ATP-Binding Cassette Expression Profiling Revealed its Role in Development and Regulating Stress Response in Potato (*Solanum Tuberosum*)

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Abstract

The ATP-binding cassette (ABC) transporter gene family play vital role in substance transportation including heavy metals ion, secondary metabolites and phytohormone across membranous structure. Despite its essential role in crops, still uncovered in potato (*Solanum tuberosum* L), grown world widely as a 3rd important food crop. Current study identified a total of 54 ABC transporter genes (StABC) in potato genome. The accumulative phylogenetic tree of StABC with Arabidopsis, divided into 8 groups (ABCA to ABCH). Among these, ABCG was the largest family covering 90% of StABC genes followed by ABCB group. The number and architecture of exon-intron varied from gene to gene. However, the sequence alignment and motif analysis demonstrated similar functional and structural sites and motif in all StABC. The syntenic blocks presented segmental duplication among intra and inter genomes. In addition, the presence of stress responsive element in the regulatory regions depicted their role in environmental adaptation. Furthermore, the tissues-specific and stress-specific expression profiling of StABC genes and their validation through Real-Time-qPCR (RT-qPCR) analysis revealed their role in development and stress. The presented results provided useful information for further functional analysis of StABC and can also use as reference study for other important crops.

Introduction

The ATP-Binding Cassette (ABC) transporter is one of a largest transporter proteins family, involved in substance transportation across inter and intra cellular membrane. (Jones and George, 2004). In many genome the ABC family of gene is known as a wide family of transporters, distributed into 8 groups, designated ABCA through ABCH, although human has only 7 groups (ABCA–ABCG)(Sánchez-Fernández et al., 2001). On the other hand plants don't have ABCH but they have ABCI (Lopez-Ortiz et al., 2019). These documented proteins normally have two conserved domains, nucleotide binding domain (NBD) and transmembrane domain (TMD). The NBD bind and hydrolyzes ATP for production of energy, needed for TMD to generate pore for substrate translocation across membrane (Sharom et al., 2011; Zhiyi et al., 2015).

In cellular efflux, the ABCG play a vital role in phytohormone transportation and possessed both domains (NBD-TMD)(Borghi et al., 2015). Literature demonstrated that *AtABCG25* and *AtABCG40* performed have great contribution in the transportation of abscisic acid (ABA) in *Arabidopsis* (Kang et al., 2010). Similarly, another gene, *AtABCG14* is associated with cytokinin pathways (Ko et al., 2014). It was also observed that ABCG member are involved in tissue growth and development. For instance, *AtABCG26/WBC27* and *OsABCG15* has significant role in growth of pollen in *Arabidopsis* (Qin et al., 2013; Quilichini et al., 2010).

A genome-wide analysis is a detailed method for the identification of genes in a specific family. By this method important information including evolutionary pathway, genetic diversity and relationships among genes and proteins can be obtained, which are helpful for future functional analysis. Several Genome-wide studies carried and identified different number of ABC genes in various land plants including Arabidopsis (Sánchez-Fernández et al., 2001) [15], rice (Garcia et al., 2004)[16], maize (Pang et al., 2013)[17], *Lotus japonicus* (Sugiyama et al., 2006)[18], grapes (Çakır and Kılıçkaya, 2013)[19], pineapple (Chen et al., 2017)[20] and *Hevea brasiliensis* (Zhiyi et al., 2015)[4]. However, such research is required in potato genome to explore its role in Solanaceae family.

Potato (*Solanum tuberosum*) is the fourth largest food crop in the world(Zaynab et al., 2017b). Because of its rich carbohydrate nutrients, high yield and short life cycle, several countries have been using it as staple crop for long time. Different environmental stresses like salinity, alkali, drought and waterlogging, heat and cold are effecting on the elongation of potato roots, potato respiration and photosynthesis, which are compromising the yield and growth of potato (Li et al., 2019b). In addition to abiotic factors, there are several biotic stresses caused by viruses, fungi and nematode (Dai et al., 2007). So, in order to developed potato plant immunity against these biotic and abiotic stresses, scientist are trying to explore stress responsive genes and their implementation in advance molecular biology including genetic engineering, genome editing and marker assisted breeding and selection to develop new resistant varieties. As ABC gene family is one of stress responsive family involved in plant adaptation. Therefore identification, characterization, expression profiling and structural and functional conserved motifs prediction is necessary in potato genome which is fulfilled by current study results. These results are important for functional validation of StABC in potato crop.

