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Original article

Expression profiling of *DUF599* genes revealed their role in regulating abiotic stress response in *solanum tuberosum*

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ABSTRACT

The proteins containing the domain of unknown functions (DUFs) have significant roles in stress response and the growth of plants. A comprehensive genome-wide analysis and expression profiling of *DUF599* was conducted to identify their roles in potato growth and response to stressed conditions. A total of nine *DUF599* genes were identified, located on five chromosomes in the potato genome. The phylogenetic analysis divided *StDUF599* genes into three groups in accordance with gene conserved motifs and gene structure distribution patterns. The *StDUF599* promoters comprised several *cis*-acting factors responsive to plant hormones and abiotic stresses. The present study revealed that *StDUF599* genes also possessed MBS, LTR, ABRE, and anaerobic induction responsive elements, indicating their importance in coping with abiotic stresses. *StDUF599* genes were the target of several families of micro-RNAs also identified in this study. Purifying selection pressures lead to the duplication of the *StDUF599* genes. Expression analysis of *StDUF599-6* and *StDUF599-9* in various tissues illustrated their vital role in developmental processes. It was found that *StDUF599-7* and *StDUF599-9* were highly expressed against heat and salt stresses. Expression profiling revealed that the *StDUF599-8* gene has a significant role against GA3 and IAA. Lastly, this article forecasted that the *DUF599* genes could enhance plant tolerance against several abiotic stresses.

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1. Introduction

Several abiotic stress factors negatively affect plant growth and decrease yield (Sade et al., 2018). For instance, environmental deformations cause 70 % yield losses annually (Zaynab et al., 2020). Salinity, temperature, and drought are the significant abiotic stress factors influencing the geographical privilege of plants, curbing crop production, and menacing food safety. In general, plants do not possess structures that are directly involved in discerning environmental changes but could show responses to environmental aberrations (Hamant and Haswell, 2017). Under stress conditions, plants demonstrate comprehensive defence retributions at cellular and molecular levels to counter cell damage (Kim et al., 2014). Easy accessibility to plant genomes empowers us to characterize and identify several gene families against abiotic stress factors (Zaynab et al., 2021b). Various members of these families have been well recognized in plant genomes, with a reasonable number

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of them have been functionally characterized, including NAC, bHLH, MAPK, bZIP and HSF transcription factor families (Waseem et al., 2021). Moreover, plant genomes possess numerous genes expressed against stresses, comprise highly conserved domains, and carry out crucial tasks in different metabolic processes of plants during stress. Genes possessing these potential hypothetical domains are recognized as the domain of unknown functions (DUFs) (Zaynab et al., 2021c). Researchers have reported that DUF family members perform an imperative role against abiotic stress factors in different plants (Zhao et al., 2021). They reported different gene families, such as DUF936, DUF810, DUF1618, DUF866, and DUF221 in rice and gene families DUF724 and DUF581 in Arabidopsis have a significant role against abiotic stress (Waseem et al., 2021).

Specific genes were also linked to abiotic stresses, including *OsDUF810, OsDSR2,* and *SIDP361*, which have been regulated through dehydration and nutritional status during the developmental stage. The reduction in *MsDUF* gene expression in *Medicago sativa* was noticed when plants were treated with NaCl, abscisic acid, gibberellic acid, and PEG6000, indicating its negative regulatory role against stress responses (Albornos et al., 2017). The wild-type Arabidopsis contains the *TaSRHP* gene that possessed the DUF581 domain, induced during salinity stress. Enhanced *TaSRHP* gene expression illustrated resistance to drought and salinity. Likewise, enhanced expression of *CiDUF4228-3* in the genome of *Caragana intermedia* enabled plants to maintain their normal growth under low temperature, dehydration, and drought, suggesting its critical tasks under stressed conditions (Leng et al., 2021). A previous Arabidopsis study exhibited that the *ESK1* gene (*AT3g55990*), a DUF231 family member, works as a pessimistic regulator for cold acclimatization in plants (Xin et al., 2007). The *OsSIDP366* gene (DUF1644) significantly regulates the retaliation against drought and salt stress factors in rice. Moreover, the transgenic rice overexpression of the *OsSIDP366* gene shows tolerance against drought and salt (Li et al., 2016). In addition, the competent characterization of *DUF* genes regarding tolerance against various stresses is presently delineated in model plants only. In contrast, extensive investigations on the DUF family members in different plant species have seldom been reported. This indicates that genes comprising DUF-domain may directly or indirectly participate in plant tolerance mechanisms.

Potato is an imperative economic crop, extensively utilized as food almost everywhere (Zaynab et al., 2021a). However, potato yield is also affected by various environmental stresses (Zaynab et al., 2021c). Therefore, to understand the function of *DUFs*, we executed a detailed study of the *DUF599* gene members in the potato genome. Further, we investigate the structure of genes, their location on chromosomes, phylogenetic relationships of genes, conserved domains of proteins, and gene expression profiling in various tissues. Moreover, we analyzed the expression of *StDUF599* genes in response to heat, salinity, and several hormonal stresses. The present study has the ability to dispense a base for functional ratification of these *DUF599* genes and their functions in the growth and developmental mechanisms of potato plants under stressed environments.

2. Materials and methods

2.1. Identification of DUF599 genes in S. tuberosum

For the identification of *DUF599* genes in the *S. tuberosum* genome, the HMM (Hidden Markov Model) and BLASTP methods were used. Thus, the potato (*S. tuberosum*) genome was retrieved from the Phytozome 12.0 (http://phytozome.jgi.doe.gov) database (Goodstein et al., 2012). In addition, the amino acid sequences of 9 *AtDUF599* genes were attained from TAIR (https://www.arabidopsis.org/) (Goodstein et al., 2012; Rhee, 2003) database. The 9 AtDUF599 amino acid sequence data were utilized for the BLASTP query in the potato genome. In addition, the HMMER 3.1 program (http://www.hmmer.org/) (Finn et al., 2015) was utilized to find out the *DUF599* genes with standard limitations. The HMM files with Pfam ID (PF04654) were attained through the Pfam site (http://pfam.xfam.org/) protein domain dataset (El-Gebali et al., 2019). Finally, nine *StDUF599* gene members were identified after using two procedures in the potato genome (Supplementary Table S1). Similarly, six *DUF599* genes were also found in *Z. mays* using the same process. Moreover, its genome sequences were attained using Phytozome JGI 12.0 database (http://phytozome.jgi.doe.gov). The physico-chemical properties were resolute by ProtParam (https://web.expasy.org/protparam) (Gasteiger et al., 2005). Therefore, the StDUF599 proteins' subcellular localizations have been forecasted through CELLO v2.5 (http://cello.life.nctu.edu.tw/). We used the TBtools software (V1.068; https://github.com/CJ-Chen/TBtools) to determine the *StDUF599* genes structure. Moreover, StDUF599 proteins conserved motifs were identified using the MEME (https://memesuite.org/meme/db/motifs) website with the following parameters; such as motifs number = 10 (Bailey et al., 2009).

