell membrane
CcABCG8	KYP71088	LOC109796083	20447829-20456456	1448	164560.21	8.28	3	20	Cell membrane
CcABCG9	KYP71089	LOC109796084	20469958-20479590	1444	163478.51	8.27	3	20	Cell membrane
CcABCG10	KYP71090	LOC109796805	20488744-20497576	1454	164868.63	7.95	3	20	Cell membrane
CcABCG11	KYP68041	LOC109798752	1617897-1630220	718	80600.64	9.1	4	9	Cell membrane
CcABCG12	KYP68044	LOC109798893	1641751-1647610	686	76872.23	8.88	4	10	Cell membrane
CcABCG13	KYP67437	LOC109799601	355237-358794	814	91841.52	8.95	5	6	Cell membrane
CcABCG14	KYP67863	LOC109799748	5000555-5003051	643	72113.02	7.88	5	1	Cell membrane
CcABCG15	KYP66379	LOC109800436	13422611-	721	81114.25	8.97	6	3	Cell membrane

			13426469						
CcABCG16	KYP66524	LOC109801031	15158990-15162297	663	74678.78	8.6	6	5	Cell membrane
CcABCG17	KYP66999	LOC109800523	19843419-19853680	1092	121456.99	8.87	6	14	Cell membrane
CcABCG18	KYP61725	LOC109804023	4405565-4412603	704	78466.57	8.83	8	10	Cell membrane
CcABCG19	KYP61105	LOC109804765	8122601-8126214	632	70593.09	9.02	9	4	Cell membrane
CcABCG20	KYP61155	LOC109805263	8663643-8668819	658	73428.07	7.99	9	8	Cell membrane
CcABCG21	KYP61157	LOC109805167	8722014-8728825	679	75362.12	8.36	9	8	Cell membrane
CcABCG22	KYP61159	LOC109805166	8746304-8752313	681	75328.34	8.65	9	8	Cell membrane
CcABCG23	KYP61160	LOC109804802	8756197-8761747	648	71982.39	8.81	9	9	Cell membrane
CcABCG24	KYP58337	LOC109805773	11158-17707	1427	161183.4	7.36	10	21	Cell membrane
CcABCG25	KYP59575	LOC109805875	14201296-14246400	1431	161968.54	7.97	10	21	Cell membrane
CcABCG26	KYP60108	LOC109805696	19992101-20040472	1104	123273.83	8.89	10	16	Cell membrane. Chloroplast.
CcABCG27	KYP53996	LOC109808825	1626589-1635183	1429	162016.24	7.32	11	22	Cell membrane
CcABCG28	KYP54148	LOC109808608	3046631-3056753	1482	167729.07	8.19	11	23	Cell membrane. Chloroplast.
CcABCG29	KYP54262	LOC109807460	4014294-4034321	1500	170370.83	6.75	11	26	Cell membrane. Chloroplast.
CcABCG30	KYP55666	LOC109807699	19989558-20004511	1418	161820.65	8.9	11	26	Cell membrane
CcABCG31	KYP56982	LOC109809098	34818776-	741	81702.72	9.05	11	12	Cell membrane

			34826902						
CcABCG32	KYP57733	LOC109807641	43015398-43024752	1449	164070.17	7.67	11	24	Cell membrane
CcABCG33	KYP57734	LOC109809552	43027240-43038856	1445	163218.52	8.03	11	24	Cell membrane
CcABCG34	KYP57934	LOC109807560	45075476-45077364	609	68159.95	8.65	11	1	Cell membrane
CcABCG35	KYP53034	LOC109810595	391122-395936	681	75466.37	8.91	Un	5	Cell membrane
CcABCG36	KYP53068	LOC109810569	840758-848796	1115	123587.99	9.16	Un	14	Cell membrane
CcABCG37	KYP52287	LOC109811196	2121-13637	1229	138171.93	8.81	Un	20	Cell membrane
CcABCG38	KYP52347	LOC109811227	785413-787392	651	73181.09	8.45	Un	1	Cell membrane
CcABCG39	KYP51780	LOC109811628	147015-153318	723	80384.61	8.89	Un	12	Cell membrane
CcABCG40	KYP51380	LOC109811918	541176-543014	612	68365.89	9.39	Un	1	Cell membrane
CcABCG41	KYP49769	LOC109813109	127353-133820	683	77708.58	9.05	Un	10	Cell membrane
CcABCG42	KYP49445	LOC109813363	17796-28760	744	82372.18	8.91	Un	12	Cell membrane
CcABCG43	KYP48955	LOC109813737	400317-424748	1358	154173.34	8.61	Un	21	Cell membrane
CcABCG44	KYP48157	LOC109814303	92212-96948	678	75455.58	9.06	Un	5	Cell membrane
CcABCG45	KYP47060	LOC109815112	59170-61921	755	83546.16	9.22	Un	1	Cell membrane
CcABCG46	KYP41536	LOC109818995	187197-197961	1445	163678.86	8.75	Un	24	Cell membrane
CcABCG47	KYP39219	LOC109788852	72316-76120	624	69345.94	8.87	Un	4	Cell membrane
CcABCG48	KYP37548	LOC109790064	6674-12941	1087	120433.13	8.96	Un	14	Cell membrane
CcABCG49	KYP36564	LOC109790672	14766-22348	1457	164896.42	8.25	Un	24	Cell membrane
CcABCG50	KYP35274	LOC109791517	44302-58642	1473	167555.55	7.74	Un	20	Cell membrane
CcABCG51	KYP32835	LOC109793033	5717-9684	628	70573.41	8.74	Un	4	Cell membrane

Note.

Aa, Amino acid; Mw, Molecular weight; pI, Isoelectric point; Chr, Chromosome; Sub-loc, Subcellular localization.

3