Material And Methods

Identification of ABC transporter gene in potato genome

Solanum tuberosum (Potato) genome was downloaded from phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>). In order to find ABC genes in potato, we used Arabidopsis ABC transporter as reference protein, retrieved from TIAR (<https://www.arabidopsis.org/>). The Blastp program was used for sequence similarity analysis. The Pfam (<https://Pfam.sanger.ac.uk/>) and CDD (<https://www.ncbi.nlm.nih.gov/cdd/>) databases were included to validate functional domains. The protein statistics including molecular weight (MW) and iso-electric point (PI) were described through an online ProtParam tool located at ExPASy (<https://web.expasy.org/protparam/>) server (Gasteiger et al. 2005).

Phylogenetic analysis and sub-cellular localization

The phylogenetic tree of selected amino acids sequences was constructed with Arabidopsis ABC transporter proteins using MUSCLE program for alignment and MEGA7 for phylogenetic tree with neighbor-joining-method using parameters: Partial deletion 90%, poisson correction and 1000 boot-strap replicate. The Subcellular localization of *S.tuberosum* ABC transporter gene was projected through CELLO v2.5(<https://cello.life.nctu.edu.tw/>).

Chromosomal locations, synten, and selective pressure analysis

Information regarding to all known *StABCs* was retrieved from the PGSC website. The comparative synten analysis for the visualization of genome conservation was executed through circoletto Tool (tools.bat.infspire.org/circoletto/). Duplicated gene coding sequences were arranged with MEGA7 by using the Muscle (codon) method. The synonymous and non-synonymous substitution rate (Ka; No. of nonsynonymous substitutions per nonsynonymous site, Ks; No. of synonymous substitutions per synonymous site) was calculated with KaKs_Calculator 2.0 software through the MYN method. Furthermore, the divergence time “t = Ks/2r” was calculated through exchange rate $r = 2.6 \times 10^{-9}$ (Li et al., 2019a).

Intron-exon Distribution And Conserved Motif Identification

The intron-exon distribution was conducted using Gene Structure Display Server (<http://gsds.cbi.pku.edu.cn/>) (Guo et al., 2007). For the ABC transporter proteins, the conserve motifs were discovered by online MEME server (<http://meme-suite.org/>) with the given parameters: optimum width range of > 6 to < 200, number of motif; 20. The distribution of motif along amino acid sequence constructed using TB tools (<https://github.com/CJ-Chen/TBtools>) software.

Prediction of Cis-element in regulatory regions

In order to find the *cis*-acting sites in the regulatory regions of StABC genes, we retrieved the promoter sequence (2000 bp upstream of the initiation codon 'ATG') from PGSC server. The *cis*-regulatory elements were detected using Plant *Cis*-acting Regulatory DNA Elements (PlantCARE) (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

Tissue-specific expression profiling of StABC genes

The tissue specific expression analysis was conducted by using publicly accessible RNA-seq data.. The collected data was compiled according to highly expressed tissues such as root, stem and leaf. The original Fragments Per Kilobase Million (FPKM) values were used to draw a heatmap with the help of TBtools (Chen et al., 2020)..

Plant material and sample collection

Potato plant seeds were taken from National Agriculture Research Center (NARC) Islamabad and planted in a glasshouse under optimum conditions at National Institute for Genomics and Advanced Biotechnology (NIGAB), NARC Islamabad. After 25 days of post-germination, leaf, stem, and roots were collected in replicates and stored in liquid nitrogen for RNA extraction.

Extraction Of Rna And Rt-qpcr

Total extraction of RNA for leaves, stem and root were performed by using the RNA isolation Kits (Huayueyang, Beijing, China) according to instructions of company. The 1st strand of cDNA was produced by cDNA Kits, ReverTra® Ace.