2.2. Phylogenetic Tree, Synteny, chromosomal location, and selection pressure analysis

A phylogenetic tree was constructed among *Z. mays*, *A. thaliana*, and *S. tuberosum* protein sequences to study the evolutionary connection of *StDUF599* genes. This study utilized MEGA 7 software (https://megasoftware.net/home) (Kumar et al., 2018) for sequence alignment. The neighbor-joining method was used to build a phylogenetic tree with 1,000 bootstrap repetitions. Moreover, the iTOL program (https://itol.embl.de/) was used to beautify the tree. In addition, the synteny relationships among *Z. mays*, *A. thaliana*, and *S. tuberosum* was executed through the Circoletto software tool (http://tools.bat.infspire.org/circoletto/) (Chen et al., 2020). The data associated with the chromosomal location were obtained through the GFF (general feature format) file of the potato genome (Potato Genome Sequencing Consortium (PGSC) Ref seq v4.03) (Potato Genome Sequencing Consortium, 2011). MEGA 7 software was used to align coding sequences for the duplicated genes. The Ka/Ks value of *StDUF599* duplicated genes was estimated employing the KaKs calculator 2.0 tool (https://sourceforge.net/projects/kakscalculator2/). Additionally, divergence time (t) = Ks/2r was calculated with r = $2.6x10^{-9}$ (Li et al., 2019).

2.3. cis-regulatory elements and miRNA prediction analysis

For the investigation of the *cis*-elements in the *StDUF599* gene promoters, the 2 Kb upstream regions from the start codon were extracted. Moreover, the PlantCARE database site (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to study the *cis*-factors of every promoter and figured out through TBtools (Bailey et al., 2009; Lescot, 2002). The psRNATarget database (http://plantgrn.noble.org/psRNATarget/) (Dai et al., 2018) with default parameters was utilized to predict miRNAs. The figure of the interaction network between the *StDUF599* genes and miRNAs was created through the Cytoscape tool (v3.8.2; https://cytoscape.org/download.html).

2.4. Expression profiling of StDUF599 genes

The *StDUF599* gene expression of different tissues was obtained from the publicly accessible source with BioProject ID: PRJEB2430. The transcriptome data of *StDUF599* genes under abiotic (heat and

salt) and hormones (abscisic acid, Gibberellic acid, and indole-3-acetic acid) stress were accessed from NCBI with accession number SRA029323. The expression of tissues (stem, flower, stolon, shoot apex, leaf, petiole, young tuber, tuber cortex, stamen, root, tuber pith, tuber peel, tuber cortex, tuber sprout, mature tuber, and whole *in-vitro* plant) and abiotic (heat and salt) stress treatments are depicted with details in Supplementary Table S5. Abiotic stress conditions were carried out by 24 h treatment of plants with heat (35 °C) and salt (50 mM NaCl). The hormone stress response of plants was induced by treatment through ABA (abscisic acid, 50 μM), IAA (indole-3-acetic acid, 10 μM), and GA3 (gibberellic acid, 50 μM) for 24 h. The abundance of transcripts was computed through FPKM (Fragment per kilobase million).

3. Results

3.1. Identification and characterization of StDUF599 genes

BLASTP and HMM methods were utilized in the present study to identify *DUF599* genes in the *S. tuberosum* genome. Finally, nine *DUF599* genes with Pfam ID (PF04654) were identified after using two methods. These genes were named "*StDUF599-1* to *StDUF599-9*", and Table 1 shows details of all nine *StDUF599* genes. The length of the amino acid (AA), MW (molecular weight), pI (isoelectric point), subcellular localization, and exon–intron numbers were investigated in this analysis. The amino acid numbers for StDUF599 proteins varied from 124 (*StDUF599-7*) to 252 (*StDUF599-5*); isoelectric point values were in the range of 5.62 (*StDUF599-8*) to 9.54 (*StDUF599-6*); while their molecular weights varied from 14.61 (*StDUF599-7*) to 28.50 kDa (*StDUF599-5*) (Table 1). The number of exons varied from one (*StDUF599-4,* and *StDUF599-7*) to two (*StDUF599-1, StDUF599-2, StDUF599-3, StDUF599-5, StDUF599-6, StDUF599-8,* and *StDUF599-9*) and number of introns were from zero (*StDUF599-4* and *StDUF599-7*) to one (*StDUF599-1, StDUF599-2, StDUF599-3, StDUF599-5, StDUF599-6, StDUF599-8,* and *StDUF599-9*). Furthermore, only two genes lack introns, and seven others have one intron. The subcellular localization results showed that all StDUF599 proteins were present on the cell membrane (Table 1). Meanwhile, nine genes in *A. thaliana* (*AtDUF599-1 to AtDUF599-9*) and six in *Z. mays* (*ZmDUF599-1* to *ZmDUF599-6*) were also identified in this study (Supplementary Table. S1).

3.2. Phylogenetic relationship of DUF599 genes

The evolutionary relationships among *ZmDUF599, AtDUF599,* and *StDUF599* genes were observed by constructing a phylogenetic tree. The multiple sequence alignment was done by DUF599 protein sequences from A. thaliana, *Z. mays,* and *S. tuberosum* (Fig. 1). In the present study results, *DUF599* genes of 3 plants (*Z. mays, A. thaliana,* and *S. tuberosum)* were grouped into three major clades I, II, and III (Fig. 1). The *DUF599* genes from three species were spotted in all three clades indicating that *DUF599* gene members were distinguished prior to the partition of their ancestral plant species. The results showed that group I comprised 4 *DUF599* family members (1 *AtDUF599,* 1 *ZmDUF599*, and *2 StDUF599*). Group II comprised 10 *DUF599* members (3 *AtDUF599*, 3 *ZmDUF599,* and 4 *StDUF599*). Group III contains 10 *DUF599* members (5 *AtDUF599*, 2 *ZmDUF599*, and 3 *StDUF599*) (Fig. 1; Supplementary Table S1). The *DUF599* genes clustered in the identical group might continue to perform identical functions. In the present study results, *StDUF599* distributed in groups II and III possessed a higher number of *StDUF599*. The findings revealed that groups II and III have ten *DUF599* members each.

Table 1
Physicochemical properties of *StDUF599* genes.

