



Article

Identification and Expression Analysis of the BTB/POZ Gene Family in *Solanum tuberosum*

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Abstract: The BTB/POZ (broad-complex, tramtrack, and bric-a-brac) family of proteins is widespread in plants and animals and plays important roles in growth, development, metabolism, and environmental responses. There are few reports on *BTB* family genes in potato. In this study, 34 sequences containing conserved BTB domains were obtained from the potato gene database, and the phylogenetic, physical, and chemical properties, gene structure, conserved motif, domain, and chromosomal localization of the potato BTB protein family were analyzed via bioinformatics methods. In addition, we used qRT-PCR to detect 12 selected *StBTB* genes. The results confirmed that these genes are involved in cold, ABA, salt, hydrogen peroxide (H₂O₂), drought, and low-nitrogen stress, which is highly important for elucidating BTB family members and studying stress response and tolerance mechanisms. This study provides a theoretical basis for the study of the function and expression of potato BTB and lays a solid foundation for further understanding the molecular mechanism of the potato *BTB* gene under various environmental stresses.

Keywords: *Solanum tuberosum*; BTB protein family; bioinformatic analysis; expression analysis; abiotic stress



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1. Introduction

There are many transcription factor families in plant genomes that generally activate or inhibit the expression of target genes by binding to their promoters and subsequently regulate plant growth and development. The BTB (broad-complex, tramtrack, and brick-a-brac) domain was originally thought to be a conserved motif found in *Drosophila melanogaster*; it is an evolutionarily conserved protein–protein interaction motif consisting of approximately 120 amino acid residues and has a wide range of functions in development and homeostasis, as well as in a wide range of interactions [1,2]. Members of the BTB family are also a class of transcription factors. This domain is composed of five α -helices and three β -sheets, and each family member of the BTB protein family has one or more BTB structures [3]. The BTB protein family contains not only the BTB protein domain but also several other protein domains. These proteins include TRAF homology (MATH), ankyrin repeats (ANK), armadillo (ARM), zinc finger protein (TAZ), nonphototropic hypocotyl 3 (NPH3), BTB and C-terminus Kelch (BACK), tetratricopeptide repeat (TPR), proteins containing only the BTB domain, and other proteins, thus forming different subfamilies of BTB proteins and playing different roles [4–7].

The BTB protein has not only been extensively studied in animals but also plays an important role in many fields such as plant growth and development, stress resistance, protein ubiquitination and degradation, cell structure, ion channel, and cell cycle regulation [8,9]. Members of the BT subfamily negatively regulate nitrate absorption genes and NUE in plants, providing a basis for the improvement of NUE. This subfamily also responds to nutrition, stress, and hormones, and BTs' proteins can interact with the E3 ligase CULLIN3. The nonexpressor of pathogenesis-related (NPR) subfamily is also a disease resistance

protein that plays an important role in regulating broad-spectrum resistance and is a key gene for disease resistance [10–14]. In plants, many members of the BTB protein family are involved in mediating plant-related signal regulation, such as salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA), and gibberellin (GA3) [15].

Potato (*Solanum tuberosum*) is an annual herb of the Solanaceae genus *Solanum* and is the most important tuber crop and the fourth largest crop in the world [16,17]. In recent years, members of the BTB family have been identified in many plant species such as *Arabidopsis thaliana* [18], rice [19], and tomato [20]. However, there are few studies on the potato BTB family. In this paper, the BTB family of potato was excavated, and the bioinformatics analysis of the potato genome was carried out. On this basis, qRT-PCR verification was performed. The results showed that the BTB members in potato have high homology with the BTB members in *A. thaliana* and may have similar functions under different stresses. However, under different abiotic stresses, the gene expression is also different, indicating that the *StBTB* gene plays an important role in response to abiotic stresses. In summary, the results of this study preliminarily explored the function of potato *StBTB* gene, which laid a foundation for further study of its role and molecular mechanism in potato, and provided new ideas for the screening and breeding of highly tolerant potato plants.

2. Materials and Methods

2.1. Test Materials

The potato tissue culture-generated plants (Chuanyu No. 10 varieties) used in this study were preserved and provided by the Potato Research Center of Sichuan Agricultural University. High-quality potatoes were selected for tissue culture and stress treatment on ordinary MS medium. The potato culture condition was 28 °C, and the photoperiod was 16/8 h. Three potato seedlings at 8–9 leaf stage were selected for each treatment.

2.2. Search and Identification of Potato BTB Family Member Genes

The reference genome data of potato were derived from the Potato Genomics Resource (<http://spuddb.uga.edu/>, accessed on 15 December 2023), and the BTB/POZ (PF00651) from the Pfam database (<https://pfam.xfam.org/>, accessed on 15 December 2023) was used as the HMM file. The whole potato protein database was retrieved using Hmmer3.0 software. Then, incomplete and erroneous sequences were removed through the National Center for Biotechnology Information (NCBI) online website Conserved Domains Database.

2.3. Analysis of Basic Physical and Chemical Properties of Potato BTB Family Proteins

The online software EXPASy ProtParam 3.0 (<https://web.ExPASy.org/protparam/>, accessed on 15 December 2023) was used to analyze the basic physical and chemical properties of potato BTB gene family members, and the subcellular localization of the gene was predicted with ProtComp 9.0 (<http://linux1.softberry.com>, accessed on 15 December 2023).

2.4. Homology Analysis of BTB Family Proteins

MEGA7.0 software [21] was used to analyze 34 *StBTB* genes, and a phylogenetic tree was successfully constructed via the neighbor-joining method with 1000 replicates. Bootstrap value and position in the phylogenetic tree was used to assign 34 *StBTB* genes to appropriate clusters. Next, the constructed phylogenetic tree was beautified using the ChiPlot (<https://www.chiplot.online/>, accessed on 15 December 2023) [22].