To observe ABC genes expression level in the three tissues, a qRT-PCR was performed. The amplification of cDNA was performed with SYBR® Green Real time PCR Master Mix (TOYOBO QPK-210, Shanghai, China) by DNA engine Opticon three thermal cycler (Bio-Rad) with 20 µL volume. Protocol for amplification have primary step at 95°C for one mintue and by following this step fourty cycle of denaturation of 15 second at 95°C was performed. The step of annealing was performed for 15 second at 55°C. The extension step lasts for 15 second at 72°C. Target gene magnification was checked regularly in every single cycle with the help of SYBR-green fluorescence dye. The amplification of qRT-PCR specificity was regulated by managing a melting-curve-analysis routinely. Analysis of data were performed by $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001) and the outcomes were explained on the basis of relative level of expression of gene. For a house-keeping genes the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was utilized (Pimentel et al., 2010). For each experiment three replicates were performed. All the sequence of primer is listed under (Table.S1).

Results

Physico-chemical properties and ABC Transporter gene identification

In the present study, we have identified a total of 54 proteins with ABC transporter domains in the genome of *S. tuberosum*. Same genes with different transcripts were not considered. The details of 54 ABC transporter proteins, including genomic location, chromosome number, description, domain organization, molecular weight, protein length and isoelectric point (pI) were listed in (Table 1). The encoded protein have amino acid range from 84 to 1580aa, PI range from 5.91 to 9.69 and the molecular weight range from 9261.82 to 178764.6kDa.

Phylogenetic Analysis For Abc Transporter Genes

For the investigation of evolutionary relationships of the 54 ABC transporters, by using MEGA7.0 software a phylogenetic tree was designed by method of neighbor-joining. The multiple sequence alignment demonstrated important conserved residues in ABC transporter proteins (Fig. 1). The phylogenetic tree was divided into 7 sub-groups with named as ABCA, ABCB, ABCC, ABCE, ABCF, ABCG and PGP1. Of these, ABCG was the largest subgroup. In case of StABCs, all genes were distributed into two sub-groups mainly ABCG and ABCB (Fig. 2).

Sub-cellular localization for StABC proteins

Sub-cellular localization of 54 StABC genes were predicted with the help of 2 diverse tools and the higher confidence prediction were determined in the Table 1. Many number of ABC transporter proteins in potato were predicted to localized in plasma membrane although two genes; *Stabct3* and *Stabct20* localized in mitochondria while only one genes; *Stabct7* was found in cytoplasm. In addition, *Stabct10*, *Stabct16* and *Stabct54* were in nucleus and single gene (*Stabct2*) was in inner membrane (Table.1). These suggest that these genes may require for the coordinated expression in both nucleus and other organelles.

Intron-exon structure of ABC transporters in *S.tuberosum*

The gene structure of StABC genes was examined to define the proteins diversity. The exons number was ranged between 1 to 28. A total of nine genes possessed one exon while only one genes; *Stabct4* gene had 28 exon. Some genes such as *Stabct11*, *Stabct12*, *Stabct15* and *Stabct2* had simpler structure with 1–2 exons. The gene structure was differing only in length and numbers of exons and introns. In addition some genes carried equal number of exons e.g. *Stabct23* and *Stabct29* had four exons in each gene (Fig. 3).

Conserved motifs analyses

The proteins structures are directly associated with their function and the conserved motifs have great potential in maintaining the structure and activity of proteins. Therefore, we presented conserved motifs and their patterns in StABCs. A total of ten conserve motifs were evaluated in order to study the architecture of ABC transporter of *S.tuberosm*. Motif 1st was observed in all genes of *S.tuberosm* ABC transporter except the two genes *Stabct16* and *Stabct20* (Fig. 3). Motifs 2nd to 3rd were present in most of the genes. Some motifs were distinctive with respect to 1 or 2 sub-families. Each sub-family member showed some common and some unique motifs. Motif 6th, and 7th consist of 50 amino acids residues, and 2nd and 10th motif was comprised of approximately 29 amino acids (Fig. 4).

Chromosomal distribution and Cis-element of StABC genes

The chromosomal distribution of StABCs demonstrated that, all genes were distributed unequally on all chromosomes. Highest number of genes were localized on Chr5 and Chr6 with 9 genes followed by Chr11 (8 genes), Chr8 and Chr12 (7 genes), Chr1 (5 genes), Chr3,4,7,8 and Chr9 (4 genes) and Chr2 (1 gene)(Table.1). On the basis of significance of *cis*-element in promoters region on gene regulation the identified *cis*-elements were categories into different groups including stress responses, development and phytohormone response. Forinstqance, the most common *cis*-actingf site was MeJRE and anaerobic induction. Followed by ABRE, GR and SARE (Fig. 5).

