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Gene expression profiles of Hsp family members in different poplar taxa under cadmium stress

Esra Nurten YER ÇELİK[1,*], Mehmet Cengiz BALOĞLU[2,3], Sezgin AYAN[1]

[1]Department of Silviculture, Faculty of Forestry, Kastamonu University, Kastamonu, Turkey

[2]Department of Genetics and Bioengineering, Faculty of Engineering and Architecture, Kastamonu University, Kastamonu, Turkey

[3]Department of Agronomy, University of Florida-IFAS, Gainesville, USA

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Abstract: Heat shock proteins (Hsps), also known as stress proteins, are expressed by living organisms. Hsp genes play key roles in the regulation of change in response to various abiotic stresses (e.g., salinity, drought, heavy metal, and extreme temperatures). In our previous studies, all Hsp family gene members were determined and named using bioinformatics approaches. We also examined their expression profiles under different stress conditions. In this study, the aim was to indicate the expression pattern of Hsp family genes under cadmium (Cd) stress in different poplar taxa which are resistant to various stresses. Firstly, transcriptome data including RNA-seq and microarray were evaluated to select Hsp gene members that were suitable targets for the cadmium stress response. Then, the expression analysis of selected genes was studied with qRT-PCR (real-time quantitative reverse transcription PCR) in different poplar taxa. Under cadmium stress conditions, the expression profiles of genes including *PtsHsp-44*, *PtsHsp-54*, *PtHsp40-117*, *PtHsp60-06*, *PtHsp60-12*, *PtHsp70-21*, *PtHsp70-28*, *PtHsp90-02*, *PtHsp90-10*, *PtHsp90-12*, *PtHsp100-22*, and *PtHsp100-71* were observed. In the future, *N.03.368A* and *I-214* taxa may be used for plantation in Cd-contaminated areas and studied under subsequent long-term observation. This study yielded preliminary information about Cd-stress-related molecular mechanisms that will be utilized for future projects. In addition, the genes responsive against Cd stress can be used for gene cloning and functional analyses, which could open new perspectives for improving Cd-tolerant plants or trees.

Key words: Cadmium stress, gene expression analysis, heat shock proteins, heavy metal, poplar, qRT-PCR

1. Introduction

The development of bioinformatics has led to an increase in studies of the complex mechanisms of abiotic stress tolerance in plants. In these studies, *Arabidopsis thaliana* was initially selected as a model plant species. Since then, studies in molecular biology of forest tree responses to abiotic stresses have focused on the response mechanisms of *Populus trichocarpa* Torr. & A. Gray ex. Hook., a model tree species whose genome sequence was completed in 2006 (Tuskan et al., 2006). During the subsequent 10 years, the *Populus* genus has been the focus of three quarters of 'omics' studies (e.g., genomics, transcriptomes, proteomics, and metabolomics); the remaining studies involved eucalyptus, pine, and to a lesser extent, oak, beech, and Douglas fir species. Such omics-based methods have been used to determine the stress response mechanisms of plants (Taylor, 2002; Chinnusamy et al., 2004; Tuskan et al., 2006; Ashraf and Foolad, 2007; Kosova et al., 2011). Because of the effects of these abiotic stress factors, particularly at high concentrations, both essential and nonessential metals can cause cell membrane and DNA structural damage, alter enzyme specificity, and impair cellular function. Toxic levels of metal accumulation can affect areas such as riparian buffers and arable lands negatively (Sanita et al., 1999; Hall, 2002; Benavides et al., 2005; Abolghassem et al., 2015). In these areas, the poplar taxa have been recommended for phytoremediation purposes (Kramer, 2010). Poplar species are preferred because of their rapid growth, deep root systems, and short rotation periods (Di Lonardo et al., 2011). In this study, we have focused on heat shock proteins (Hsps) due to their important roles in the abiotic stress response. Hsps are the companion proteins known as molecular chaperones because of their physiological responsibilities and helper functions in the cell. Hsps repair and stabilize proteins that have denatured in response to stress. They prevent the formation of protein aggregates by preventing incorrect binding (Hendrick and Hartl, 1993). A unique similar phrase is characterized by the discovery of *Hsps* (Ritossa, 1962; Trent, 1996; Wang et al., 2004; Gupta et al., 2010;

* Correspondence: esranurtenyer@gmail.com

Wang et al., 2014; Zafar et al., 2016). Many researchers classify Hsps into five main families by molecular weight: *Hsps*, *Hsp60*, *Hsp70*, *Hsp90*, and *Hsp100*. In some studies, members of the *Hsp40* gene family provided protection in the plant genome (Schlesinger, 1990; Schöffl et al., 1998). We have determined and named all Hsp family gene members in the poplar genome in our former studies and checked their expression levels under salt- and drought-stress conditions (Yer et al., 2016; Yer et al., 2018). This study aimed to indicate the expression pattern of Hsp family genes under cadmium (Cd) stress in different poplar taxa which are resistant to various stresses. This research will enable gene cloning and functional analysis that can be used for the protection of general resources and to establish resistant forests.

