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SHORT COMMUNICATION

Mining sequences with similarity to *XTH* genes in the *Solanum tuberosum* L. transcriptome: introductory step for identifying homologous *XTH* genes

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ABSTRACT

The *xyloglucan endotransglucosylase/hydrolase* (*XTH*) genes in *Arabidopsis thaliana* (L.) Heynh. form part of a group of mechano-stimulated genes and play an important role in abiotic stress tolerance. Mining the RNAseq transcriptomic database of 40,430 potato (*Solanum tuberosum* L.) genes based on functional annotation and homology search, our objective was to discover potentially homologous *XTH* genes. A Gene Ontology-based *XTH* homology search and functional annotation discovered, from among the 33 *A. thaliana* (*AtXTH*) and 25 tomato (*Solanum lycopersicum* L.) (*SlXTH*) *XTH* genes, 35 gene sequences corresponding to 20 *AtXTH* genes and 40 gene sequences corresponding to 21 *SlXTH* genes, respectively. Thirteen sequences corresponding to 11 putative *XTH* genes in potato, named as *StXTH* after SlXTH genes, were significantly up- or down-regulated in response to ultrasound. These putative *StXTH* genes in potato can serve for future functional genetic analyses.

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Introduction

Plants are constantly exposed to environmental effects and stresses from which they cannot escape but which can modify their growth and development. Therefore, the ability of plants to perceive and respond to different environmental signs is of evolutionary importance.[1]

Sound vibration (SV), including sound and ultrasound (US), is one group of physical stimuli originating from the surrounding environment.[2,3] Depending on the frequency and intensity of SV and US, they can positively or negatively affect different biological functions of plants, such as germination, the cell cycle, shoot, root and callus growth and development, signal transduction systems, activities of various enzymes and plant hormones, and gene expression.[4-7] Natural SVs produced by birds (chirping), bees (buzzing) and other animals cause different changes in plant gene expression patterns[8,9] and accelerate seed germination rates,[10] Collins and Foreman (2001)[11] using different frequencies (500, 5,000, 6,000, 12,000 and 14,000 Hz) of sound vibration on common bean (*Phaseolus vulgaris* L.) observed frequency-specific responses in which beans showed maximum growth at 5000 Hz. Qin et al. (2003)[12] found improved growth in Chinese cabbage (*Brassica rapa* L.) and cucumber (*Cucumis sativus* L.) in response to 20,000 Hz. Our group has worked on trying to better understand the biochemical and transcriptomic changes that take place in *in vitro* potato (*Solanum tuberosum* L.) explants exposed to ultrasound (US; 20 min; 35 kHz; 70 W) transmitted by air (air-based ultrasonication, AB-US) or liquid (liquid-based ultrasonication by piezoelectric ultrasound generator, PE-US). Those studies[5,7,13] concluded that both AB-US and PE-US-generated US waves represent abiotic stresses and that both US treatments modified the gene transcription of the *in vitro* potato plants. Changes in the transcription profile of both ultrasonicated plant materials resulted in modified and stimulated growth response.

Mechanostimulation-induced genes of plants play a role in the response to sound waves or US.[4,8] Almost three decades ago, five "touch" (*TCH*) genes (*TCH1-TCH5*) were discovered in *Arabidopsis thaliana* (L.) Heynh. ecovar Colombia in response to rain, wind and touch, which the authors then attributed to genes related to calmodulin.[14] As neatly summarized by Braam (2005),[15] several experiments conducted over more than a decade elucidated that *TCH1* encodes a calmodulin protein, CAM2, *TCH2* encodes a calmodulin-like (CML) protein, CML24, *TCH3* encodes CML12, while *TCH4* encodes a xyloglucan endotransglucosylase/hydrolase, XTH22. Johnson et al. (1998)[16] observed strong induction of *TCH4* (*XTH*) which modified the cell wall after exposure to sound vibrations (SVs) at 50 Hz, 30 min. Curiously, no additional studies after the 1990 study were ever conducted on *TCH5*, which is not listed on NCBI as a gene. XTHs are cell wall-loosening enzymes, thereby affecting cell expansion and growth[17] and also play a role in a plant's adaptation to abiotic stresses.[18] Lee et al. (2005)[19] found that 589 genes out of 22,810 genes on an Affimetrix microchip were touch-inducible or 2.58% of the genome, whereas 171 genes (0.75%) were down-regulated in response to touch. In their study of the 33 *XTH* genes, only four (*XTH17, XTH22, XTH25*, and *XTH31*) were significantly over-expressed in response to touch. *A. thaliana* Col–0 seedlings, in response to sound stimulus, showed strong