Gene duplications of StABC

The value of Ka/Ks for each duplicated gene pair was estimated to calculate the molecular evolution rate. The Ka/Ks > 1 considered as + ve selection effect, while Ka/Ks < 1 as purifying selection and Ka/Ks = 1 with neutral selection among the duplicated genes (Yang and Bielawski, 2000). The cosmic majority of StABC genes were presented the Ks value greater than 0.52, while the corresponding deviation time can be more than 100 million years ago (MYA). Fascinatingly, the Ks value of the duplicated pair genes (*Stabct30/Stabct52*) was 2.94, while the corresponding duplication time may be 93.98 million years ago (MYA) (Table 2).

Comparative synteny of StABCs

Comparative synteny analysis among Arabidopsis (*Arabidopsis thaliana*), potato (*S.tuberosum*), and tomato (*Solanum lycopersicum*) demonstrated a remarkable relationship regarding gene evolution, expansion, duplication, triplication, and function. It was noticed that the tomato Solyc05g053570.2 gene sequence illustrated synteny among potato gene sequence *Stabct48*. Similarly, potato gene sequence *Stabct19* illustrated synteny with Solyc12g100190.1 (Fig. 6).

Tissue-specific expression profiling

The tissues specific expression profiling results suggested that different tissues had different number of differential genes. For instance, twenty one ABC transporter genes showed relatively high transcriptional abundance in leaves. However, some genes did not show any expression, whereas others showed tissue-specific expression. For instance, *Stabct17*, *Stabc18*, *Stabc6*, *Stabc19* showed high transcript level in leaf, root and root, respectively. Some genes, expressed in more than one tissues like, *Stabc21* and *Stabc50* had similar expression in root and stem. Most of the gene did not show any expression (Figure. 7A).

Stress-specific Expression Profiling Of Stabcs

In order to find the putative role of StABCs under different abiotic stresses we have also analysis the RNA-seq data of StABCs under heat, Indole acetic acid (IAA), abscisic acid (ABA) and *P. infestant* stresses. A total of twenty nine StABC genes showed expressed under heat stress. The expression of some genes enhanced under heat stress *i.e.* *Stabc3*, *Stabc10*, *Stabc36*, *Stabc51*, and *Stabc41*. Similarly, some genes had remarkable expression under *P. infestation* while some had in ABA and IAA. (Fig. 7B)

RT-qPCR Analysis

The RT-qPCR analysis also validated the expression analysis of *StABC6*, *StABC17*, *StABC18*, *StABC21*, and *StABC50* genes in roots, stems, and leaves. The relative expression pattern demonstrated that *StABC6* and *StABC18* genes had a higher expression in the roots compared to stems and leaves. The genes *StABC17*, *StABC21*, and *StABC50* had higher expression in the leaf (Fig. 8).

Discussion

Potatoes are an important component of the world's food supply (Zaynab et al., 2017b). During their growth and developmental process, potatoes are subjected to a variety of stresses (Quandahor et al., 2021). In order to develops plant immunity, several stress responsive genes were identified. Of these ABC transporter is one of an important genes family that are involved in various regulatory processes such as growth and development and response to various biotic and abiotic stresses (Rehman et al., 2020). In current study we have identified a total of 54 StABC genes in potato genome which is quite similar to other crops like one hundred thirty five member in *Vitis vinifera* (Çakır and Kılıçkaya, 2013) (Çakır and Kılıçkaya, 2013), one hundred thirty five member in maize (Pang et al., 2013) (Pang et al., 2013), one hundred twenty one member in rice (Garcia et al., 2004) (Garcia et al., 2004), one hundred thirty one member in *Arabidopsis thaliana* (Sánchez-Fernández et al., 2001).

The phylogenetic anlaysis demonstrated that most of the identified StABCs were belong to ABCG groups followed by ABCB group, showing their abundance in potato genome. The accomulaitve phylogenetic tree divided into 8 groups (ABCA-ABCI). Literature study demonstrated that AtABCB19 and AtABCB1 in arabidoposis have a strong role in post-embryonic organs and cytoplasmic flow with a synergistic role [52,53]. Similarly, the AtABCG11 is involved in the transportation of grease substance and paraffin wax and found in leaf, stem and floral organ. So, the StABCB and ABCG may have similar role in potato genome.