2. Materials and methods

2.1. Examination of sequence read archive

Sequence read archive (SRA) data were downloaded from the NCBI[1] and DnaNexus[2] databases and used to explore the expression levels of Hsp genes in different poplar taxa under various abiotic stress conditions such as aridity, salinity, and heavy metal exposure. For this analysis, RNA-seq data from SRP018922 (Ariani et al., 2015) and microarray data from the AFFY-131 ArrayExpress studies (Jiali et al., 2013) were retrieved from open source sequence databank archives. All readings were downloaded as raw sequence data files (.sra format) and converted to the fastq format. After elimination of low-quality readings [Phred quality (Q) score < 20], a FastQC analysis was conducted to evaluate the qualities of all remaining clean readings in terms of sequence quality, quality score, nucleotide content, and sequence duplication levels for each base. All readings were transformed and normalized using CLC Genomic Workbench software, version 10.1. After determining reads per kilobase million (RPKM) values, a hierarchical clustering heat map was created using Permut Matrix software (Baloglu, 2014a; Baloglu et al., 2014b; Baloglu et al., 2014c; Baloglu et al., 2014d; Baloglu et al., 2015).

2.2. Plant materials and application of cadmium stress

Different poplar taxa were kindly provided by the poplar populentums at Ankara Behiçbey Forest Nursery Garden and used as experimental material. These poplar taxa were obtained via stem cutting, a vegetative reproduction method. The taxa of *Populus tremula* L. (named *TK*): *I-214* (natural cross-breed of European and American black poplars; *Populus euramericana* and black poplar); I-77/51, *Populus deltoides* Bartr. of *Samsun* (named *Samsun*); *Populus nigra* L. of *Geyve* (named *Geyve*); and *N.03.368.A* (N.03.368.A) were used to evaluate the expression levels of Hsp genes under Cd stress. A liquid plant layering hormone [indol-3-butyric acid (IBA)], fungicide, boric acid, and Farmatalk-8000 ppm were diluted with sterile water. This application provides support to root formation from stems. The stems were exposed to this mixture for 10 s. After hormone application, the stems were planted in 25 cm × 40 cm sapling bags filled with solid medium comprising a 1:3 peat:perlite mixture. Once the saplings reached a height of 80–100 cm, cadmium stress was applied based on previous studies (He et al., 2013; Baccioa, 2014). The stress application involved the daily application of a 200 mM $CdSO_4H_2O$ solution to the plants during the cool evening hours. According to the weights of the sapling bags, poplar taxa in the stress and control groups were watered with 100–250 mL of the $CdSO_4H_2O$ solution and water, respectively. Leaf samples were collected from both groups on the 28th day (28d) after the beginning of stress application. Three biological samplings were collected and stored at –80 °C for species-specific gene expression evaluations.

2.3. RNA isolation and quantitative real-time PCR analysis

Total RNA was extracted using our previously described CTAB procedure (Yer et al., 2016; Yer et al., 2018). RNA samples from the control and stress treatments were treated with DNase I (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA) to remove DNA contaminants. The quality and integrity of the isolated RNA were evaluated using agarose gel electrophoresis and the Multi Scan Go device (Thermo Fisher Scientific, Waltham, MA, USA), respectively. The RNA samples were then converted into cDNA using kits and reagents purchased from Fermentas (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA). For qRT-PCR analyses, Hsp genes were selected based on expression levels in RNA-seq data obtained from previous studies (Yer et al., 2018). Subsequently, Hsp genes exhibiting strong or differential expression under cadmium stress in transcriptome data were selected for qRT-PCR. *Hsp* gene-specific primers were designed and checked using the NCBI Primer Blast tool[3]. The GC content, Tm values, and hairpin formation of the primers were also evaluated using the Beacon Designer™ database[4]. Poplar *18SrRNA*, which has been frequently used in the literature, was selected as an internal control gene; the forward and reverse primer sequences were

[1] (http://www.ncbi.nlm.nih.gov/sra)

[2] (http://sra.dnanexus.com)

[3] (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)

[4] (http://www.premierbiosoft.com/qOligo/Oligo)

5′-TCAACTTTCGATGGTAGGATAGTG-3′ and 5′-CCGTGTCAGGATTGGGTAATTT-3′, respectively (Tang et al., 2015). A list of primers used is given in Table S1 (supplementary material 1). All selected Hsps were first amplified using conventional polymerase chain reaction (PCR) for melting temperature (Tm) optimization. Next, gene-specific primers were used to conduct a qRT-PCR analysis. Three biological replicates and three technical replicates were performed for each sample. The SYBR Green master mix and Light Cycler 480 Real-Time PCR system (Roche Applied Science, Penzberg, Upper Bavaria, Germany) were utilized for specific gene expression analyses (Chun et al., 2020). Relative gene expression levels were calculated using *18SrRNA* gene expression as a reference. The ΔCT and ΔΔCT calculations (Livak and Schmittgen, 2001) were performed as indicated in our previous studies (Baloglu, 2014a; Baloglu et al., 2014b; Yer et al., 2016; Celik Altunoglu et al., 2016; Celik Altunoglu et al., 2017; Yer et al., 2018).