Gene Name	Transcript Name	Chromosome Name	Renamed	Strand	Gene Start (bp)	Gene End (bp)	Protein (aa)	MW	pI	Subcellular localization
PGSC0003DMG400031770	PGSC0003DMT400081285	ST4.03ch02	*StDUF599-1*	1	33132572	33133877	240	27770.48	8.48	Plasma Membrane
PGSC0003DMG400000549	PGSC0003DMT400001484	ST4.03ch03	*StDUF599-2*	−1	46654461	46655258	237	26931.61	9.08	Plasma Membrane
PGSC0003DMG400000550	PGSC0003DMT400001485	ST4.03ch03	*StDUF599-3*	−1	46651719	46652492	233	26353.03	8.6	Plasma Membrane
PGSC0003DMG400012818	PGSC0003DMT400033369	ST4.03ch03	*StDUF599-4*	−1	1726382	1726777	131	15127.81	9.18	Plasma Membrane
PGSC0003DMG400014849	PGSC0003DMT400038472	ST4.03ch08	*StDUF599-5*	−1	2573534	2578039	252	28507.56	8.53	Plasma Membrane
PGSC0003DMG400031231	PGSC0003DMT400080235	ST4.03ch10	*StDUF599-6*	−1	48797620	48799064	226	25131.57	9.54	Plasma Membrane
PGSC0003DMG400034106	PGSC0003DMT400084511	ST4.03ch12	*StDUF599-7*	−1	39706885	39707259	124	14611.25	9.02	Plasma Membrane
PGSC0003DMG400029374	PGSC0003DMT400075537	ST4.03ch12	*StDUF599-8*	1	58025470	58028813	188	20888.59	5.62	Plasma Membrane
PGSC0003DMG400015823	PGSC0003DMT400040908	ST4.03ch12	*StDUF599-9*	1	40211451	40213089	234	26819.43	9.01	Plasma Membrane

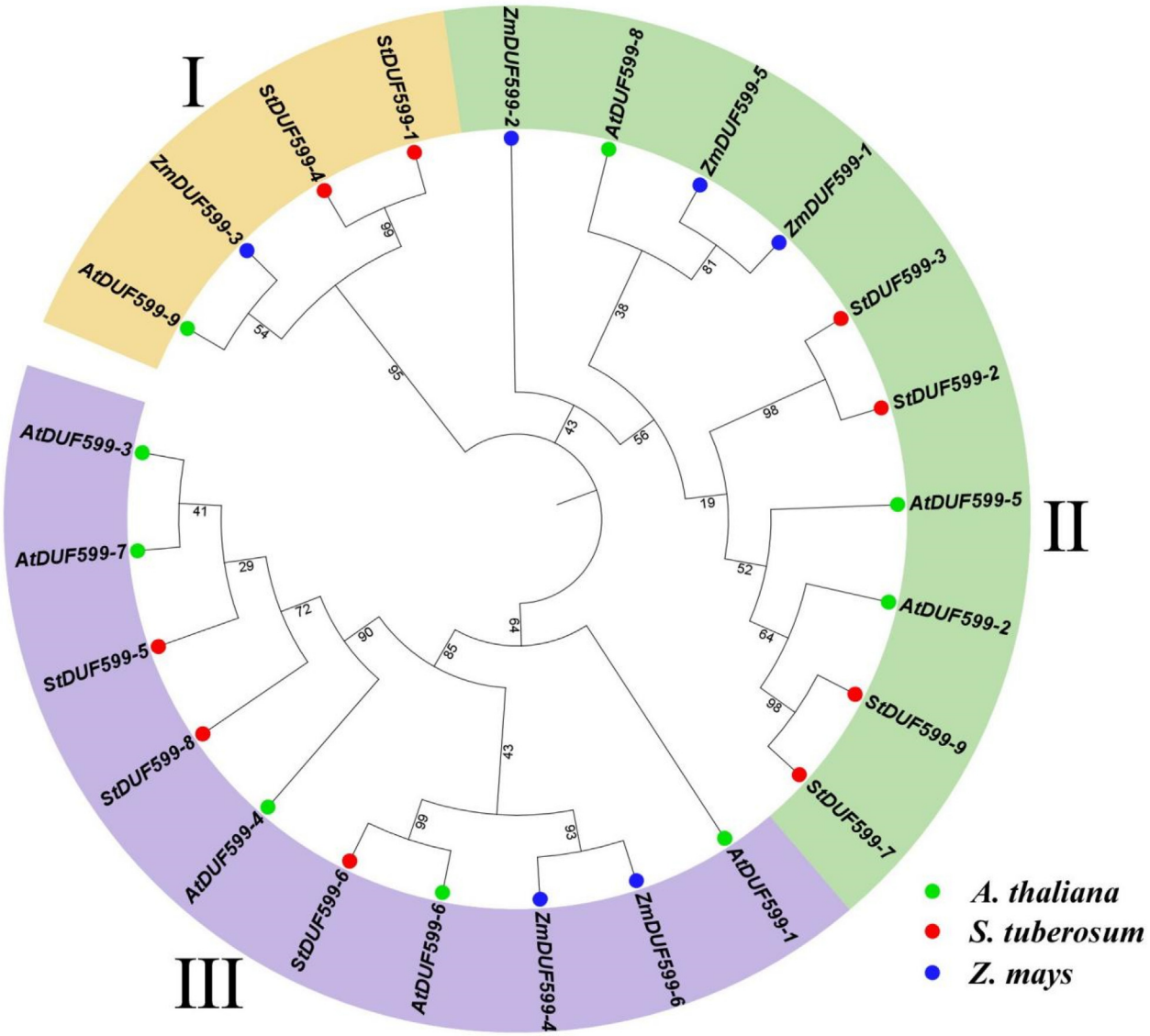

Fig. 1. A neighbor-joining phylogenetic tree assessment of DUF599 genes from A. thaliana, Zea mays, and *S. tuberosum*. Overall, 9 *At*DUF599 from A. thaliana (green circles), 6 *Zm*DUF599 from Z. mays (blue circles), and 9 *St*DUF599 from *S. tuberosum* (red circles) were clustered into three major classes, denoted by exclusive colors.

3.3. cis-acting elements analysis and chromosomal locations

The *cis*-acting elements control gene expression and functions. We investigated *cis*-acting elements in *StDUF599* gene promoters to learn about their regulatory and functional activities. Supplementary Table S2 contains all the information on *StDUF599* promoter *cis*-regulatory elements. We found that the TCA- element, CGTCA-motif, ABRE, TGACG-motifs, P-box, TATC-box, and GARE-motif are the most enriched elements of the four plant hormones, including salicylic acid, methyl jasmonate, gibberellins and abscisic acid (Supplementary Table S2). Furthermore, several phytohormone-correlated components were widely distributed, emphasizing the significance of these genes in phytohormone-arbitrating processes. The MBS, LTR, and ARE were identified as stress-responsive factors (low temperature, drought, and anaerobic induction), suggesting their involvement in stress stimulation. The MBS (drought stress-responsive element) was found in three *StDUF599* genes (*StDUF599-1, StDUF599-7,* and *StDUF599-9*). Further, three *StDUF599* genes (*StDUF599-1, StDUF599-4,* and *StDUF599-9*) contained low-temperature responsive element (LTR) (Supplementary Table S2). Total eight *StDUF599* genes (*StDUF599-1*, *StDUF599-2*, *StDUF599-3*, *StDUF599-4*, *StDUF599-5*, *StDUF599-6*, *StDUF599-7*, and *StDUF599-9*) had anaerobic responsive elements (ARE) (Fig. 2A; Supplementary Table S2). According to these findings, the expression pattern of *StDUF599* genes may differ under phytohormones or abiotic stress. The chromosomal location of *StDUF599* genes depicted that all chromosomes have an unequal number of genes. Moreover, a maximum of three genes were present on the chr12 and chr3, while a single gene was present on the chr2, chr8, and chr10 each (Fig. 2B).