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up-regulation of *XTH22*.[20] These studies indicate that *XTH* genes, including the *TCH4* gene, form part of a wider subset of mechanostimulation-induced genes in *A. thaliana*.

Saladié et al. (2006)[21] identified 25 tomato (*Solanum lycopersicum* L. cv. Alisa Craig) *XTH* genes (*SlXTH1-25*) (http://labs.plantbio.cornell.edu/XTH) but they did not study its expression intensity change in response to mechanostimuli, such as touch or SV. However, the phylogenetic analyses of *A. thaliana* and tomato *XTH*s based on full-length protein sequences revealed four distinct clades. Considering that potato and tomato are closely related species belonging to the same genus of *Solanum*, it is of importance to assess the similarity and potential homology for *SlXTH*s, as well.

In our previous AB-US and PE-US studies,[5,7] we found some significantly differentially expressed genes (DEGs) that could not be functionally categorized by gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Therefore, we were interested to mine these DEGs of *S. tuberosum* L. genes to assess whether there might be similar or homologous sequences to *XTH* genes of *A. thaliana* (*AtXTH)* and/or *S. lycopersicum* (*SlXTH*), and thus putative *XTH* genes in potato, i.e., *StXTH* genes.

Methods

RNA-seq datasets originated from two different experimental studies[5,7] were used for bioinformatics analysis. In both studies, single-node-stem explants cut from 4-week-old *in vitro* plantlets of potato (*Solanum tuberosum* L. cv. Desirée) were ultrasonicated with 35 kHz and 70 W US for 20 min. AB-US was applied to explants immediately after placing them horizontally onto solid hormone-free MS[22] medium and they were ultrasonicated in an ultrasonication unit where the ultrasound was transmitted by air, as described in Dobránszki et al.[5,13] In the case of PE-US, single-node explants were placed into beakers surrounding by distilled water and containing liquid hormone-free MS medium. After ultrasonication in an Elmasonic X-tra 30 H ultrasonicator (ElmaSchmidbauer GmbH, Singen, Germany)[7] explants were cultured on the same medium and under the same *in vitro* growing conditions (63.5 µmol m^{-2} s^{-1}, 16-h photoperiod, 22 ± 2°C, for 4 weeks) as for AB-US.[5,7,13] Samplings were made five times during the 4-week-long subculture, at 0 hour (h), i.e. immediately after the ultrasonication, and then at 24 h, 48 h, 1 week (w) and 4 weeks after the US treatment.

RNA-seq datasets

In the present study, we used our previous RNA-seq datasets gained from our earlier studies.[5,7,23] The RNA-seq projects were uploaded to the Gene Expression Omnibus (GEO) under GSE123176, GSE123037 and GSE135294. The RNA-seq raw FASTQ files were uploaded as GSM3498049, GSM3498050, GSM3498051, GSM3498052, GSM3498053, GSM3498054, GSM3498055, GSM3498056, GSM3498057, GSM3498058, GSM3494235, GSM3494236, GSM4002842, GSM4002843, GSM4002844, GSM4002845, GSM4002846, GSM4002847, GSM4002848, GSM4002849, GSM4002850 and GSM4002851 onto the Sequence Read Archive (SRA).

Bioinformatic analysis

The raw FASTQ files were trimmed using TrimGalore v0.5.0 (https://github.com/FelixKrueger/TrimGalore) with one addition, i.e., 10 bp was removed from the 5′ end of the reads.[24] HISAT2[25] was used for alignment to the *Solanum tuberosum* L. reference genome (SolTub 3.0: https://plants.ensembl.org/Solanum_tuberosum/Info/Index). Alignments were quantitated in SeqMonk v1.42.0 (https://github.com/s-andrews/SeqMonk). The quantitated values were reads per million reads of input and were log2 transformed. The χ^2 probe was used to detect significant DEGs based on the changes in intensity of gene expression between any two pairs of control (non-ultrasonicated) and ultrasonicated samples.