2.4. Statistical analysis

All gene expression analyses were carried out using three biological replicates with technical replicates. Standard error of mean (SEM) and one-way ANOVA analyses were performed with Minitab version 17. The statistical difference between stressed samples and control samples was indicated as follows: "*" $P < 0.1$; "**" $P < 0.05$; "***" $P < 0.01$.

3. Results

3.1. Evaluation of transcriptome data

RNA-Seq data were obtained from a database (SRA-Sequence Read archive) by selecting poplar species for heavy metal and Cd stresses. The results of the SRP018922 experiment were utilized for Cd stress. Based on these analyses, the control group scores and different levels of expression of the genes *PtsHsp*, *PtHsp40*, *PtHsp60*, *PtHsp70*, *PtHsp90*, and *PtHsp100* in response to Cd stress were shown. In addition, A-AFFY-131 ArrayExpress[5] data for the hybrid type Populus × canescens (*Populus alba* × *Populus tremula*) were used to evaluate Cd stress. Related readings were downloaded from the database. The E-MEXP-3741 coded microarray results were checked and compared with the access numbers of all Hsp genes. However, only data for *Hsp40* could be obtained. For other Hsp gene families (*sHsp*, *Hsp60*, *Hsp70*, *Hsp90*, and *Hsp100*), no access numbers were found to match the existing array results. The levels of *PtsHsp* gene expression varied between the Cd stress-exposed and control groups. Under Cd stress, 26% of the evaluated genes exhibited an upregulation pattern according to the SRP018922 transcriptome data. Moderate changes in gene expression were observed for 15% of all *PtsHsp* genes. By contrast, 35% of the *PtsHsp* genes were downregulated (supplementary material 2). Similarly, 26% of *PtHsp40* genes were expressed at high levels according to SRP018922 transcriptome data. A total of 60% of *PtHsp40* genes showed a reduction in gene expression level under stress conditions relative to the control group (supplementary material 3). When the microarray results were examined, the *PtHsp40-48*, *-74*, *-92*, *-101*, *-109*, *-113*, *-117*, *-129*, and *-137* genes were responsive to Cd-stress conditions (supplementary material 4). According to the SRP018922 transcriptome data, *PtHsp60* genes under Cd stress indicated a similar ratio in terms of upregulation and downregulation. About 47% of the genes responded moderately to stress conditions (supplementary material 5). Increases in gene expression levels for *PtHsp70-01*, *-02*, *-07*, *-08*, *-13*, *-21*, and *-28* were observed under Cd stress based on SRP018922 transcriptome data. By contrast, 62% of the genes exhibited a downregulation expression pattern in *PtHsp70* gene family members. Furthermore, expression of the *PtHsp70-25*, *-29*, *-31*, and *-34* genes did not vary between the control and stress application groups (supplementary material 6). According to the SRP018922 transcriptome data, the expression levels of the *PtHsp90-04*, *-05*, *-10*, and *-12* genes increased under Cd-stress conditions. Furthermore, the *PtHsp90-01*, *-02*, *-03*, *-06*, *-08*, *-09*, and *-11* genes appeared to develop a response mechanism to conditions of short-term stress (supplementary material 7). Under Cd stress, a total of 47% of the *PtHsp100* genes were upregulated based on SRP018922 transcriptome data. By contrast, 38% of the genes exhibited a downregulation expression pattern under stress conditions (supplementary material 8).

3.2. Evaluation of qRT-PCR analysis

To examine Cd stress responses of *PtHsps* genes in different poplar taxa, 24 *PtsHsp* genes were selected according to transcriptome data, and their expression levels were checked via qRT-PCR. *PtsHsp-13* and *-44* were selected for the experimental step, because both genes were expressed at relatively high levels in the transcriptome data. Additionally, *PtsHsp-03* and *-54* were selected as representative genes for indication of reduced gene expression (supplementary material 2). In this experimental analysis of the selected *PtsHsp* genes, which belonged to *N.03.368A* and *I-214* taxa, the expression of *PtsHsp-44* and *-54* increased under stress compared to the control group. These taxa can be deemed sensitive to Cd stress (Figure 1).

PtHsp40-36 and *-117*, which were expressed at high levels according to the transcriptome data, were selected for this experimental step. In addition, *PtHsp40-17* and *-69* were selected because these genes exhibited downregulation expression (supplementary materials

[5] (http://www.ebi.ac.uk/arrayexpress/)

3 and 4). In an experimental analysis of the selected *PtHsp40* genes, the *N.03.368*A poplar taxon developed a Cd stress response mechanism. In contrast, the *Geyve*, *I-214*, *Samsun*, and *P. tremula* (*TK*) poplar taxa may be susceptible to stress (Figure 2).