3.4. Gene duplications and motif analysis of DUF599 genes

This study showed that both segmental and tandem duplication events were present in *StDUF599* genes (Table 2). The gene pairs *StDUF599-1*/*StDUF599-4* and *StDUF599-5*/*StDUF599-8* were segmentally duplicated, while the *StDUF599-7*/*StDUF599-9* and *StDUF599-2*/*StDUF599-3* have tandem duplication (Table. 2). Thus, the results illustrated that the segmental duplication events and

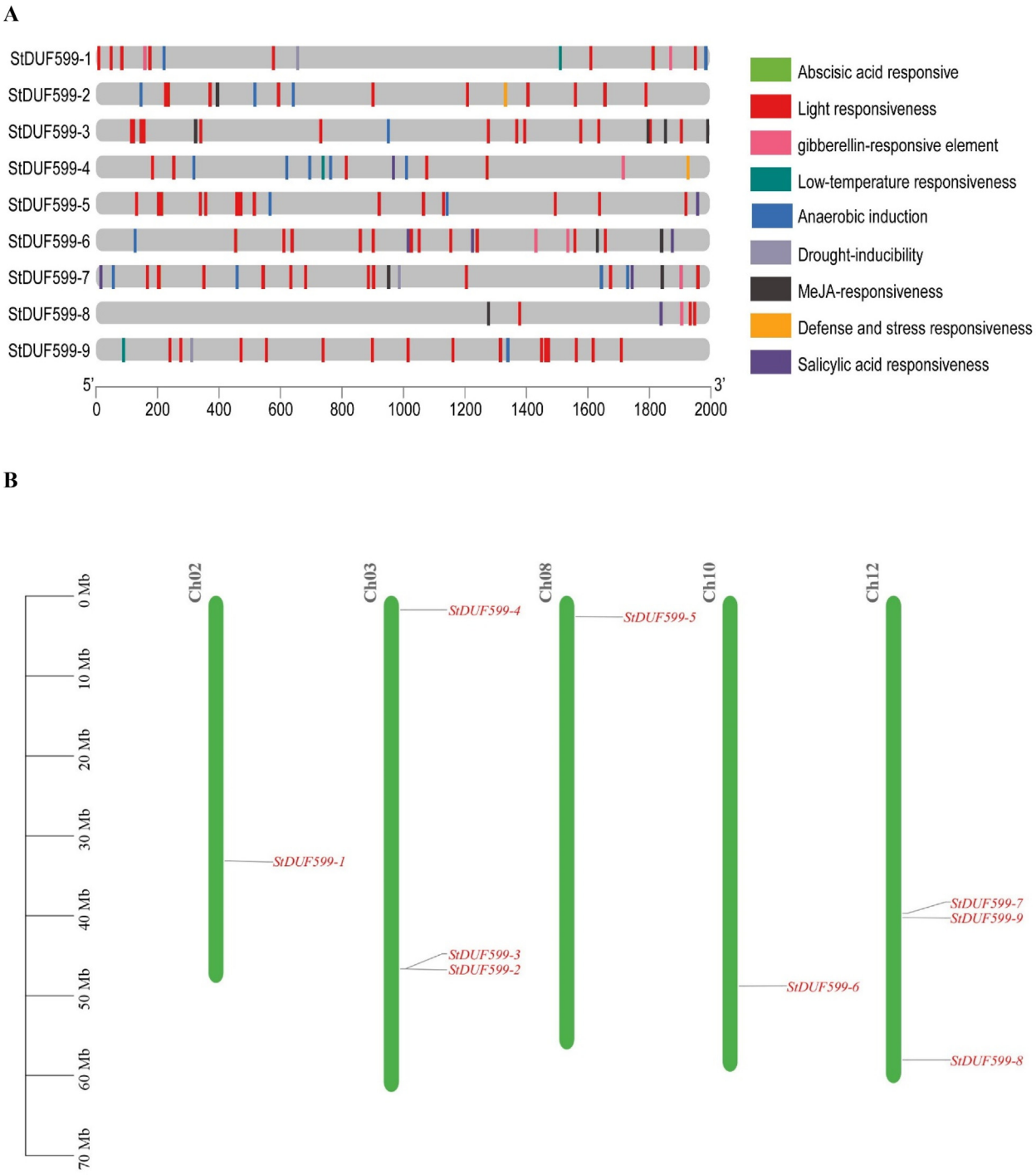

Fig. 2. (A) cis-elements in the promoter regions of the *StDUF599* genes are linked with different hormone- and stress-responsive elements. Different color boxes show different identified elements. See Supplementary Table S2 for more information. (B) Chromosomal distribution of *StDUF599* genes. The green color showed Chromosomes; red color genes.

Table 2
Ka/Ks calculations for *StDUF599* genes.

Seq_1	Seq_2	Ka	Ks	Ka_Ks	Time (MYA)	Duplication Type
StDUF599-5	*StDUF599-8*	0.167201	0.811113	0.206138	155.9833	Segmental
StDUF599-1	*StDUF599-4*	0.154774	1.093722	0.141511	210.3312	Segmental
StDUF599-2	*StDUF599-3*	0.070768	0.545704	0.129682	104.9432	Tandem
StDUF599-7	*StDUF599-9*	0.024876	0.01173	2.120672	2.255855	Tandem