The structural analysis of StABC proteins will be helpful in functional analysis. The evolutionary tree revealed that intron-exon arrangements had shaped the evolution of the gene family (Flagel and Wendel, 2009; Moore and Purugganan, 2005). It corresponds to earlier scientific findings that some genes tend to retain in plants that may have short introns or may not have introns during evolution (Mattick and Gagen, 2001). The expression levels of genes with no and few introns are low in plant genomes (Mattick and Gagen, 2001). Furthermore, compact gene structure may allow rapid expression response to exogenous or endogenous stimulus (Jeffares et al., 2008). Our gene structure analysis results also suggested that *StABC* gene sequences presented the same number of exons/introns with similar functional features because they may have originated during evolutionary processes in the course of duplication events (Bari et al., 2018; Waqas et al., 2019).

Cis-elements studies can present an important foundation for additional functional dissection of *StABC* genes. We found that all *StABC* promoters contain one or more stress-responsive *cis*-elements such as LTR (low-temperature response element), TATC- box, and P-box (MeJA-response element), ABRE (ABA response element), and SARE (SA response element). These *Cis*-elements play a vital role in stress response by regulating stress-responsive genes (Wu et al., 2014). Therefore, these critical *cis*-acting sites in the *StABCs* suggested their response under various environmental stresses.

The genome size, gene distribution, and duplications in the genome are the main factors of genetic diversity among land plants. The genetic duplication character has long been identified during the origins of gene families' evolutionary novelty, expression, and complexity. We also found some duplication events in *StABCs*, which may perform a vital role in *StABC* amplification. Because gene duplication is an essential feature in the neofunctionalization, expansion, and diversification of gene family (Lavin et al., 2005). Similarly, the distribution and mapping of *StABC* genes at the chromosome level will help potato breeders to develop new varieties with the desired traits. These mapping can be used in marker assisted breeding and selection of desired offspring.

The expression profiling of genes in different tissues improve our understanding of the tissue-specific and dynamic expression of the ABC genes in potato. Some *StABC transporter (Stabct)* were expressed uniquely in vegetative organs, *Stabct6*, *Stabct17*, and *Stabct18* in the roots and leaves,

indicating their potential role in the vegetative tissue development in potato. The current study reported higher transcripts abundance of *Stabct*s in the leaves and such evidence was also observed in previous studies (Shi et al., 2020) and supported our results. The upregulation of *Stabct 17* in leaves tissues also validated with RT-qPCR analysis, which was presented similar result. It suggests that potato ABC transporter genes have a substantial role in plant growth, development and response to various stresses.

To date, an increasing number of studies have shown that members of the ABC transporters family can play an important role in response to different stresses (Wang et al., 2021). In potato we observed upregulation of *Stabct3* and *Stabct51* under heat stress and similar results also seen in rice (Moon and Jung, 2014). Hormones can affect the physiological and biochemical reactions of plants through a variety of signal transduction pathways (Fatima et al., 2021; Zaynab et al., 2017a). IAA and ABA are important hormones in the plant immune system. A number of studies have revealed that *ABC*s are not only involved in stress response but also in developmental and hormonal signaling (Kumar et al., 2020).. Under ABA and IAA treatment, 26 and 24 *Stabct* genes upregulated, respectively. The upregulation of these genes might have putative role in potato to regulate abiotic stresses (El Guizani et al., 2014). The expression and their clustering also highlighted a strong correlation of genes clusters and their expression under different tissues and stresses. These co-expression and co-occurrence shows their essential role in plant adaptation under diverse environmental stresses.

The *P. infestans* pathogenic stress expression profiling of *Stabct* also demonstrated up regulation of two important genes e.g. *Stabct48*, and *Stabct49*. Similar results were also observed in *Oryza sativa* (rice) ABC gene, which was transcriptionally upregulated upon blast fungus (*Pyricularia grisea*) infection. (Xiong et al., 2001). Several functional studies of ABCG gene showed their role under biotic stress in potato (Ruocco et al., 2011). Most interestingly, infection of potato plants by *P. infestans* led to the tremendous up regulation of *StPDR1* and *StPDR2* expression in leaves at 48 hrs post infection.

Conclusion

Taken together, a total of 54 ABC transporter genes were reported in potatoes. The comparative evolutionary analysis concluded that, the ABCG group was the largest ABC transporter group in potato. The conserved structural and functional motifs associated to ABC transporter domain were present in all *Stabct*s with slight variation among groups and members. The tissue-specific and stress-specific expression profiling of *Stabct*s demonstrated their putative role in potato developmental stages and environmental adaptation. These results are essential steps for potato growth and development under various environmental stresses.