Functional annotation and homology search

The *XTH* homology search was conducted with the Gene Ontology (GO) Annotation workflow in OmicsBox v1.1.164.[26] A local blast database was established in OmicsBox with the *AtXTH1-33* genes (Suppl. Table 1) from the NCBI RefSeq protein database (Release 95, https://www.ncbi.nlm.nih.gov/refseq/) based on Lee et al. (2005).[19] A local blast was performed with Blast+ v2.9.0 (NCBI] using blastX (E-value = 1.0E-10; number of blast hits = 10; blast description annotator included; word size = 3 with low complexity filter; HSP length cutoff = 33) on the SolTub 3.0 (EMBL_EBI Assembly ID: GCA_000226075.1) genome [40,430 genes). The blastX options, E-values and BitScore settings were based on Pearson (2013).[27] InterProScan v5.36–75.0[28] was employed on the EMBL-EBI servers with the following families, domains, sites and repeats (CDD, HAMAP, HMMPanther, HMMPfam, HMMPIR, FPrintScan, ProfileScan, HMMSmart, HMMTigr, PatternScan), structural domains (Gene3D, SFLD, SuperFamily) and other sequence features (Coils, MobiDBLite, Phobius, SignalPHMM, TMHMM). GO mapping was performed on the putative sequences with the 2019.07 database. Annotation mapping was performed with annotation CutOff (55 score), GO weight (5), GO was filtered by taxonomy (green plants, 33090 taxa, Viridiplantae), the E-value-hit-filter was 1.0E-10, an HSP-hit coverage cutoff of zero, and a hit filter of 500. GO-Slim was run on the putative sequences with the Plant slim file from the GO-Website (http://geneontology.org/). We used GO-EnzymeCode Mapping in OmicsBox to map the existing GO terms to enzyme codes. EggNOG Mapper v1.0.3 with EggNOG v5.0.0[29,30] was used to predict orthology for functional annotation while avoiding the transfer of annotations from paralogs. EggNOG mapper results were merged into the NCBI results with an E-value cutoff value of 1.0E-10 and a BitScore cutoff value of 60 (Suppl. Table 1).

Sequence alignment and phylogenetic tree

Full-length amino acid sequences of *StXTH, SlXTH* and *AtXTH* were obtained from the genome sequence of *S. tuberosum* (SolTub 3.0 and NCBI RefSeq protein database; Suppl. Table 1), *S. lycopersicum* (SL3.0; Suppl. Table 1) and *A. thaliana* (TAIR10: GCA_000001735.1; Suppl. Table 1), respectively. For the phylogenetic tree, we used the 11 putative *StXTH* genes which were

verified with the RNAseq dataset. The amino acid sequence alignment of *StXTH*s, *SlXTH*s and *AtXTH*s was compiled using Multiple Sequence Comparison by Log-Expectation (MUSCLE: https://www.ebi.ac.uk/Tools/msa/muscle/). The phylogenetic tree was constructed by the Maximum Likelihood statistical method (ML[31]), Nearest-Neighbor-Interchange (NNI[32]) heuristic method and neighbor-joining initial tree with 5000 bootstrap replications using MEGA X.[33] The amino acid substitution model was optimized by MEGA X based on the Bayesian Information Criterion (BIC) scores (Suppl. Tables 2 and 3[34]). The BIC is a criterion for model selection, where the lowest BIC score is preferred.[35] Based on the BIC scores, we used the WAG+G + F substitution model (WAG: Whelan and Goldman; G: gamma distributed rates; F: frequencies), which had the lowest BIC score for the ML analysis.