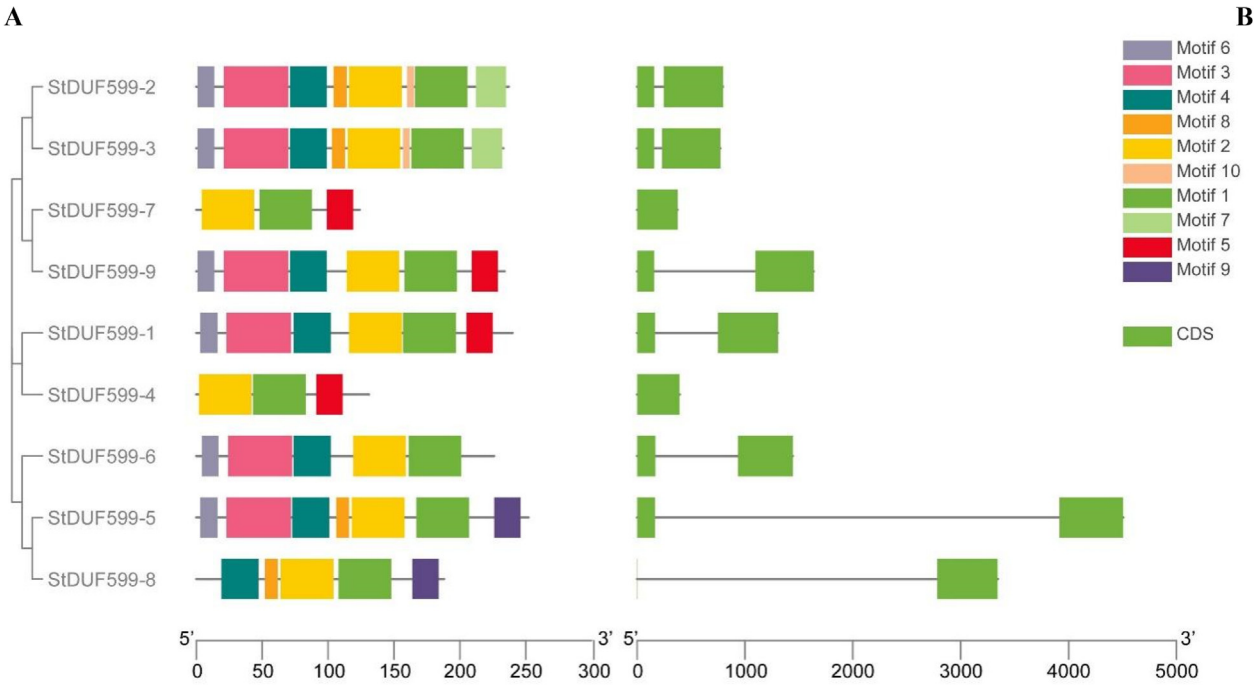

Fig. 3. The gene structure and motif analysis of *St*DUF599 genes. Based on phylogenetic relationships, the *St*DUF599 were clustered into three major classes. (A) Conserved motif compositions were detected in *St*DUF599. Different color boxes represent different motifs. (B) Gene structure of *St*DUF599 genes. The light green color denotes exon and the black horizontal line symbolizes introns.

tandem duplication participated in the *DUF599* gene family evolution in potato.

The Ka/Ks value of each duplicated gene pair has been calculated to estimate the rate of molecular evolution. A positive selection effect will be considered if Ka/Ks is greater than 1. If Ka/Ks value is lower than 1, then the purifying selection exists, and if the Ka/Ks value is equal to 1, then neutral selection lies among the duplicated genes. Moreover, our findings demonstrated that most of the duplicated *StDUF599* genes usually underwent purifying selection. Moreover, the divergence time (DT) between duplicated gene pairs has also been computed in the present study. When the *DUF599* genes exhibited the value of Ks is greater than 0.52, the DT (divergence time) might exceed 100 MYA (million years ago). In our results, the value of Ks for duplicated genes pair *StDUF599-1/StDUF599-4* was 1.09, while the expected divergence time was 210.33 MYA (Table 2). The MEME server identified ten conserved motifs in the StDUF599 proteins (Fig. 3A). All *StDUF599* genes restrained motif 1 and motif 2. All genes have motif 3 except *StDUF599-4* and *StDUF599-7*. Motifs 1 and 4 have 50 and 49 amino acid residues, respectively; motif 3 contained 47, while 41 amino acids were present in motif 2 (Supplementary Table S3).

3.5. Gene structure organization, miRNA, and synteny analysis

The gene structure analysis revealed that *StDUF599-1*, *StDUF599-2*, *StDUF599-3*, *StDUF599-5*, *StDUF599-6*, *StDUF599-8*, and *StDUF599-9* have two exons and one intron, while *StDUF599-*

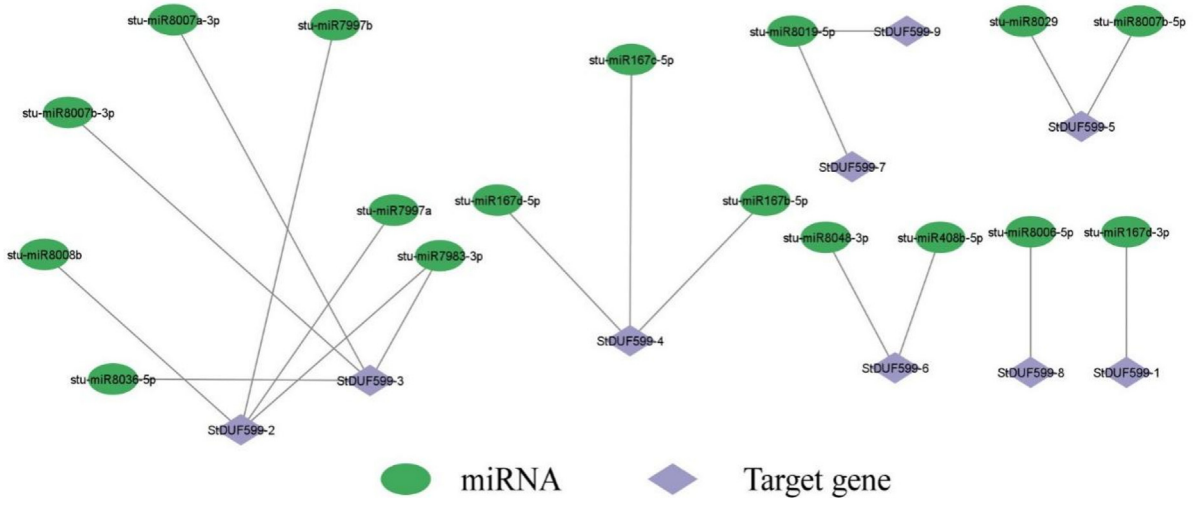

Fig. 4. miRNA targeting *St*DUF599 genes. (A) Network figure of anticipated miRNA targeting *St*DUF599 genes. Green colors correspond to miRNAs, and purple color represents *St*DUF599 genes. See Supplementary Table S4 for the detailed data of all predicted miRNAs.

4 and *StDUF599-7* genes contained a single exon (Fig. 3B). Previous studies reported that micro-RNAs are involved in various regulatory mechanisms that carry out plant responses against stresses. The comprehensive details of all genes and target sites for miRNAs are shown in Supplementary Table S4. Our findings illustrated that five stu-miR167 members target two *StDUF599-4* and *StDUF599-1* genes (Fig. 4; Supplementary Table S4). Moreover, only one gene, *StDUF599-3,* was targeted by stu-miR8036 (Fig. 4; Supplementary Table S4). Two genes *StDUF599-5* and *StDUF599-3* were the target of three mi-RNAs members (stu-miR8007b-3p, stu-miR8007a-3p, and stu-miR8007b-5p) and one gene *StDUF599-2* was targeted by stu-miR8008. Additionally, two genes, *StDUF599-3* and *StDUF599-2,* were the target of stu-miR7983. The two members of the stu-miR7997 targeted the *StDUF599-2* gene, while the stu-miR8019 targeted two other genes, including *StDUE599-7* and *StDUF599-9* (Fig. 4; Supplementary Table S4). Furthermore, two genes, *StDUF599-2, StDUF599-3,* and *StDUF599-4,* were predicted to be targeted by numerous micro-RNAs (Fig. 4; Supplementary Table S4). Synteny analysis among *A. thaliana, Z. mays, and S. tuberosum* delineated a unique link regarding the expression of genes, their evolution, functions, duplications, and triplications. It was noted that the sequence of the *ZmDUF599-5* exhibited synteny with the sequence of the *StDUF599-2* gene of *S. tuberosum.* Furthermore, syntenic relations were present between potato gene *StDUF599-5* and *A. thaliana* gene *AtDUF599-1.* Gene *StDUF599-9* of potato depicted synteny with *AtDUF599-2* of *A. thaliana* (Fig. 5). In comparative synteny analysis, intensity and color of outward and inward ribbons tangling illustrated duplication and conservation events, respectively.