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Tables

Table 1 List of identified putative *Stabcts* and their features. CDS; coding sequence, pI; iso-electric point

Gene	Location Start -end	Genomics sequence	CDS	Peptide	Chromosomes	Number of amino acids	Molecular weight	pI:	Sub-cellular localization
Stabct1	58762883..58769064	6182	2271	756	4	756	84481.71	8.89	PlasmaMembrane
Stabct2	11327196..11336370	9175	1812	603	11	603	66691.78	9.08	InnerMembrane
Stabct3	47266008..47267889	1882	1317	438	8	438	48277.62	8.98	Mitochondrial
Stabct4	41606331..41613682	7352	4743	1580	8	1580	178764.6	6.12	PlasmaMembrane
Stabct5	41628506..41631135	2630	1878	625	8	625	71347.73	7.06	PlasmaMembrane
Stabct6	60412413..60421201	8789	4503	1500	3	1500	170088.85	8.24	PlasmaMembrane
Stabct7	59678366..59679025	660	504	167	12	167	17798.29	5.93	Cytoplasmic
Stabct8	59696990..59702667	5678	3864	1287	12	1287	139652.46	6.74	PlasmaMembrane
Stabct9	40225813..40235522	9710	3864	1287	11	1287	141384.58	9.2	PlasmaMembrane
Stabct10	641972..644313	2243	1209	402	4	402	44884.16	8.9	Nuclear
Stabct11	6566287..6568374	2088	2088	695	4	695	77892.19	9.03	PlasmaMembrane
Stabct12	6560427..6562601	2175	2175	724	4	724	81057.78	9.27	PlasmaMembrane
Stabct13	55507940..55511125	3186	1081	359	6	359	39889.86	9.25	PlasmaMembrane
Stabct14	55574774..55583712	8939	3219	1072	6	1072	118757.27	8.83	PlasmaMembrane
Stabct15	52773860..52775722	1863	1863	620	7	620	69167.78	8.78	PlasmaMembrane
Stabct16	82110892..82111440	549	339	112	1	112	12193.81	9.69	Nuclear
Stabct17	82091452..82095286	3835	1890	629	1	629	69765.02	7.53	PlasmaMembrane
Stabct18	49017339..49024379	7041	4284	1427	5	1427	161056.36	7.02	PlasmaMembrane
Stabct19	48998602..49005673	7072	3738	1245	5	1245	140318.44	7.99	PlasmaMembrane
Stabct20	48992723..48993463	741	267	88	5	88	9744.33	9.25	Mitochondrial
Stabct21	50423518..50427078	3561	2124	707	3	707	79063.15	8.6	PlasmaMembrane
Stabct22	813841..815847	2007	1671	556	12	556	62046.39	8.97	PlasmaMembrane
Stabct23	808495..811827	3333	1890	629	12	629	69913.27	8.47	PlasmaMembrane
Stabct24	2753615..2761615	8061	1938	645	11	645	72455.58	8.59	PlasmaMembrane
Stabct25	46442609..46446926	4318	1959	652	5	652	72463.08	7.95	PlasmaMembrane
Stabct26	78728173..78733344	5172	2673	890	1	890	100244.39	7.16	PlasmaMembrane
Stabct27	3599414..3601252	1839	1384	462	8	462	52051.61	8.85	PlasmaMembrane
Stabct28	23010110..23018202	8093	3654	1217	12	1217	137720.46	8.52	PlasmaMembrane
Stabct29	53551639..53554802	3164	1842	613	7	613	68483.95	8.55	PlasmaMembrane
Stabct30	75147184..75150158	2975	2499	832	1	832	93962.11	8.91	PlasmaMembrane
Stabct31	5021794..5029583	7790	4341	1446	11	1446	164225.77	6.81	PlasmaMembrane
Stabct32	5013377..5020180	6804	4179	1392	11	1392	158626.74	7.53	PlasmaMembrane
Stabct33	41997222..42005704	8483	2172	723	11	723	80509.68	8.86	PlasmaMembrane
Stabct34	50049590..50051416	1827	1827	608	7	608	68618.94	8.76	PlasmaMembrane
Stabct35	44358550..44360340	1791	1791	596	3	596	66398.86	9.31	PlasmaMembrane
Stabct36	2557631..2565744	8114	2181	726	1	726	80223.77	8.84	PlasmaMembrane
Stabct37	55781265..55790061	8797	3387	1128	7	1128	125437.86	8.27	PlasmaMembrane
Stabct38	51897691..51899616	1926	1926	641	5	641	72186.33	9.09	PlasmaMembrane