RNA-seq dataset validation with RT-qPCR

Total RNA was isolated from 120 samples (three biological and three technical replicates from each AB-US and PE-US samples from different sampling time) using Direct-zol kit (Zymo Research, Irvine, CA, USA) with TRIzol reagent based on the manufacturer's protocol. We used microcapillary electrophoresis with Implen n50 nanophotometer (Implen, Munich, Germany), agarose gel electrophoresis and fragment analysis with Agilent Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA) to quality control (QC) of total RNA. The cDNA was synthetized from 120 ng total RNA with FIREScript RT cDNA Synthesis MIX (Solis BioDyne, Tartu, Estonia). RT-qPCR was performed with the 5x HOT FIREPol EvaGreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) on the ABI 7300 real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). The RNA-seq dataset was validated by RT-qPCR with gene-specific primers (Suppl. Table 4) using 13 gene sequences of the 11 predicted *StXTH* genes (PGSC0003DMG400024755, PGSC0003DMG400021398, PGSC 0003DMG400026189, PGSC0003DMG400017299, PGSC0003D MG400021877, PGSC0003DMG400017298, PGSC0003DMG400 014823, PGSC0003DMG400013975, PGSC0003DMG400007854, PGSC0003DMG400001809, PGSC0003DMG400014024, PGSC0 003DMG400000408, PGSC0003DMG400004109). The *Glycer aldehyde-3-phosphate dehydrogenase* (*GAPDH*) reference gene was selected for RT-qPCR as normalization based on Tang et al. (2017)[36] and our previous studies.[5,7] RT-qPCR primers were designed with CLC Main Workbench 7.9.2 (Qiagen, Hilden, Germany). Logarithmic fold change was used at the RT-qPCR analysis based on the $2^{-\Delta\Delta Ct}$ method. Spearman and Pearson correlation coefficients were calculated in Microsoft Office 365 Excel (Microsoft, Redmond, WA, USA).

Results

Among the 40,430 *S. tuberosum* genes, a total of 38 gene sequences were found that corresponded to 20 *AtXTH* genes (*AtXTH1, AtXTH2, AtXTH3, AtXTH4, AtXTH5, AtXTH7, AtXTH8, AtXTH9, AtXTH10, AtXTH15, AtXTH16, AtXTH22, AtXTH23, AtXTH24, AtXTH25, AtXTH28, AtXTH30, AtXTH31, AtXTH32*, and *AtXTH33*) and 21 *SlXTH* genes (*SlXTH2, SlXTH3, SlXTH4, SlXTH5, SlXTH6, SlXTH7, SlXTH8, SlXTH9, SlXTH10, SlXTH11, SlXTH12, SlXTH13, SlXTH14, SlXTH15, SlXTH16, SlXTH18, SlXTH19, SlXTH20, SlXTH23*, and *SlXTH25)* from among the 33 *A. thaliana* and 25 *S. lycopersicum XTH* genes studied and which may be putatively homologous genes in *S. tuberosum* (Suppl. Table 1). Based on sequence mining, two putatively homologous sequences (different sequences for the same gene) were identified for seven *XTH* genes (*AtXTH16, AtXTH23, AtXTH24, AtXTH28, AtXTH30, AtXTH31*, and *AtXTH32*), three putatively homologous sequences for three *XTH* genes (*AtXTH5, AtXTH8*, and *AtXTH15*), and in the case of *AtXTH22*, four putatively homologous sequences were found (Table 1) compared to *A. thaliana* and *S. tuberosum*.

When comparing *S. tuberosum* L to *S. lycopersicum*, we identified two putatively homologous sequences for four *XTH* genes (*SlXTH5, SlXTH8, SlXTH15* and *SlXTH23*), three putatively homologous sequences for four *XTH* genes (*SlXTH3, SlXTH4, SlXTH9* and *SlXTH10*), and in the case of *SlXTH6*, five putatively homologous sequences were found (Table 1). *StXTH*s were named after the *SlXTH*s.