PtHsp60-12 and *-42*, which were strongly expressed in the transcriptome data, were selected for qRT-PCR analysis. *PtHsp60-06* and *-33* were selected for their moderate expression pattern (supplementary material 5). According to the results of the analysis, the *Geyve* poplar taxon may be more sensitive to Cd stress than the other poplar taxa. *PtHsp60-12* was strongly expressed in taxon *I-214*. This gene was also upregulated in the *N.03.368A* taxon and *P. tremula* (*TK*). Figure 3 demonstrates that *Hsp60* genes formed the response to stress. The *Geyve* poplar taxon may be more sensitive to Cd stress than the other poplar taxa.

PtHsp70-09, *-21*, and *-28*, which were strongly expressed based on transcriptome data, were selected for this experimental step. *PtHsp70-24* was selected to represent only genes with decreased expression levels (supplementary material 6). In an experimental analysis of the selected *PtHsp70* genes, the *Geyve, N03.368A*, and *I-214* taxa may produce a response against Cd-stress conditions. The *Samsun* and *P. tremula* (*TK*) taxa appeared to be more Cd-sensitive than other poplar taxa. The expression levels of *Hsp70* genes are shown in Figure 4.

PtHsp90-10 and *-12*, which were expressed at high levels according to transcriptome data, were chosen for this experimental step. In addition, *PtHsp90-07* was selected as an example of moderate expression. *PtHsp90-02* was selected as an example for downregulated expression (supplementary material 7). The poplar taxa *Geyve*, *Samsun*, and *P. tremula* (*TK*) are sensitive to Cd stress. The taxa *N.03.368A* and *I-214* formed responses against stress. Among the poplar taxa in this study, the *N.03.368A* taxon exhibited high levels of gene expression under Cd-stress conditions (Figure 5).

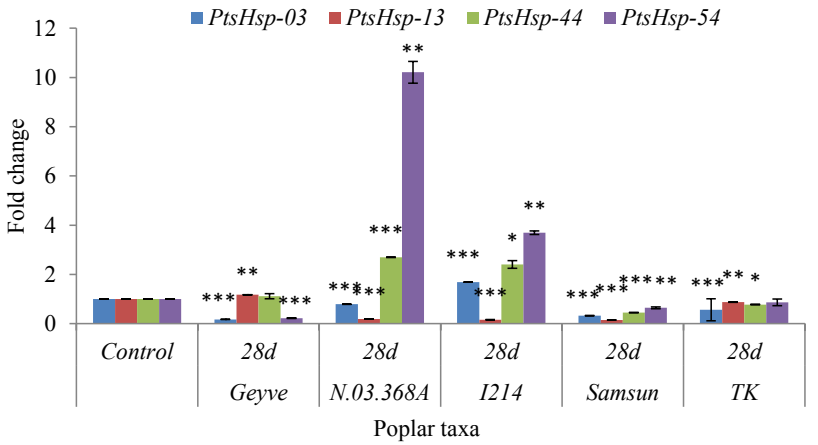

Figure 1. Expression profile of *PtsHsp* genes.

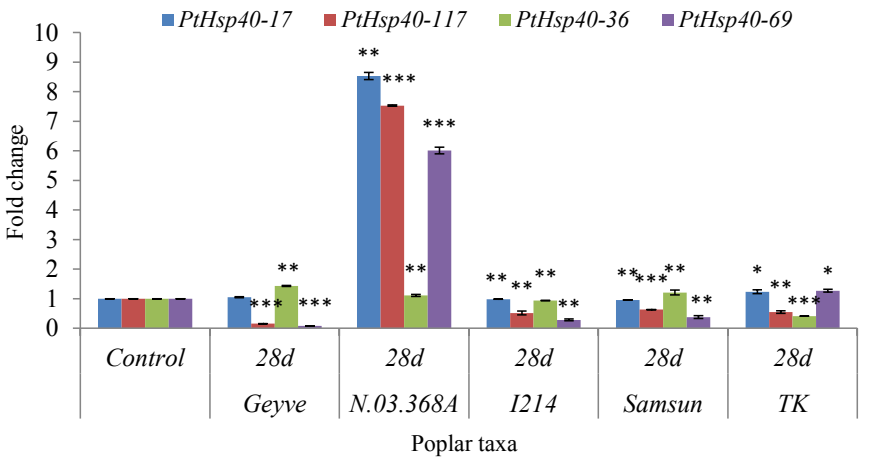

Figure 2. Expression profile of *PtHsp40* genes.