2.5. Analysis of BTB Motifs, Gene Structures, and Domain in Potato

The structural information of the required *StBTB* gene was screened by TBtools (v1.120) software [23], and the motif of the gene was predicted using the MEME [24] (<http://meme.sdsc.edu/meme/memeintro.html>, accessed on 15 December 2023) online website. Finally, TBtools (v1.120) was used to visualize the genes. The parameter settings were as follows: motif maximum, 10; motif length range, 6~100. The conserved domains of potato BTB

family were analyzed and visualized by NCBI conserved domain search, InterproScan and Pfam domain prediction tools.

2.6. Analysis of Chromosomal Location, Gene Duplication

The chromosome location information of each *StBTB* gene was obtained from the Spud DB database (<http://spuddb.uga.edu/>, accessed on 15 December 2023), and the gene location was visualized by TBtools (v1.120). At the same time, the collinear relationship of BTB family in potato was visualized.

2.7. Collinearity Analysis of the BTB Family

TBtools (v1.120) software was used for data preparation, and MCScanX was used to visualize the collinearity between potato and *A. thaliana*.

2.8. Gene Expression Analysis of Potato BTB Family Members

The potato transcriptome data were downloaded from the Solanaceae Genomics Resource database [25], the FPKM values of the corresponding transcripts of the BTB family members were retrieved in turn, and the clustering heatmap was drawn using TBtools (v1.120) software.

2.9. Expression Verification of Screened Genes

Through the expression of potato BTB family members under different abiotic stresses via a cluster heatmap, 12 genes whose expression increased in response to stress were identified. A control group and six groups receiving stress treatment were established. The control group had no stress, and the treatment groups included low temperature (4 °C), ABA (1 µmol/L), drought (5% PEG-6000), high salt (200 mmol/L), low nitrogen (0.75 mmol/L), and H₂O₂ (10 mmol/L). After 0 h and 6 h of treatment, the plants were quickly frozen in liquid nitrogen and stored at −80 °C until further use. Total RNA was extracted using Trizol[®] reagent (Invitrogen, Carlsbad, CA, USA) according to the RNAiso plus (TaKaRa, Osaka, Japan, Code No. 9108/9109) method, and the total RNA was reverse transcribed into cDNA using PrimeScript[™] 1st Strand cDNA Synthesis Kit (TaKaRa, Osaka, Japan, Code No. 6110A). SYBR[®] Premix Ex Taq II (Dalian, China, Code No. RR820A/B) was used for real-time fluorescence quantitative PCR. The fluorescence quantitative 10 microbiome system consisted of 5 µL fluorescence quantitative enzyme, 0.5 µL upper primer, 0.5 µL lower primer, 2.5 µL ddH₂O, and 1.5 µL cDNA, and the program was set according to the instructions; three technical repetitions were used. Then, we used the $2^{-\Delta\Delta CT}$ method to calculate the relative expression of the genes [26]. The sequences of primers used in the experiment are shown in Supplementary Table S1.

3. Results

3.1. Physicochemical Properties and Subcellular Localization of Potato BTB Family

Finally, 34 *StBTB* genes of potato BTB protein family were screened out, numbered *StBPM1*~*StPHL2*, and their physical and chemical properties (isoelectric point, relative analysis quality, hydrophobicity, etc.) were analyzed for prediction. The number of amino acids (aa) encoded by 34 proteins ranged from 201 to 947, of which *StEOL1* contained the greatest number of amino acids (947) and *StBBK1* contained the least (only 201). The molecular weights ranged from 22.7 (*StBBK1*) to 107.6 (*StEOL1*) kDa. Except for a few proteins with molecular weights that were too large or too small, most of the protein molecular weights ranged from 50 to 80 kDa. The isoelectric points of 34 proteins ranged from 4.84 (*StBBK2*) to 9.18 (*StBT1*). Except for *StBTB1/2/3*, *StNPL1/7/8/10*, and *StBTB1*, the isoelectric points of the other genes ranged between 4.84 and 7.17, indicating that most of the amino acids encoded by the potato BTB family were acidic amino acids. The hydrophilicity was between −0.473 (*StBT2*) and −0.027 (*StBTB2*), and all proteins were hydrophilic and unstable. The results of subcellular localization prediction showed that most of the potato BTB family proteins were located in the nucleus (14); the remaining 10

were located in the cytoplasm and nucleus, 7 in the cytoplasm and 3 in the cell membrane, cytoplasm, and nucleus (Table 1).

Table 1. Basic physical and chemical properties of the potato BTB family.