3.6. Tissue-specific expression analysis of StDUF599 genes

The tissue-specific expression of *StDUF599* genes was observed in the shoot apex, stem, leaf, flower, stolons, petiole, young tuber, tuber cortex, root, mature tuber, tuber sprout, stamen, tuber pith, tuber peel, whole *in-vitro* plant using RNA-seq data of *S. tuberosum.* In general, these results represented that some genes illustrated expression in one tissue (*StDUF599-3)* and others genes (*StDUF599-6* and *StDUF599-9)* demonstrated expression in all tissues such as young tuber, mature tuber, root, shoot apex, stolon, flower, stem, leaf, root, tuber sprout, tuber cortex, tuber peel, tuber pith, petiole, and the entire *in-vitro* plant. *StDUF599-6, StDUF599-7, StDUF599-8,* and *StDUF599-9* have higher expression in the stolon. In young tuber, the *StDUF599-6*, *StDUF599-8*, and *StDUF599-9* genes display higher expression (Fig. 6, Supplementary Table. S5). Moreover, the expression of the *StDUF599-1* gene was significantly

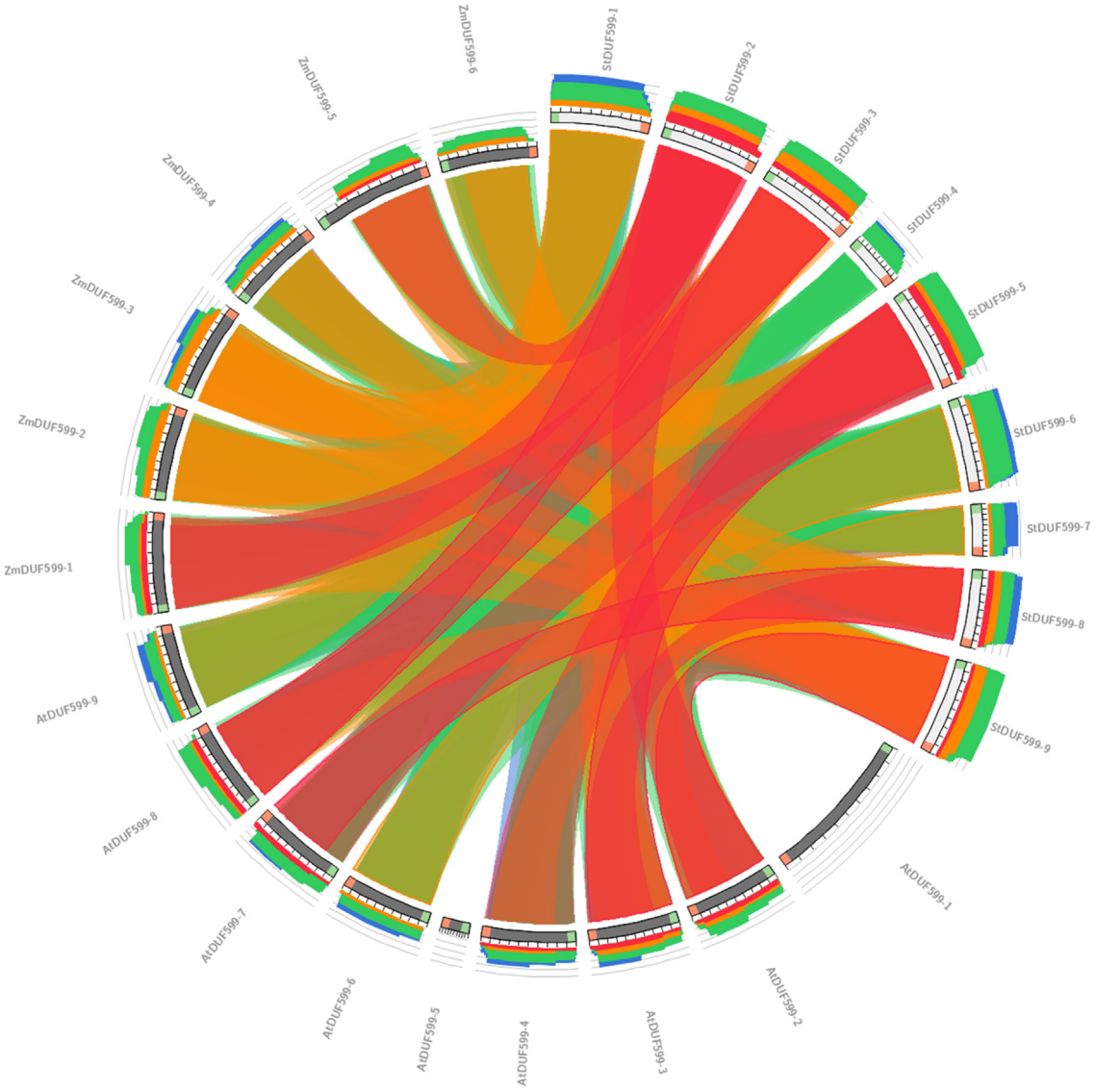

Fig. 5. Synteny map among all identified *DUF599* sequences of Arabidopsis, Zea mays, and *S. tuberosum.*