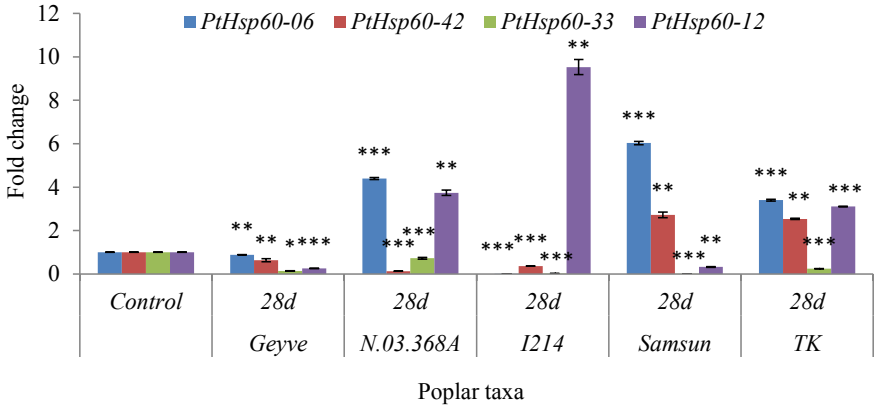

Figure 3. Expression profile of *PtHsp60* genes.

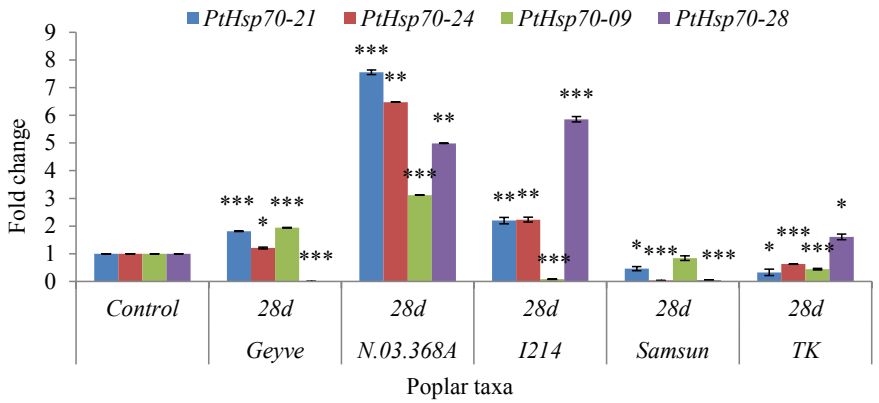

Figure 4. Expression profile of *PtHsp70* genes.

PtHsp100-29, *-51*, and *-71* had high levels of expression according to the transcriptome data and were selected for this experimental step. *PtHsp100-22* was selected as the representative gene with downregulated expression (supplementary material 8). However, as shown in Figure 6, the *N.03.368A* and *Samsun* taxa exhibited upregulated *PtHsp100-22* expression. Compared to other poplar taxa, the *N.03.368A* taxon exhibited increased expression of all selected genes against Cd stress (Figure 6).

4. Discussion

Plants activate stress responsive pathways in order to tolerate the damaging effects of abiotic stress factors. Heat shock proteins are one of these responses and accumulate rapidly in the cell center. These proteins have many functions. To protect against stress-damage effects, they provide chaperon activity for correct protein folding (Wang et al., 2014; Zafar et al., 2016). This study indicated that Hsp genes played a protective role against Cd stress as well. The above-described investigation of transcriptome data and experimental results regarding Hsps gene family sequences suggests that the response mechanisms against Cd stress in the poplar genome vary depending on differences in the poplar taxa and *PtHsp* genes. Generally, the results of qRT-PCR and RNA-Seq were consistent. Our study demonstrated that the different responses of *Hsps* genes to Cd stress were attributable to genotypic variation. In addition, findings from a chemical, morphological, and proteomic investigation of Cd stress in poplar species, which determined that Cd-stress–related damage occurred in leaf tissues, is consistent with this study (Marmiroli et al., 2013). *At4g27670* (*Hsp21*), an ortholog of *PtsHsp-54*, was involved in responses to heat stress (Kim et al., 2012). In our study, we observed relatively high gene expression levels in the *N.03.368A* and *I-214* poplar taxa under Cd stress. *At5g59720*, an orthologue of the *PtsHsp-13* gene in *A. thaliana*, was also expressed at high levels under heat stress (Ruan, 2012). The *At2g17880* in *A. thaliana*, an orthologue of *PtHsp40-17*, did not exhibit a different stimulation pattern under epigenetic stress relative to the control (Najafi and Farahani, 2013). These results were consistent between the transcriptome data and our experimental results. This finding may be related to the indirect role these genes play in the stress tolerance mechanism. Based on the transcriptome data, *PtHsp40-117* was expressed at higher levels under saline stress, another abiotic stress

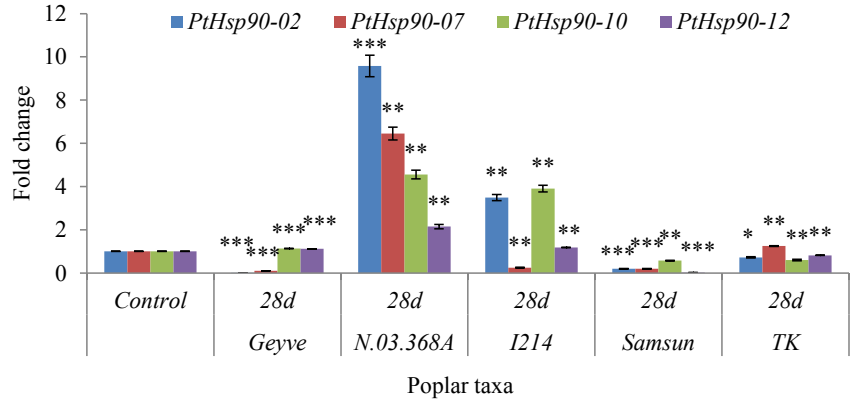

Figure 5. Expression profile of *PtHsp90* genes.