ID	Sequence ID	Number of Amino Acids	Molecular Weight	pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Localization Prediction
PGSC0003DMT400058456	StBPM1	405	44,974.76	6.07	40.55	81.33	−0.291	Nuc
PGSC0003DMT400011115	StBPM2	407	45,182.59	6.02	35.53	90.79	−0.111	Nuc
PGSC0003DMT400069942	StBPM3	408	45,178.55	5.86	42.19	86.03	−0.162	Nuc
PGSC0003DMT400009802	StBPM4	408	45,235.32	6.02	32.49	83.16	−0.181	Nuc
PGSC0003DMT400070468	StBPM5	408	44,966.77	5.66	35.64	82.48	−0.218	Nuc
PGSC0003DMT400039384	StBT1	345	39,521.12	9.18	61.43	84.75	−0.43	Cyt/Nuc
PGSC0003DMT400069392	StBT2	349	40,831.39	8.81	61.42	84.64	−0.473	Cyt/Nuc
PGSC0003DMT400064939	StBT3	403	45,248.57	8.89	48.9	91.14	−0.124	Cyt/Nuc
PGSC0003DMT400073584	StNPL1	554	63,575.64	8.98	40.07	96.19	−0.24	Nuc
PGSC0003DMT400066161	StNPL2	626	69,014.65	6.78	34.07	85.99	−0.303	Cel/Nuc
PGSC0003DMT400083737	StNPL3	659	74,285.54	7.02	44.17	86.15	−0.408	Nuc
PGSC0003DMT400008211	StNPL4	629	69,867.04	5.74	45.44	87.14	−0.284	Cel/Nuc
PGSC0003DMT400033866	StNPL5	601	67,427.69	6.18	41.26	95.21	−0.184	Cel/Nuc
PGSC0003DMT400031245	StNPL6	576	64,374.17	7.17	33.72	94.69	−0.137	Nuc
PGSC0003DMT400015699	StNPL7	687	78,154.78	8.39	55.12	90.52	−0.286	Cel/Cyt/Nuc
PGSC0003DMT400022573	StNPL8	615	69,021.61	8.92	42.21	92.07	−0.256	Cel/Cyt/Nuc
PGSC0003DMT400010359	StNPL9	578	64,675.52	5.86	42.78	96.31	−0.108	Nuc
PGSC0003DMT400013928	StNPL10	610	67,523.02	8.38	33.78	87.38	−0.291	Cel/Nuc
PGSC0003DMT400001154	StNPL11	631	70,448.94	6.79	44.55	91.17	−0.128	Cel/Cyt/Nuc
PGSC0003DMT400021076	StNPR1-1	575	64,579.71	5.66	44.6	92.42	−0.345	Cyt
PGSC0003DMT400054660	StNPR1-2	581	64,360.48	6.03	37.36	89.12	−0.213	Cyt
PGSC0003DMT400027591	StNPR1-3	461	50,646.89	6.03	50.55	94.32	−0.116	Cyt
PGSC0003DMT400076710	StNPR1-4	487	53,415.76	6.14	52.27	91.48	−0.199	Cyt
PGSC0003DMT400002412	StNPR1-5	490	54,288.74	6.28	54.16	90.35	−0.234	Cyt
PGSC0003DMT400051860	StARM1	709	78,098.49	5.79	45.24	104.01	−0.11	Cyt
PGSC0003DMT400033530	StARM2	708	78,339.6	5.86	47.84	104.31	−0.131	Nuc
PGSC0003DMT400071906	StBBK1	201	22,742.1	5.05	29.77	100	−0.138	Nuc
PGSC0003DMT400016452	StBBK2	577	65,471.59	4.84	50.44	89.64	−0.268	Cyt
PGSC0003DMT400057328	StBBK3	328	37,372.73	5.65	41.26	96.43	−0.179	Nuc
PGSC0003DMT400041081	StEOL1	947	107,692.49	5.68	44.71	80.7	−0.361	Nuc
PGSC0003DMT400055245	StBTB1	488	54,586.29	8.34	54.18	91.91	−0.3	Nuc
PGSC0003DMT400032077	StBTB2	459	52,632.78	5.94	42.77	95.19	−0.027	Cyt/Nuc
PGSC0003DMT400000261	StPHL1	552	62,965.09	5.18	55.2	74.09	−0.387	Cyt/Nuc
PGSC0003DMT400049926	StPHL2	558	63,419.06	5.39	50.71	76.99	−0.291	Cyt/Nuc

Note: Nuc: nucleus; Cyt: cytoplasm; Cel: cell membrane.

3.2. Phylogenetic Analysis of Potato BTB Protein

To elucidate the phylogenetic relationship of potato BTB proteins, multiple sequence alignment and phylogenetic tree analysis were performed on 34 potato BTB proteins (StBTBs) and 80 *A. thaliana* BTB proteins (AtBTBs) (Figure 1). The potato BTB proteins were mainly divided into nine subfamilies. The MATH group contained five *StBTB* members (*StBPM1*, *StBPM2*, *StBPM3*, *StBPM4*, and *StBPM5*). The TAZ group consisted of three *StBTB* members, *StBTB1/2/3*. Group NPH3 had more BTB members, namely, *StNPL1/2/3/4/5/6/7/8/9/10/11*. The ANK group was mainly composed of *StNPR1-1/2/3/4/5*. The ARM group contained *StARM1* and *StARM2*. Group BACK contained *StBBK1*, *StBBK2*, and *StBBK3*. The TPR group had only *StEOL1*. The BTB-ONLY group contained *StBTB1* and *StBTB2*. The OTHER group contained *StPHL1* and *StPHL2*. Within the subfamily, there was a significant difference in the BTB protein content between potato and *A. thaliana*. Among these different subfamilies, potato and *A. thaliana* have similar structural types. The NPH3 subfamily had the largest number of members among all the subfamilies, covering 11 potato BTB family genes and 30 *A. thaliana* BTB family genes. Among all TPR subfamilies, the number was the lowest, containing only one potato BTB family gene and three *A. thaliana* BTB family genes.