A total of 13 sequences corresponding to candidate 11 *XTH* genes were identified (Table 2) that were significantly up- or down-expressed after US treatments in different comparisons. RT-qPCR for the candidate 11 *XTH* genes validated RNA-seq data, indicating that all of the chosen DEGs were truly positively up- or down-regulated DEGs. A strong positive correlation (r = 0.95 and 0.98 with Spearman and Pearson correlations, respectively) between SeqMonk LFC and RT-qPCR LFC was found (Suppl. Table 4). The different comparisons correspond to the AB-US and PE-US compared to their respective controls, as well as AB-US vs PE-US comparisons, each at five sampling times, namely 0 h, 24 h, 48 h, 1 w and 4 w, corresponding to five growth stages of *in vitro* potato as described in Dobránszki et al.[5,7,23] After the AB-US treatment, which caused a weaker stress than PE-US treatment,[7] the expression intensity of five sequences differed. Up-regulation of *StXTH16* and *StXTH12* occurred immediately after ultrasonication (0 h), while *StXTH9-1* and *StXTH25* were up-regulated at 24 h. Later, at 48 h, *StXTH9-1* was then down-regulated. Moreover, *StXTH3* was down-regulated at 4 w (Table 2). However, the expression intensity of seven sequences belonging to six putative *StXTH* genes (*StXTH1, StXTH3, StXTH2, StXTH6-2, StXTH7*, and *StXTH10*) were down-regulated between 24 h and 4 w after PE-US treatment (Table 2). The most affected gene by PE-US was *StXTH6-2* which was up-regulated at 4 w following its down-regulation at 24 h and 1 w. When PE-US to AB-US was compared at different sampling times, seven sequences of five putative *StXTH* genes (*StXTH1, StXTH5, StXTH6, StXTH7*, and *StXTH9*) were down-regulated at different sampling times (Table 2).

We calculated 56 different amino acid substitution models with the ML method based on the amino acid sequences of *StXTH* and *AtXTH* genes. The mtREV24 + I model had the highest BIC score (219172.5) while the WAG+G + F model had the lowest BIC score (207467.2) (Suppl. Table 2). To construct the phylogenetic tree, the substitution pattern estimation was calculated for amino acids under the WAG+G + F model (Suppl. Table 3). Based on the phylogenetic tree (Figure 1) and cluster analysis, we identified *StXTH1* gene in the same cluster with *SlXTH1* and close to the *AtXTH5-1*. *StXTH2* gene was in the same cluster with *SlXTH19* and *SlXTH2* genes and

Table 1. Putatively homologous *XTH* genes from the *Solanum tuberosum* RNAseq database.