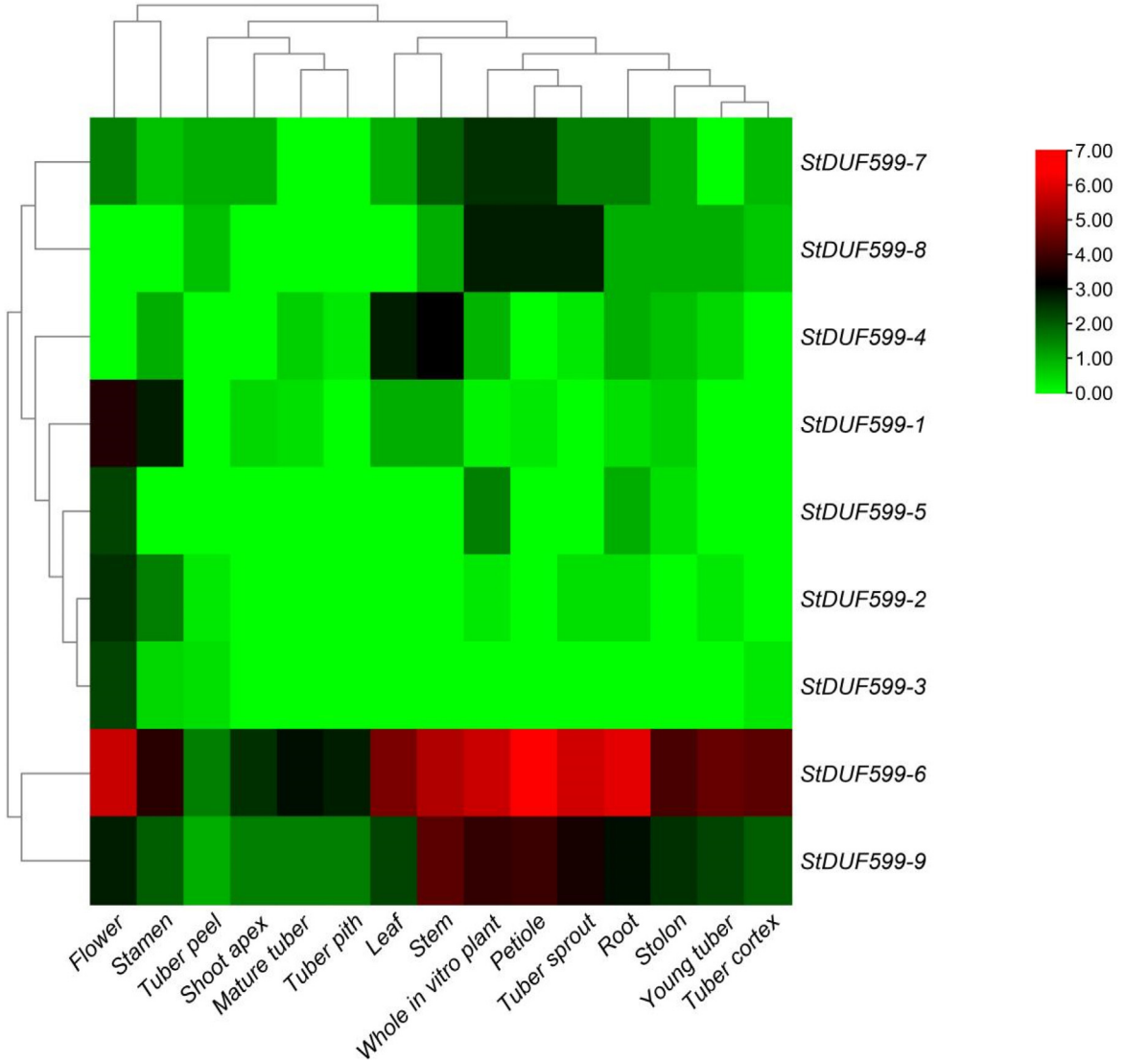

Fig. 6. Expression profiling of *St*DUF599 genes in various developmental tissues. The red, black, and green colors display high to low expression. levels.

expressed inside the flower, leaf, stem, and stamen, while the *StDUF599-2* gene had high expression in the stamen and flower. In the present study results, the *StDUF599-3* gene has only higher expression in flowers, while *StDUF599-4* has higher expression in leaf, stem, root, and stamen (Fig. 6, Supplementary Table S5). Gene *StDUF599-5* display higher expression in flower, root, and whole *in vitro* plants. Likewise, the *StDUF599-7* gene showed higher expression in stolon, shoot apex, stem, leaf, flower, petiole, root, tuber peel, tuber sprout, and whole *in vitro* plant. Furthermore, the *StDUF599-8* gene showed higher expression in stolon, young tuber, petiole, stem, root, tuber sprout, and whole *in vitro* plant (Fig. 6, Supplementary Table S5). Moreover, our findings revealed that only a few genes expressed in all observed tissues might significantly participate in the growth process.

3.7. StDUF599 genes expression patterns under heat, salt, and phytohormones stress

RNA-seq-based *StDUF599* gene expression was observed under abiotic (heat and salt) and phytohormones (ABA, GA, IAA) stresses. It revealed that *StDUF599* genes might carry out significant tasks in potatoes to regulate their response to salt and heat stress. Two *StDUF599* genes illustrated high expression against salt stress. After the salt stress, *StDUF599-7* and *StDUF599-9* showed expression compared to the control (Fig. 7, Supplementary Table S5). At the same time, four *StDUF599* genes were expressed against heat stress. When plants were treated to heat, *StDUF599-5*, *StDUF599-6*, *StDUF599-7*, and *StDUF599-9* genes were significantly enhanced, while more expression was observed in *StDUF599-5* compared to control (Fig. 7, Supplementary Table S5). In the current study, GA3, ABA, and IAA were designated to observe the expression pattern of *StDUF599* genes later than phytohormones treatment. Moreover, for the *StDUF599* gene's expression profiling against GA3, leaves were treated with GA3. In response to GA3 treatment, the *StDUF599-8* gene showed maximum expression levels compared to the control. Similarly, leave tissues were treated with IAA for the *StDUF599* gene's expression analysis. Overall, *StDUF599-8* was highly expressed when IAA was used. The leave tissues were treated through ABA to observe the expression responses against ABA. In response to ABA, no gene showed expression compared to control (Fig. 7, Supplementary Table S5). Furthermore, *StDUF599-1, StDUF599-2, StDUF599-3,* and *StDUF599-4* did not show expression against any stress, while *StDUF599-5* showed significant expression only to heat stress. However, *StDUF599-8* was identified to be more active during IAA and GA3 treatment.