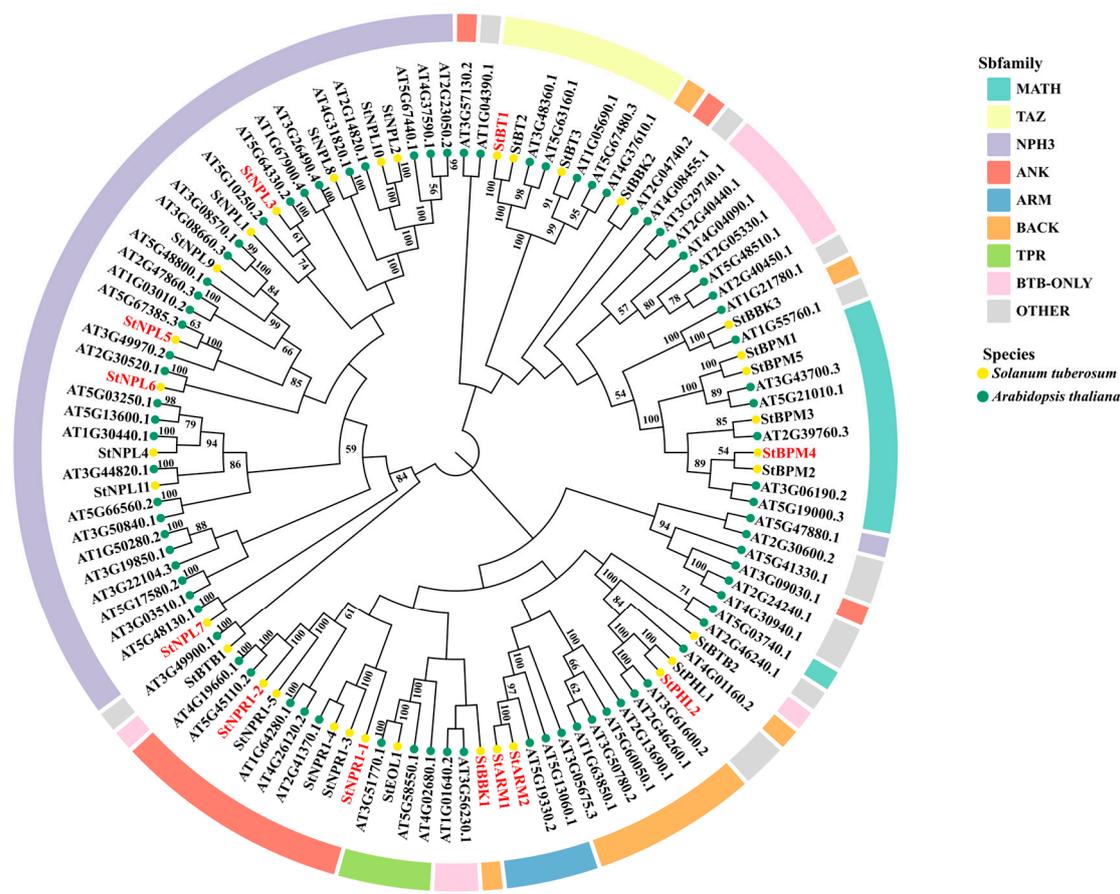


Figure 1. Genetic evolution analysis of potato BTB family proteins. Note: Genes in red were used in subsequent experiments.

3.3. Analysis of the Conserved Motif, Gene Architecture, and Domains in Potato BTB Family Proteins

The conserved motif composition among members was not the same, but the motif composition, location, and number among subtribes exhibited collinearity. Among them, the conserved motif 1 is shared by all members; motif 9 is present in most *StBTB* protein sequences. At the same time, we analyzed the structure of the BTB family genes based on the annotation files. Most *StBTB* genes have 2–6 exons (*StARM1* and *StARM2* have 19 exons). In addition to *StBTB2*, *StBBK2*, *StBT3*, *StNPL11*, and *StBPM3*, most *StBTB* genes (29 out of 34 genes) have untranslated regions (Figure 2).

The BTB protein domains in the subfamily were similar in arrangement and contained similar functional domains, and there were significant differences in different domains between each subfamily (Figure 3).

3.4. Chromosome Localization and Collinearity Analysis of Potato BTB Gene

The *BTB* gene family members are unevenly distributed on the 11 chromosomes of ch01–ch11. Six genes, *StBPM1*, *StBPM2*, *StPHL1*, *StNPL1*, *StNPL3*, and *StNPL2*, were collected from the potato ch01 chromosome. There were five *StBTB* genes on ch07 (*StNPL6*, *StNPR1-5*, *StNPL7*, *StBBK2*, and *StBBK3*) and ch10 (*StPHL2*, *StNPL10*, *StEOL1*, *StNPR1-4*, and *StNPR1-1*) and four *StBTB* genes on ch02 (*StNPL4*, *StNPL5*, *StNPR1-2*). There were three genes in ch06 (*StBBK1*, *StBT2*, and *StARM1*), ch08 (*StNPL8*, *StBT3*, and *StBTB2*), and ch09 (*StNPL9*, *StARM2*, and *StBPM4*), and two *StBTB* genes, *StNPL11* and *StBPM5*, were found on ch11. In addition, there was only one gene on each of the other three chromosomes (Figure 4).

Collinearity analysis within the potato BTB family is helpful for understanding its evolution and predicting repetitive events. As shown in Figure 5, a total of 11 pairs of fragment repeats were identified, including *StBBK3/StBPM3*, *StARM1/StARM2*, *StBTB2/StNPL1*, *StBTB2/StPHL2*, *StPHL2/StNPL1*, *StBTB2/StNPL1*, *StNPL10/StNPL2*, *StNPR1-4/StNPR1-1*, *StBPM5/StBPM1*, *StBTB1/StNPL2*, and *StBTB1/StNPR1-2*, and no tandem repeats.