Stabct39	51014601..51019460	4860	3048	1015	5	1015	114928.23	8.36	PlasmaMembrane
Stabct40	51000420..51006217	5798	3432	1143	5	1143	129243.28	6.8	PlasmaMembrane
Stabct41	50607365..50609636	2272	2175	724	5	724	80435.99	8.98	PlasmaMembrane
Stabct42	60789857..60796562	6706	4308	1435	12	1435	162634.28	8.55	PlasmaMembrane
Stabct43	7152699..7154924	2226	2226	741	9	741	82584.39	9.3	PlasmaMembrane
Stabct44	53683922..53685907	1986	1986	661	3	661	74141.98	8.55	PlasmaMembrane
Stabct45	46111847..46120421	8575	4224	1407	6	1407	159992.74	8.78	PlasmaMembrane
Stabct46	39864565..39873867	9303	4011	1336	11	1336	151501.91	6.28	PlasmaMembrane
Stabct47	52893988..52897879	3892	1971	656	6	656	73689.67	7.6	PlasmaMembrane
Stabct48	58529046..58537413	8368	4080	1359	9	1359	153519.13	5.91	PlasmaMembrane
Stabct49	58544464..58551480	7017	4302	1433	9	1433	161618.19	6.02	PlasmaMembrane
Stabct50	37030656..37036265	5610	1359	452	11	452	49897.56	7.68	PlasmaMembrane
Stabct51	3945546..3955866	10321	2133	710	5	710	78004.55	7.03	PlasmaMembrane
Stabct52	61225165..61227965	2801	2190	729	9	729	81481.54	9.12	PlasmaMembrane
Stabct53	51148426..51155060	6635	3438	1145	12	1145	130281.63	6.92	PlasmaMembrane
Stabct54	14392117..14392497	381	255	84	2	84	9261.82	7.61	Nuclear

Table 2. Gene duplication and selection pressure of Stabctcs. Ka; No. of nonsynonymous substitutions per nonsynonymous site, Ks; No. of synonymous substitutions per synonymous site, MYA; Million years ago

Seq_1	Seq_2	Ka	Ks	Ka_Ks	Time(MYA)
Stabct21	Stabct47	0.112387323	0.855380415	0.131388703	21.61294671
Stabct13	Stabct33	0.085755124	0.418796211	0.204765758	16.49136991
Stabct16	Stabct17	0.060577816	0.394385524	0.153600506	11.64958002
Stabct24	Stabct25	0.189904664	1.059093651	0.179308661	36.52012767
Stabct8	Stabct9	0.382823768	2.245388104	0.170493362	73.61995534
Stabct15	Stabct34	0.303168999	1.309777872	0.231465965	58.30173062
Stabct30	Stabct52	0.48869732	2.942385034	0.166088841	93.98025378
Stabct11	Stabct12	0.056643031	0.253656609	0.223305956	10.89289067
Stabct37	Stabct54	0.142248336	0.427450302	0.332783333	27.35544926
Stabct3	Stabct27	0.086200038	1.020628207	0.084457825	16.57693046
Stabct22	Stabct23	0.090579633	0.288029867	0.314480001	17.41916017
Stabct31	Stabct40	0.099321903	0.58069016	0.171041133	19.10036598

Figures

Figure 1

multiple sequence alignment of AtABC and StABC

Figure 2

An accumulative phylogenetic tree containing Arabidopsis ABC and Potato ABC proteins. Numbers on the nodes are representing bootstrap values.

Figure 3

Distribution of exons, introns and UTR (untranslated regions) in Stabct genes sequence.

Figure 4

De-novo MEME motifs distributions in the Stabcts proteins.

Figure 5

Cis-acting elements distribution in the regulatory regions of Stabct

Figure 7

Expression profiling of Stabct genes (A) leaves, root and stem (B) in response of heat, IAA, ABA and pathogen.

Figure 8

Real time relative expressions of Stabcts in leaves, root and stem.

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