Sequence name	E-value	Similarity mean (%)	Bit-Score	Putative homologous *XTH* gene in *Arabidopsis thaliana*[1]	Putative homologous *XTH* in *Solanum lycopersicum* [1]
PGSC0003DMG400041434	1.11189E-22	63.66	156.4	-	*SlXTH19*
PGSC0003DMG400042833	3.41499E-36	65.63	212.2	*AtXTH1/AtXTH2*	*SlXTH7*
PGSC0003DMG400001229	1.44952E-42	60.59	286.6	*AtXTH3*	*SlXTH4*
PGSC0003DMG400002188	7.09171E-69	65.91	299.7	*AtXTH4-1*	*SlXTH4*
PGSC0003DMG400024755	8.73328E-70	66.63	294.3	*AtXTH5-1*	*SlXTH1*
PGSC0003DMG402010181	4.33809E-63	54.05	229.9	*AtXTH5-2*	*SlXTH18*
PGSC0003DMG402010918	5.90652E-64	66.53	243.0	*AtXTH5-3*	*SlXTH4*
PGSC0003DMG400021398	1.12747E-75	67.99	239.2	*AtXTH7*	*SlXTH7*
PGSC0003DMG400002084	2.91162E-59	54.69	211.1	*AtXTH8*	*SlXTH23*
PGSC0003DMG400024060	8.36804E-71	61.67	306.6	*AtXTH8*	*SlXTH15-2*
PGSC0003DMG400011281	9.85939E-16	91.43	124.0	*AtXTH8*	*SlXTH15-1*
PGSC0003DMG400026189	1.00497E-89	61.97	318.2	*AtXTH9-2*	*SlXTH16*
PGSC0003DMG400005127	4.29487E-60	59.03	144.8	*AtXTH10-1*	*SlXTH7*
PGSC0003DMG400017299	5.34064E-93	63.77	171.0	*AtXTH15*	*SlXTH10*
PGSC0003DMG400031101	6.93065E-94	65.19	171.8	*AtXTH15*	*SlXTH10*
PGSC0003DMG400021877	5.64726E-57	69.87	260.4	*AtXTH15*	*SlXTH12*
PGSC0003DMG400017298	1.58365E-53	69.82	291.6	*AtXTH16*	*SlXTH9*
PGSC0003DMG400004670	7.39946E-56	25812	260.8	*AtXTH16*	*SlXTH3*
PGSC0003DMG401018740	1.26591E-40	63.48	237.7	*AtXTH22*	*SlXTH13*
PGSC0003DMG402018740	1.08367E-55	70.22	284.6	*AtXTH22*	*SlXTH3*
PGSC0003DMG400024121	4.36905E-23	72.95	134.0	*AtXTH22*	*SlXTH10*
PGSC0003DMG400002309	2.24189E-89	62.1	161.4	*AtXTH22*	*SlXTH11*
PGSC0003DMG400014823	9.64778E-106	67.05	250.8	*AtXTH23*	*SlXTH3*
PGSC0003DMG400013975	2.05102E-53	68.65	251.9	*AtXTH23*	*SlXTH2*
PGSC0003DMG400007794	2.54537E-61	61.28	235.3	*AtXTH24*	*SlXTH9*
PGSC0003DMG400007854	9.06518E-61	61.11	236.1	*AtXTH24*	*SlXTH9*
PGSC0003DMG400018741	4.50311E-57	83.13	229.6	*AtXTH25*	*SlXTH20*
PGSC0003DMG400029503	2.13033E-67	59.27	319.3	*AtXTH28*	*SlXTH8*
PGSC0003DMG400022791	1.19207E-67	57.83	333.6	*AtXTH28*	*SlXTH8*
PGSC0003DMG400001809	5.07525E-63	58.3	337.8	*AtXTH30*	*SlXTH5*
PGSC0003DMG400009300	3.39019E-56	59.08	389.8	*AtXTH30*	*SlXTH5*
PGSC0003DMG400014024	1.64996E-40	68.94	161.4	*AtXTH31*	*SlXTH6*
PGSC0003DMG400000408	5.57005E-46	70.39	216.9	*AtXTH31*	*SlXTH6*
PGSC0003DMG400003432	1.61541E-33	65.02	256.4	-	*SlXTH6*
PGSC0003DMG400036031	3.00177E-14	62.97	236.3	-	*SlXTH6*
PGSC0003DMG400003434	5.11064E-48	50.48	117.9	*AtXTH32-1*	*SlXTH6*
PGSC0003DMG400003866	2.11514E-65	65.16	271.9	*AtXTH32-2*	*SlXTH14*
PGSC0003DMG400004109	3.54934E-52	52.48	297.0	*AtXTH33*	*SlXTH25*
PGSC0003DMG400004599	1.57405E-11	60.87	265.6	-	*SlXTH23*
PGSC0003DMG400029203	2.54838E-12	72.34	326.8	-	*SlXTH23*

[1]based on NCBI and EggNOG (*St* = *Solanum tuberosum*); different numbers after several gene names indicate orthologs or putatively homologous sequences for the same gene which were identified from *S. tuberosum* RNAseq data.

close to the *AtXTH23* gene. *StXTH3-1, StXTH3-2, StXTH3-3* and *StXTH3-4* were in the same cluster with *SlXTH3* gene and close to the *AtXTH23* gene. *StXTH5* gene was in the same cluster with *SlXTH25* and *AtXTH33* genes. *StXTH6-1* and *StXTH6-2* were in the same cluster with *SlXTH6* gene and close to *AtXTH32-1, AtXTH32-2* and *SlXTH14* genes. *StXTH7* gene was in the same cluster with *SlXTH7* and close to the *AtXTH6* gene. *StXTH9-1, StXTH9-2* and *StXTH10* genes were in the same cluster with *SlXTH9* and *SlXTH10* genes and close to the *AtXTH15* and *AtXTH16* genes. *StXTH12* gene was in the same cluster with *SlXTH12* and close to the *AtXTH15* and *AtXTH16* genes. *StXTH16* gene was in the same cluster with *SlXTH16* and close to *AtXTH9-1* and *AtXTH9-2*. The *StXTH25* gene was in the same cluster as the *SlXTH5* gene and close to the *AtXTH30* gene.