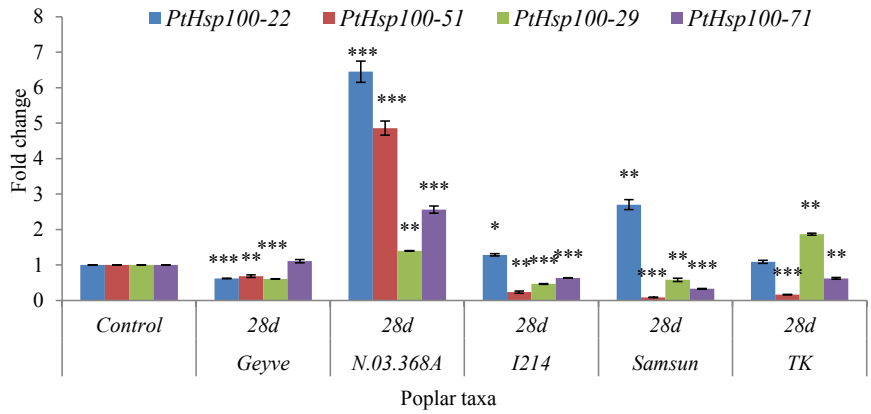

Figure 6. Expression profile of *PtHsp100* genes.

that we are currently studying based on the results of the E-MEXP-3741 array. Notably, the *At2g42750.1* gene was similarly upregulated under stress conditions in *A. thaliana* (Liu et al., 2015). In our study, we found that this gene was only involved in the Cd-stress–response mechanisms of the *N.03.368A* taxa. *PtHsp60-12*, as well as its *A. thaliana* orthologue *At3g23990.1* gene and *Oryza sativa LOC_Os03g04970.1* gene, played important roles in protein folding under normal conditions and were strongly upregulated under various stress conditions (Demirevska et al., 2008). Those results are consistent with our study findings. This suggests that *PtHsp60-12* plays an important functional role under both normal and stress conditions. Regarding the selected *PtHsp70* genes, our experiments demonstrated that the *Geyve*, *N03.368A*, and *I-214* taxa responded to Cd stress. The *Samsun* taxon and *P. tremula* (*TK*) appeared to be more susceptible to stress than the other poplar taxa. A previous study of *A. thaliana* found that the *PtHsp70-21* orthologue *At2g32120* was stimulated by exposure to high salt stress and dehydration (Liu and Zhu, 1998). Another study found that gene expression increased in response to high temperature stress (Livak and Schmittgen, 2001). Furthermore, previous studies also observed increased *PtHsp70-28* expression under stress conditions, which is consistent with our findings (Lim et al., 2006; Yang et al., 2014). Several studies found that *O. sativa Hsp* gene LOC_Os05g23740, which is orthologous to *PtHsp70-09*, was stress responsive (Wei et al., 2009). Similarly, the expression levels of *PtHsp70-09* orthologues including *At5g49910* (*A. thaliana*), *Sb08g009580* (*Sorghum bicolor* L.), *Pgl_GLEAN_10002651* (*Pennisetumglaucum* L.), and *LOC_Os12g14070* (*O. sativa* L.) increased under heat and drought stress (Ghatak et al., 2016). *Oryza sativa LOC_Os05G38530*, an orthologue of *PtHsp70-24*, was temperature-stress responsive (Jeong and Jung, 2015). Our study findings are consistent with these studies. Specifically, the *N.03.368A* and *I-214* taxa are stress responsive. Relative to the other tested taxa, *N.03.368A* exhibited high levels of expression for all selected genes against Cd stress. Previous studies of the *PtHsp90-02* orthologues *At5g56000* in *A. thaliana* (91% similarity) and *LOC_Os08g39140* in *O. sativa* (89%) demonstrated the expression of both genes under drought-stress conditions (Hasegawa et al., 2006; Landi et al., 2017). In