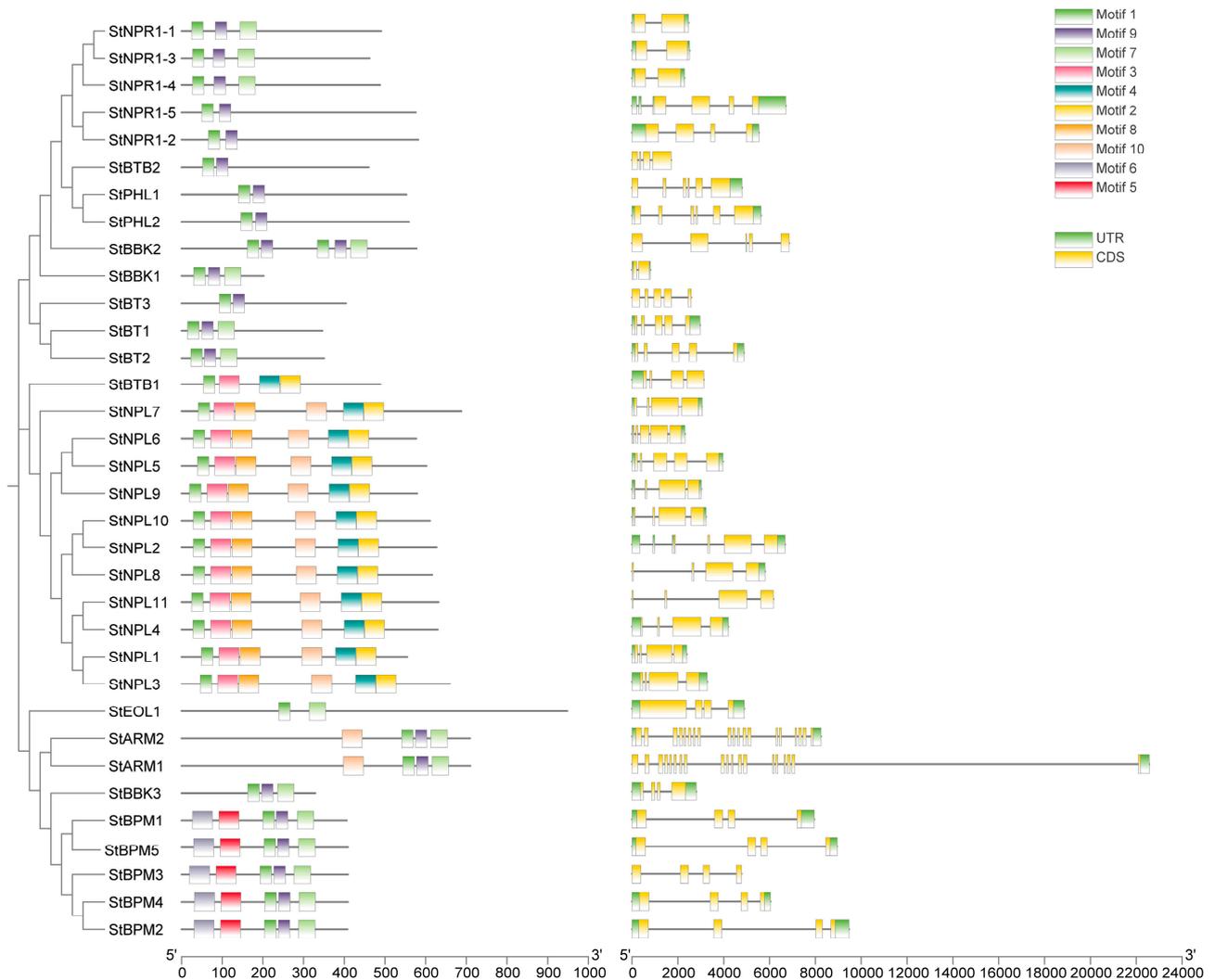


Figure 2. Conserved motifs and structural analysis of potato BTB family proteins.

3.5. Collinearity Analysis of BTB Family Genes between Potato and *A. thaliana*

The genes of the gene family are relatively conserved among species, and genomic collinearity analysis between species can confirm the degree of homology of the gene family between species. Therefore, we analyzed the collinearity between the members of the BTB family of potato and *A. thaliana* and found that the BTB family between the two species exhibited 34 pairs of collinearity (Figure 6).

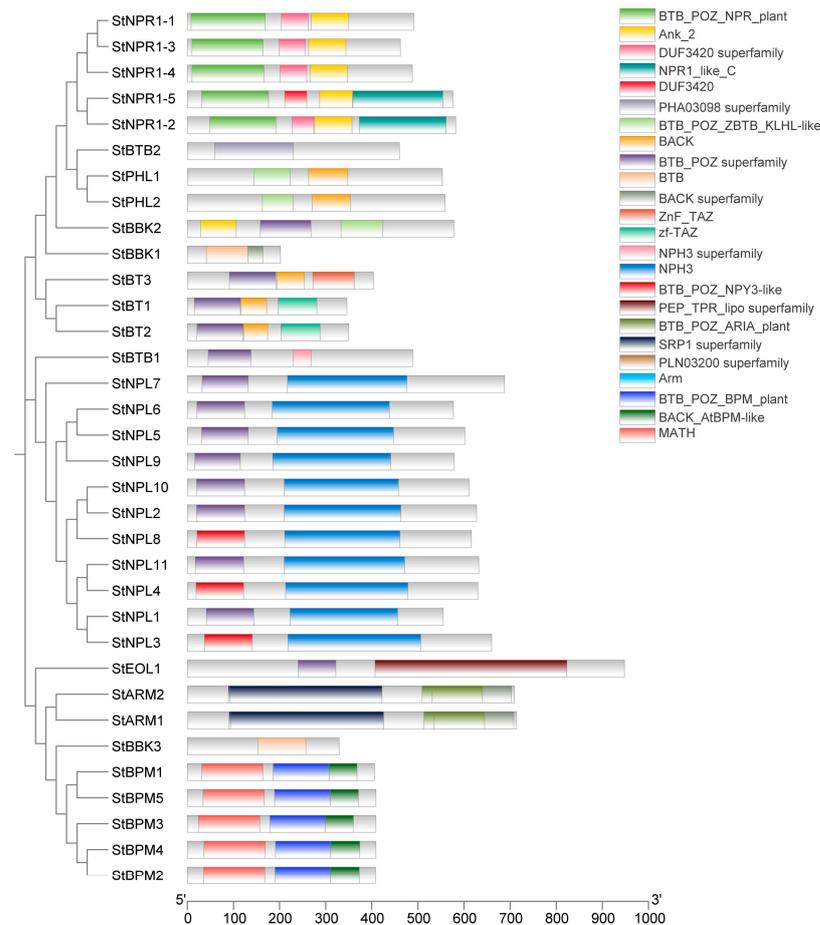


Figure 3. Domain analysis of potato BTB family proteins. Domain accession numbers: BTB_POZ_NPR_plant(cd18310); Ank_2(PF12796); DUF3420 superfamily(cl13357); NPR1_like_C(PF11900); DUF3420(PF11900); PHA03098 superfamily(PF11900); BTB_POZ_ZBTB_KLHL-like(cd18186); BACK(cl28903); BTB_POZ superfamily(cl38908); BTB(PF00651); BACK superfamily(cl28903); ZnF_TAZ(cl02660); zf-TAZ(PF02135); NPH3 superfamily(cl03827); NPH3(PF03000); BTB_POZ_NPY3-like(cd18312); PEP_TPR_lipo superfamily(cl37187); BTB_POZ_ARIA_plant(cd18352); SRP1 superfamily(cl34886); PLN03200 superfamily(cl33659); Arm(PF00514); BTB_POZ_BPM_plant(cd18280); BACK_AtBPM-like(cd14736); MATH(cd00121).