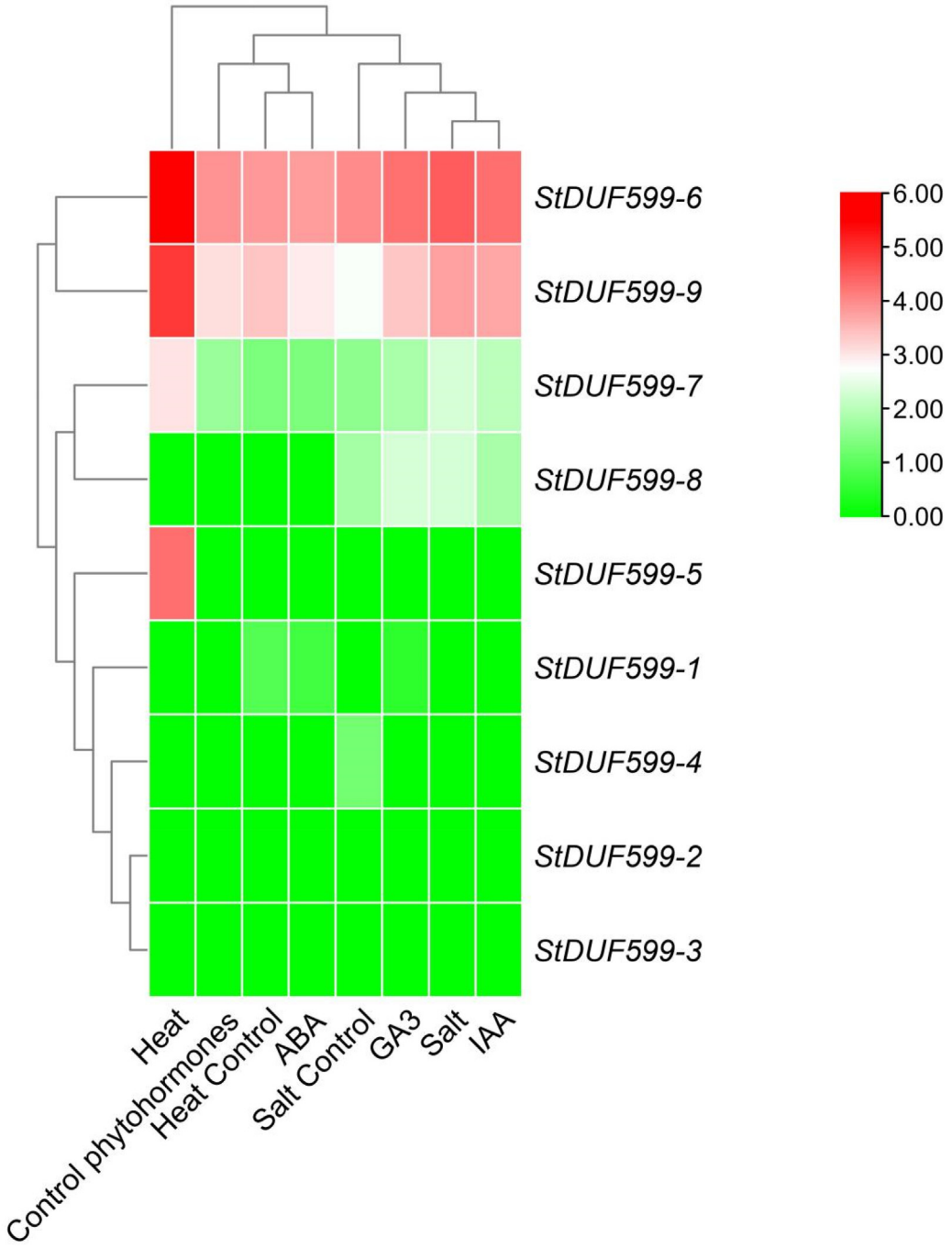

Fig. 7. Expression patterns of the StDUF599genes under different abiotic (heat and salt)stress conditions and phytohormones (ABA, GA3, and IAA). The red, black, and green colors display high to low expression levels.

4. Discussion

Potato is a diet source with various nutrients imperative for health and substantial food security worldwide (Zaynab et al., 2017b). Therefore, enhancing potato production will enable us to satisfy the nutritional needs of the world population. Potato exhibits sensitivity toward the various stress factors. Its ability to tolerate different abiotic and biotic stresses is imperative to sustain its productivity and fulfilling food demands in the coming time. Several gene families with the *DUF* domain have been discovered, and their genome resources are available inside the Pfam database (Bateman et al., 2010). While the origin, variability, and biological duties of these gene families (*DUF*) were extensively explored in several studies, there is very small information on the biological roles of these genes in potatoes. At present, the database of Pfam possesses 17,939 gene families, of which around 22 % (3,961) are DUFs (Chalupska et al., 2008). *DUF599* genes were identified in recent research, and their significant role in potatoes is against abiotic and phytohormones stress. cis-acting factors in *DUF599* gene promoter regions are also linked to abiotic stress conditions. The current research identified St*DUF599* genes promoter, including stress-associated cis-acting elements, such as LTR, TATC-box, P-

box, ABRE, and SARE. ABRE is associated with ABA and salt stress. CGTCA-motif, LTR, and ARE were affiliated with low-temperature stress factors (Maestrini et al., 2009), anaerobic induction response (Geffers et al., 2001), and response to methyl jasmonate (MeJA) (Xu et al., 2018). These findings indicate that *cis*-acting elements perform a significant role against stress by regulating stress-associated genes. Our results propose that St*DUF599* genes may be involved in response to abiotic stress factors. Moreover, genome size, gene duplication, and gene distribution are essential factors in plants' genetic diversity. Moreover, gene duplication is an important feature during the gene families' expansion, diversification, and neofunctionalization (Lavin et al., 2005). Here we also noticed some duplication events, which carry out the critical task of *StDUF599* evolution. Furthermore, *DUF599* gene distribution at the chromosomal level will provide a deep knowledge to form new varieties with desired traits. In recent decades, extensive research has been executed to recognize miRNAs in plant genomes (Buhtz et al., 2008). The current analysis identified different miRNAs from various families that target several *StDUF599* genes. It was earlier observed that miR167 carries out several critical tasks in response to stress conditions (Khraiwesh et al., 2012). Briefly, these researches strengthen our findings and endorse that miRNAs may perform crucial tasks against various stress factors by amending the transcription levels of *StDUF599* genes. Waseem et al. (2021) reported the link between ZmDUF and AtDUF proteins and stresses (Waseem et al. 2021). That's why expression profiling of *StDUF599* genes is helpful for the functional study of the *S. tuberosum*. In a recent analysis, nine *StDUF599* genes' tissue-specific expression was assessed in various tissues using publicly available RNA-seq data. This study demonstrated that *StDUF599* genes illustrated expression in different developmental tissues. Waseem et al. (2021) investigated *DUF* gene expression of different plant tissues, including leaves, roots, fruits, and flowers, at varying developmental stages of tomato (Waseem et al., 2021). Waseem et al. (2021) reported that *DUF221* genes illustrated high expression in specific tissues (Waseem et al., 2021). Moreover, these findings illustrated that these *DUF221* genes played a critical role in tissue development (Waseem et al., 2021). Yang Q; (2020) investigated the *ATDUF4228* genes expression in various tissues and presented significant variability. According to findings, some *ATDUF4228* genes showed high expression in most tissues while others showed lower expression in some tissues. The highly expressed *ATDUF4228* genes in tissues have a significant role in plant growth (Yang et al., 2020). These consequences have the same results as our findings, where *DUF* genes exhibited significant expression at the tissue level, representing that these expressed genes may be involved in potato development and growth. Consequently, more research on *DUF* genes established their notable roles against different abiotic stress (Leng et al., 2021). Two genes (*StDUF599-9* and *StDUF599-7)* are highly expressed when potato plants treated with salt and heat may have a significant role against abiotic stress. Several hormones participating in signal transduction may alter physiological and biochemical mechanisms (Zaynab et al., 2017a). Hormones like IAA and GA3 are imperative for the immunity of plants. Several studies reported that *DUF* genes regulate stress response and carry out various developmental and hormonal signalling processes. Furthermore, to observe the *DUF599* genes expression in response to hormonal signalling, the leaves of *S. tuberosum* were treated with IAA and GA3. After the hormone treatments, the expression of the *StDUF599-8* gene suggests that *DUFs* have an essential function in the hormone-induced immune response. According to previous studies, after the treatments of GA3 and IAA, *DUF* genes upregulation revealed that GA3 and IAA performed a vital function in the immune system (Zaynab et al., 2022). In addition, Yang et al. (2020) revealed that the *DUF* genes were significantly expressed during the hormonal treatment, revealing that hormones have a significant role in immunity (Yang et al., 2020).

5. Conclusion

In the current study, nine *DUF599* genes were recognized inside the *S. tuberosum* (potato) genome after using BLASTP and HMM techniques. The structure of genes, *cis*-regulatory factors, phylogenetic analysis, and conserved motifs analysis of *DUF599* genes were executed to get insights into *DUF599* genes in potato. Moreover, the *StDUF599* genes expression profiling were analyzed in various tissues to observe their significant role in development and growth. The expression analysis of *StDUF599* genes during heat, salt, and hormone treatments revealed their significant role against abiotic stress. Furthermore, our study will provide a base for further functional studies of the *DUF599* genes under unfavourable or stressful conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors have direct intellectual contribution to the manuscript. The authors are in complete agreement to make submission with journal of King Saud University Science.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2022.102368.

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