our experimental study, we observed a strong expression profile of *PtHsp90-02* in the *N.03.368A* and *I-214* taxa. Furthermore, the expression of *PtHsp90-10P* and its orthologue *AT3G07770* (*A. thaliana*) increased (Zhang et al., 2016a). However, another study found that *LOC_Os12g32986* (*O. sativa*), an orthologue of *PtHsp90-10*, was downregulated under drought stress (Zhang et al., 2016b). The expression of *At2g04030* (*A. thaliana*), an orthologue of *PtHsp90-10*, increased in response to high salinity (Taji et al., 2004). These observations may be attributed to the ability of these genes to respond differently to various types of stresses at varying levels. The roles of different Hsp genes in stress tolerance have been identified, but more research needs to be done to detail the functions of these genes. The functional genomic assays conducted can serve as a basis for various projects intended to clarify the metabolic roles of *Hsp* genes under stress conditions. According to our RNA-Seq and microarray data, the following *Hsp* genes were selected and were more strongly upregulated under Cd-stress conditions (relative to other genes): *PtsHsp-44*, *PtsHsp-54*, *PtHsp40-117*, *PtHsp60-06*, *PtHsp60-12*, *PtHsp70-21*, *PtHsp70-28*, *PtHsp90-02*, *PtHsp90-10*, *PtHsp90-12*, *PtHsp100-22*, and *PtHsp100-71*. This study will provide information for the isolation, cloning, and use of these genes as stress-response biomarkers, particularly in plant transformation and genomics. In the future, trial plantations of *N.03.368A* and *I-214* taxa could be subjected to Cd stress and subsequent long-term observation.

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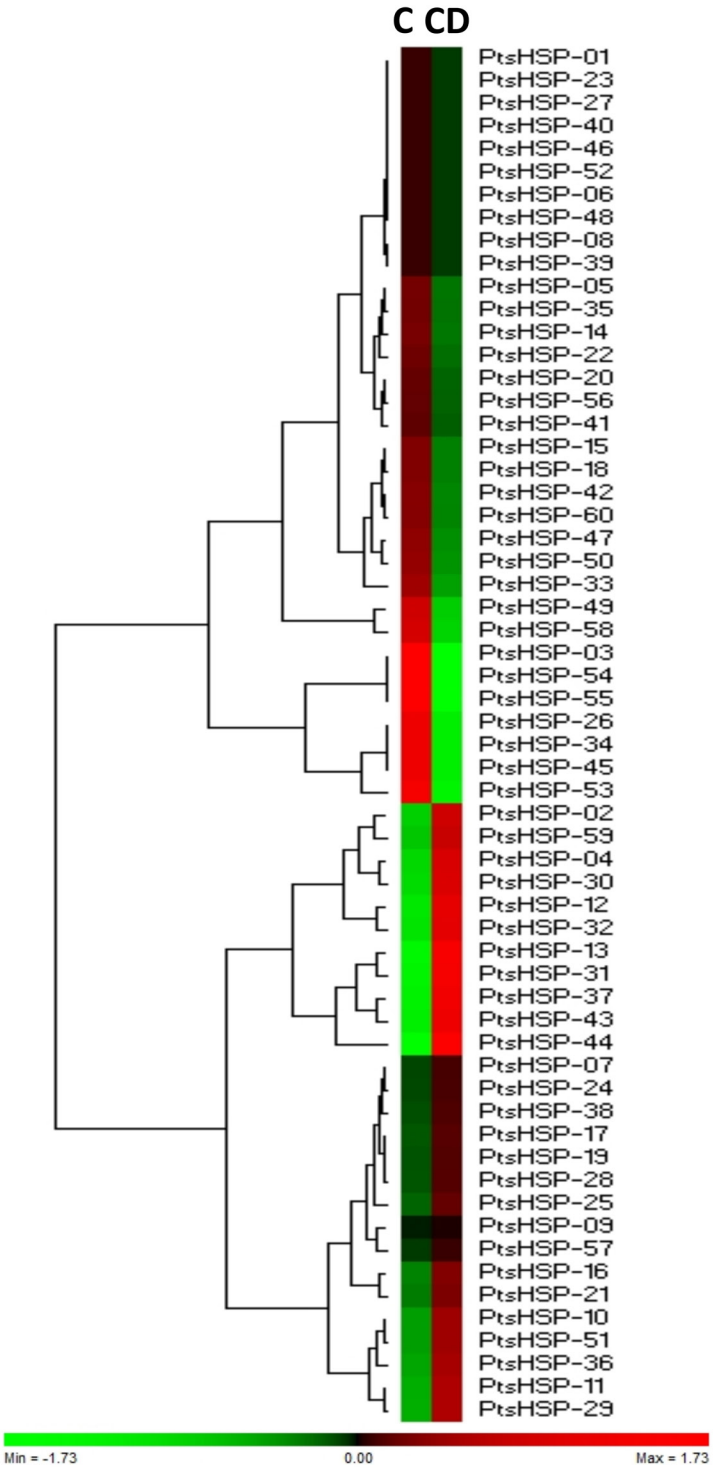
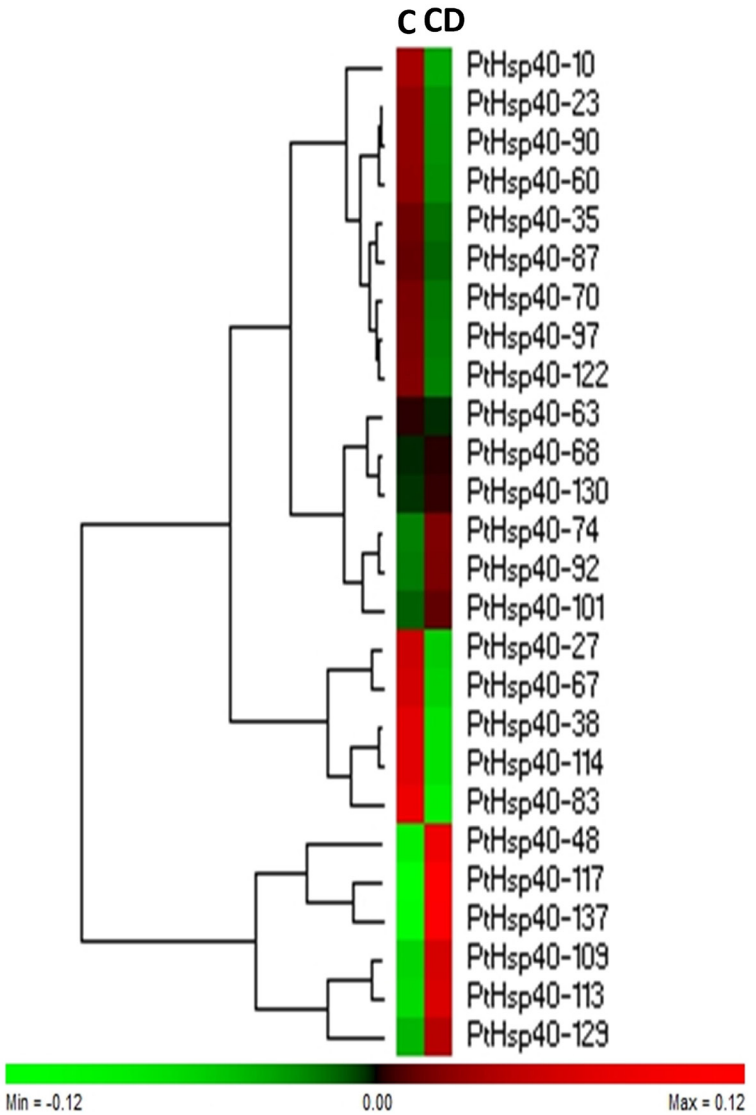
Supplementary material 1. List of primer sequences used for gene expression analysis by qRT-PCR