3.6. Expression Profile Analysis of Potato BTB Family Genes

To analyze the specificity and function of potato *BTB* gene family expression, a clustering heatmap was drawn based on transcriptome data (Figure 7). The results showed that potato *StBTB* genes exhibited tissue-specific expression, mainly in roots. Some genes were mainly found in buds, calluses, stamens, sepals, petioles, carpels, and petals, suggesting that they play important roles in root growth and development and regulation. In addition, the expression of the *StBTB* gene was significantly upregulated under abiotic stress, especially under GA3 and salt stress, and the expression of some genes significantly increased under mannitol, ABA, and drought treatment. There is a certain linear relationship between the expression changes in some genes under these abiotic stresses. There may be interactions between different *StBTBs*, which play different physiological roles in different environmental response processes in potato.

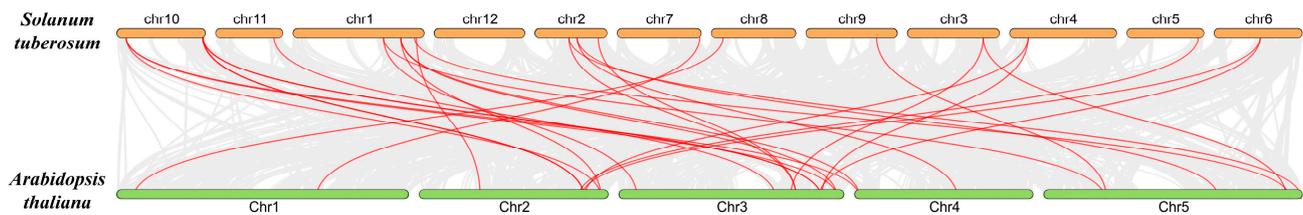


Figure 6. Collinearity analysis of the BTB family genes in *A. thaliana* and potato.

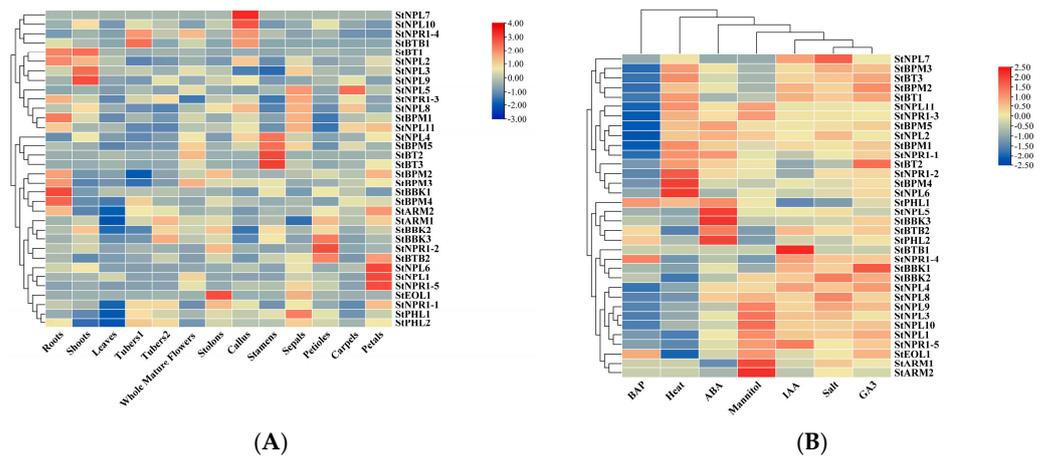


Figure 7. Expression profile of *BTB* genes in potato: (A) expression profile in different tissues; (B) expression profile under different abiotic stresses.

3.7. Validation of Potato *BTB* Family Gene Expression under Abiotic Stress

To verify the response of the 12 genes of the *BTB* family under abiotic stress, the relative expression of genes under the six stress treatments was analyzed by qRT-PCR. Figure 8 clearly shows that under cold treatment, the expression of the *StPHL2* gene did not significantly change, that of the *StBT1*, *StNPL3*, *StNPR1-1*, and *StARM1* genes significantly decreased, and that of the other members significantly increased. Under ABA treatment, the *StBPM4*, *StNPL7*, *StNPR1-1*, *StNPR1-2*, and *StARM2* genes were significantly upregulated and the other genes were significantly downregulated. Under salt stress, the expression of the *StBPM4* and *StPHL2* genes did not significantly change, the expression of the *StNPL6*, *StNPR1-2*, *StARM2*, and *StBBK3* genes significantly increased, and that of the other genes significantly decreased. Under H_2O_2 treatment, the expression of the *StBPM4*, *StNPL5*, *StARM1*, and *StBBK3* genes did not significantly change, the expression of the *StNPL3*, *StNPR1-2*, and *StARM2* genes significantly decreased, and that of the remaining genes significantly increased. Under drought stress, the *StBT1*, *StNPL3*, and *StNPL6* genes were significantly downregulated and the other genes were significantly upregulated. Under low-nitrogen stress, the expression of the *StNPL6* and *StNPR1-1* genes did not significantly change, that of the *StNPL5* and *StBBK3* genes significantly increased, and that of the other genes significantly decreased.