Discussion and conclusion

In the RNAseq transcriptomic database of *S. tuberosum*, we detected 35 gene sequences that are putative homologs of 20 *AtXTH* and 21 *SlXTH* genes, based on sequence homology and functional annotation. Lee et al. (2005)[19] found that four *A. thaliana XTH* genes [*AtXTH17, AtXTH22, AtXTH25*, and *AtXTH31*) were over-expressed in response to touch, while,[20] detected strong over-expression of *AtXTH22* in response to sound waves. Over-expression of *StXTH6-2*, a putative homolog of *AtXTH31* in *A. thaliana*, was detected by 4 w after ultrasonication transmitted by liquid (PE-US), which caused more stress to plants than ultrasonication transmitted by air (AB-US).[7] In contrast, none of the four genes described in earlier studies [*AtXTH17, AtXTH22, AtXTH25*, and *AtXTH31*[19,20]] were over-expressed in the present study, in response to mechanostimulus caused by the air-transmitted ultrasonication (AB-US), either directly after ultrasonication or later during subculture, as an after-effect. However, we detected the over-expression of four other genes (*StXTH16, StXTH12, StXTH9-1*, and *StXTH25*), potential homologues to *AtXTH9, AtXTH15, AtXTH16* and *AtXTH33*, directly (0 h) or 24 h after ultrasonication. The up-regulation of these four genes was not observed in response to touch or sound stimulus in previous studies.[19,20]

Table 2. The response of the putatively homologous *StXTH* genes to different ultrasound treatments.

Sequence name	Putative *XTH* gene in *Solanum lycopersicum* [1]	Response to different ultrasound treatments[2]	
		Up-regulation	Down-regulation
PGSC0003DMG400024755	*StXTH1*		(2.74) AB-24 h vs. PE-24 h (2.26) CPE-4 w vs. PE-4 w
PGSC0003DMG400013975	*StXTH2*		(4.06) CPE-1 w vs. PE-1 w
PGSC0003DMG400014823	*StXTH3-1*		(3.32) CPE-4 w vs. PE-4 w
	StXTH3-2		(1.87) CAB-4 w vs. AB-4 w
	StXTH3-3		
	StXTH3-4		
PGSC0003DMG400001809	*StXTH5*		(1.26) AB-1 w vs. PE-1 w
PGSC0003DMG400014024	*StXTH6-1*		(6.77) AB-24 h vs. PE-24 h
PGSC0003DMG400000408	*StXTH6-2*	(−1.43) CPE-4 w vs. PE-4 w	(3.01) AB-24 h vs. PE-24 h (1.51) AB-1 w vs. PE-1 w (2.03) CPE-24 h vs. PE-24 h (3.27) CPE-1 w vs. PE-1 w
PGSC0003DMG400021398	*StXTH7*		(2.66) AB-48 h vs. PE-48 h (1.66) CPE-48 h vs. PE-48 h
PGSC0003DMG400017298	*StXTH9-1*	(−2.45) CAB-24 h vs. AB-24 h	(3.86) AB-24 h vs. PE-24 h (3.53) AB-1 w vs. PE-1 w (3.86) CPE-1 w vs. PE-1 w (4.20) CAB-48 h vs. AB-48 h
PGSC0003DMG400007854	*StXTH9-2*		(2.57) AB-24 h vs. PE-24 h
PGSC0003DMG400017299	*StXTH10*		(2.54) CPE-4 w vs. PE-4 w
PGSC0003DMG400021877	*StXTH12*	(−2.69) CAB-0 h vs. AB-0 h	
PGSC0003DMG400026189	*StXTH16*	(−2.50) CAB-0 h vs. AB-0 h	
PGSC0003DMG400004109	*StXTH25*	(−1.82) CAB-24 h vs. AB-24 h	

[1]based on NCBI and EggNOG (*St* = *Solanum tuberosum*); different numbers after several gene names indicate orthologs or putatively homologous sequences for the same gene which were identified from *S. tuberosum* RNAseq data (Table 1). Names of the *StXTH* genes are based on the corresponding *SlXTH*s.
[2]AB, air-based US;[5,23] C, control (no US treatment); h, hours; PE, liquid-based US;[7] US, ultrasound; w, weeks; number in brackets indicate the change in expression intensity in the different US treatment comparisons.
h, hour; w, week

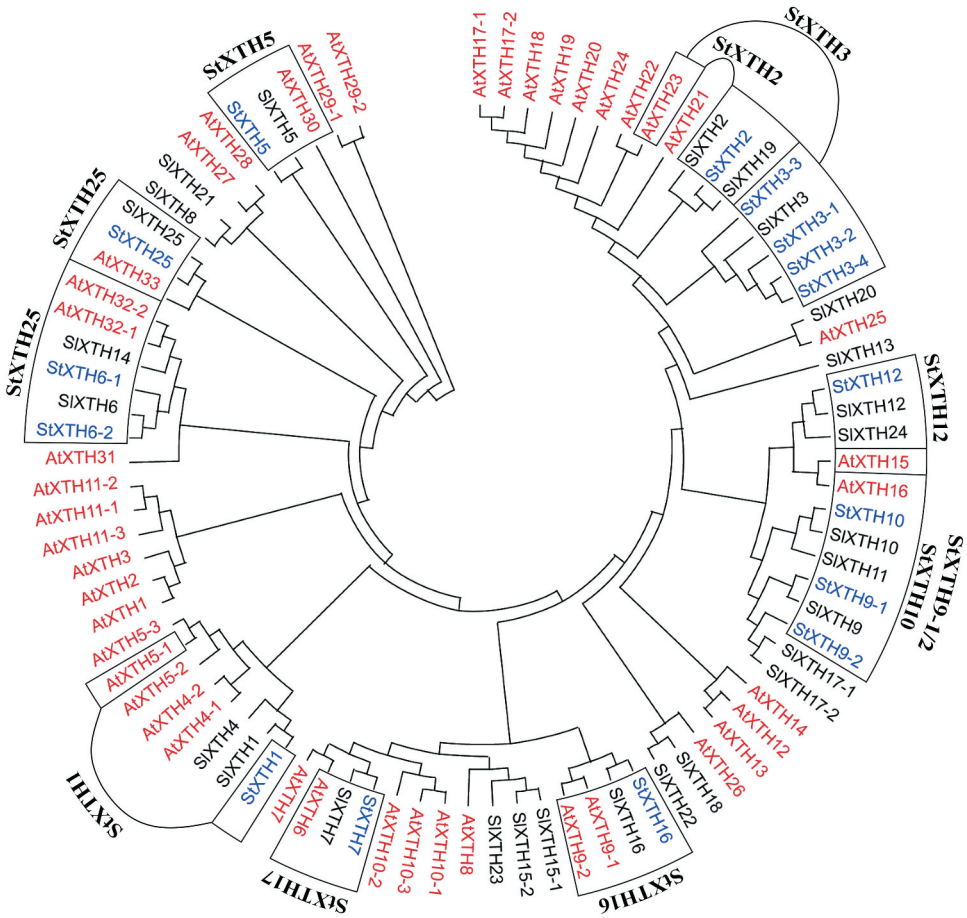

Figure 1. Phylogenetic tree of *XTH* genes from *Arabidopsis thaliana* (L.) Heynh. (*AtXTH), Solanum lycopersicum* L. (*SlXTH*) and putative *XTH* genes from *Solanum tuberosum* L. (*StXTH*). The phylogenetic tree was constructed by the neighbor-joining method with 5000 bootstrap replications using MEGA X.

Ultrasonication caused strong abiotic stress to *in vitro* potato plants, i.e. ultrasound transmitted via liquid, causing down-regulation of gene expression of six putative *XTH* genes, which was detected between 24 h and 4 w. To the best of our knowledge, down-regulation of *XTH* gene expression in response to touch or sound stimuli has not been reported previously.

Given the importance of *XTH* genes in their intricate involvement in plant mechanostimulus responses[19,20] and abiotic stress tolerance in plants,[18] this set of putative *StXTH* genes in potato and the change in their expression intensities in response to different ultrasonication treatments will be a useful dataset and a starting point for functional analyses of these sequences to determine their precise function.

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Conflicts of interest

The authors declare no conflicts of interest regarding this paper.

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