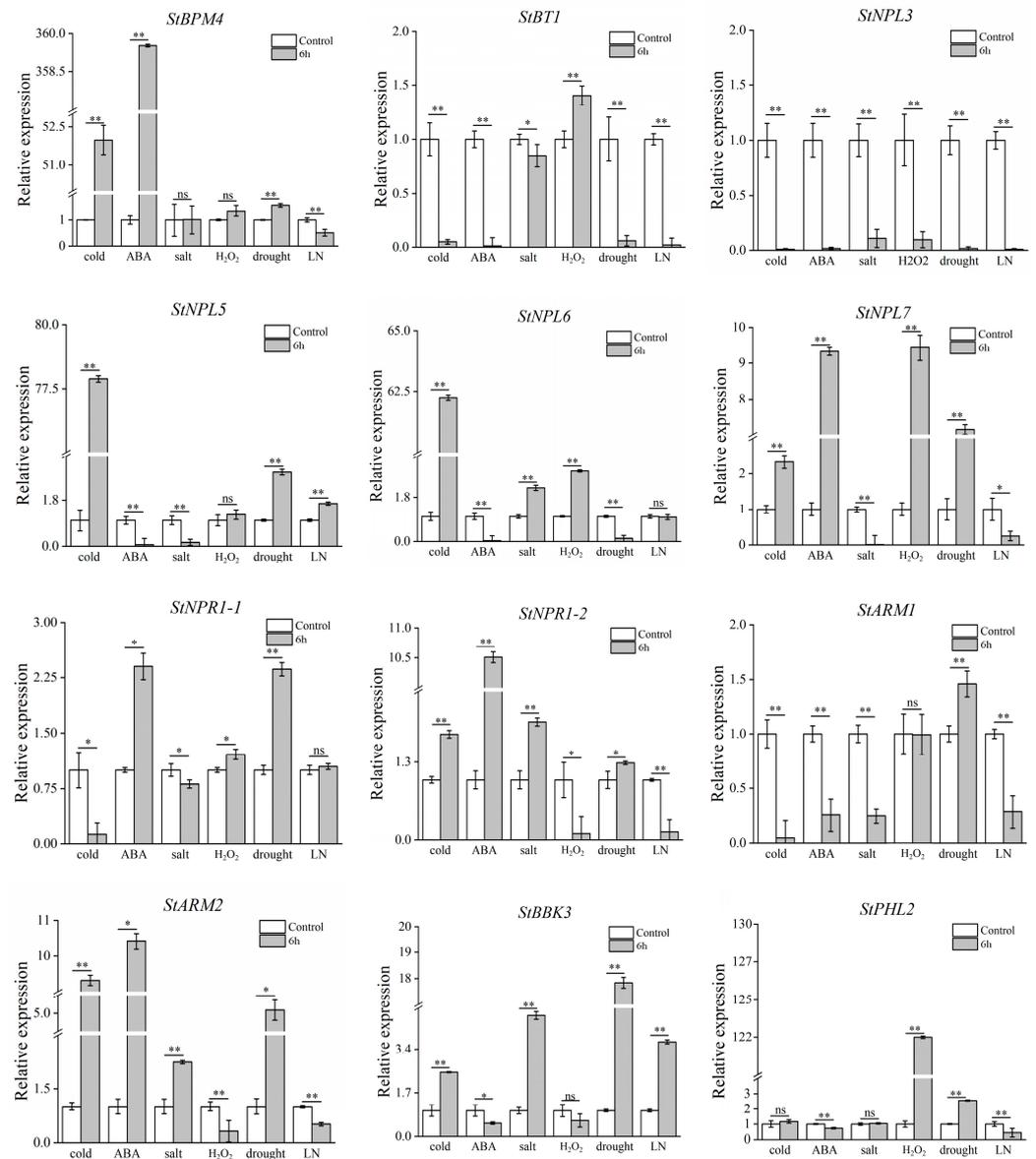


Figure 8. Expression analysis of 12 *StBTB* genes selected under abiotic stress. * signifies the differences in gene expression as determined by the *t*-test (* $p < 0.05$, ** $p < 0.01$, ns: not significant). LN is low-nitrogen stress.

4. Discussion

The BTB/POZ protein-encoding gene family is characterized by its BTB domain, which mediates protein–protein interactions [27]. Although the BTB domain proteins are widely distributed in animals, plants, and microorganisms, they are highly conserved throughout evolution [28]. At present, research on the BTB/POZ protein has focused mainly on humans and animals (fruit flies [29] and mice [30]), while research on plants is relatively lacking, and there are few reports on plant abiotic stress, which has great potential.

The 34 potato BTB proteins identified in this study all contain this functional domain in the amino acid sequence. Therefore, it can be inferred that these plants belong to the typical plant BTB family. At the same time, most of the identified BTB protein genes also covered other types of functional domains. Based on the presence of other domains, we classified the identified potato BTB protein genes into nine subfamilies, namely, the Ankyrin, Armadillo, MATH, NPH3, BACK, TAZ, TPR, BTB-only, and other subfamilies. Different subfamilies of BTB protein genes may have different functions [31].

However, as a Solanaceae plant, there are few reports on the BTB family in potato. During the process of evolution, gene replication is accompanied by a certain loss, which leads to a certain difference in the number of gene families between species. According to previous studies, *A. thaliana* and rice contain 80 and 149 BTB proteins, respectively [32,33], only a few of which have been studied in detail. A total of 34 BTB family genes were identified from the potato genome database, which was significantly less than the number of BTB family genes in *A. thaliana* and rice, indicating that there was a large difference in the number of BTB proteins among different species.

According to the phylogenetic analysis of the BTB protein in potato and *A. thaliana*, 34 *StBTB* genes were divided into nine subfamilies (Figure 1). It was also found that most BTB protein genes had high homology with those of these two species. It is speculated that there may be similar functions between the two species, so the function of *BTB* genes in potato can be speculated according to the clustering relationship between the two species. According to the conservation of the BTB family and the distribution of genes with similar functions or the same functions in the same subfamily, the BTB protein not only has high conservation in different species but also has evolutionary conservation. The structure is associated with gene expression, and the domain is associated with the exercise of protein function. Next, we analyzed 10 conserved motifs and found that these *StBTB* motifs had different distributions within subfamilies but similar distributions within subfamilies. Most BTB proteins have motif 1, and different motifs determine the structure and function of genes [34]. Moreover, the structure of the BTB protein was analyzed. The structure of introns and exons affects the structure and function of proteins, thus affecting the growth and development of plants, and plays an important role in gene expression and regulation [35,36]. Most of the BTB protein genes have untranslated regions, and there are 2–6 exons. There was a certain correlation between the structure of the *BTB* gene and the arrangement of conserved motifs, which verified the classification within the BTB subfamily (Figure 2). We analyzed the domains contained in each gene, which may imply that different domains and combinations of domains have different functions (Figure 3).

As shown in Figure 3, except for ch12, 34 *StBTB* genes were unevenly distributed on 11 chromosomes, suggesting that the family members may be widely involved in physiological activities to maintain the life activities of potato. Gene replication is important for providing the original genetic material for biological evolution [37]. Gene families are usually extended through fragment replication and tandem replication, which is also the main mode of plant evolution [38]. In Figure 4, collinear relationships between gene pairs are shown. The genes of the same subfamily are basically similar in structure, and it is speculated that they may have similar functions in various processes of plant growth and development. At the same time, we analyzed and identified 11 pairs of fragment repeats and no tandem repeats. The results showed that 11 pairs of genes were formed by genome-wide replication, which may be the reason why potatoes have fewer *BTB* genes than other species. Figure 5 shows 34 collinearity pairs between *A. thaliana* and potato. It is suggested that the BTB family genes were strongly correlated between the two species during evolution, and it is speculated that there may be some similarity in gene structure and function.

The expression of genes in tissues is closely related to their functions [39,40]. According to the cluster heatmap in Figure 6, the expression of 10 *StBTB* genes was the highest in the roots and the remaining genes were mainly distributed in the buds, callus, stamens, sepals, petioles, carpels, and petals. It is speculated that the tissue expression specificity of the *StBTB* gene in potato may play different roles in regulating its growth and development.

Losses caused by abiotic stress conditions can lead to significant reductions in crop yields [41]. Previous studies have shown that BTBs are essential for plant growth and development and are involved in abiotic stress responses [42–45]. With climate and environmental changes, cultivating potato varieties resistant to abiotic stress is beneficial for improving yields and quality. Abscisic acid (ABA) plays a mediating role in the signaling pathway involved in the plant response to stress by initiating adaptive responses that help

reduce crop yield losses [46]. Plant growth and development require appropriate temperatures, and plant cell structure and osmotic balance are disrupted by low-temperature stress, resulting in cell metabolism disorders [47]. Salt is an important environmental factor affecting plant growth and yield; high salt levels can cause plant yield reduction or death, and salt stress can damage the cell membrane and destroy plant ion homeostasis [47]. One of the main factors of plant damage under drought stress is the overproduction of reactive oxygen species (ROS) in organelles, which leads to the degradation of lipid catalase proteins and nucleic acids in cell membranes [48]. H_2O_2 is a component of intracellular ROS and is also an important signaling molecule involved in various physiological metabolic processes in plants. Excessive H_2O_2 can destroy biological macromolecules and poison cells [49]. Currently, in response to low-nitrogen conditions, nitrogen affects development, physiology, and metabolism, so it is important to study low-nitrogen stress [50]. Consequently, examining how plants withstand abiotic stress is essential.

Each impact is accompanied by the expression of the genes under it. Under low-temperature stress, the expression of the *StBPM4*, *StNPL5*, *StNPL6*, *StNPL7*, *StNPR1-2*, *StARM2*, and *StBBK3* genes significantly increased. The expression of the *StBPM4*, *StNPL7*, *StNPR1-1*, *StNPR1-2*, and *StARM2* genes was significantly upregulated under ABA stress. The expression of the *StNPL6*, *StNPR1-2*, *StARM2*, and *StBBK3* genes significantly increased under salt stress. The expression of the *StBT1*, *StNPL6*, *StNPL7*, *StNPR1-1*, and *StPHL2* genes was significantly upregulated under H_2O_2 stress. The expression levels of the *StBPM4*, *StNPL5*, *StNPL7*, *StNPR1-1*, *StNPR1-2*, *StARM1*, *StARM2*, *StBBK3*, and *StPHL2* genes were significantly upregulated under drought stress. The expression of the *StNPL5* and *StBBK3* genes significantly increased under low-nitrogen stress. Similarly, the expression levels of *StNPL7*, *StNPR1-1*, *StNPR1-2*, and *StARM2* significantly increased under ABA, salt, and drought stress. In summary, we further confirmed that *StBTB* family genes play important roles in the potato abiotic stress response.

5. Conclusions

In this study, 34 *BTB* genes were identified in potato, and these genes were analyzed in depth, including phylogeny, gene structure, conserved motifs and domains, chromosome location, gene collinearity, and expression pattern analysis. Its tissue expression and expression under stress indicate that it plays an important role in growth, development, and stress resistance. The 12 selected genes had different expression levels under different stress treatments, indicating that these genes were involved in the regulation of abiotic stress, and the expression levels of *StNPL7*, *StNPR1-1*, *StNPR1-2*, and *StARM2* were significantly increased under ABA, salt, and drought stress. These genes may be important candidate genes for potato stress resistance. In general, this study laid a foundation for the study of function and expression in potato biotic stress response and provided a basis for genetic engineering and genetic improvement of potato plants.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae10060543/s1>: Table S1: *StBTB* gene primer selection.

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