Name	Forward Primers (5′- 3′)	Reverse Primers (5′- 3′)
PtsHsp-03	CAATGTTCCTGCCTCAACTGG	ACCTGCGTAAAAACTTGCCG
PtsHsp-13	GGGTGCTTCAGATTAGCGGA	ACCCCGTTCTCCATAGAAGC
PtsHsp-44	GAGGACTCGAATAGCCACCAAC	CCTCATCCTCCTGTCCCCAATA
PtsHsp-54	GTCTTCGTCATCTGCCCCTT	AACCTCCCCTCTTGTCCTGT
PtHsp40-17	GCGTCTTTCAGCTCCTCCTC	TATTTGCGTGGCATTGCGGG
PtHsp40-36	TGAGCCAAACGAAGCCTAGC	GTTGCTGCTGTTCCACCCCTA
PtHsp40-69	AGACGGCGACTAACGAGGTA	TCCGATGGAAGCCCCAACTC
PtHsp40-117	CGATGGGAAAAGAGGCAAGC	AGCGGGAGGCTTATCAACAC
PtHsp60-06	TTTGACGGAATTGAACGGCT	AAAACCCTGCTGTCATTAACGG
PtHsp60-12	AGGTCTGTGCCATTACTCCT	TGACACTGTGACCTTAGCAG
PtHsp60-33	GGAAGCTCTGGCTCCAATCA	TAGTAGCAGTGCCCAACACC
PtHsp60-42	CCTCTTGCCTTCCCTCAACA	ACATTACGCCCGTTAGGTCC
PtHsp70-09	AGTGCTGGAGGTTGGTGATG	TCAGGGTGGTCTCAATGTGC
PtHsp70-21	GCCTTTTCCTGAACCACAGAGTC	ACCTTTGAGTCACGCAAGCAC
PtHsp70-24	CACCACCATTCCCACCAAGA	TAGGTGCTGGAGGAATGCCA
PtHsp70-28	GCCCTGAGGAGATTAGTGCC	ATCCTCGCAACTCTCACACC
PtHsp90-02	AAGAGTGGTGACGAGCTGAC	CCCACGGCGTACTCATCAAT
PtHsp90-07	GGCACAAGCACTGAGGGATA	CCCTCCTCAGCAGCATCTTC
PtHsp90-10	GTCACCCTGTGTCCTTGTGT	CATCATCCTGGTTGCTCCTG
PtHsp90-12	CCTCGTGTTGCTGCTGTTTG	CCACCTCCTCCATCTTGCTC
PtHsp100-22	CATTGGTAGCAAGCGTGGC	TTTCCTGCAGTAGAGCGTCC
PtHsp100-29	GCACATCAAAGAAGGGGTTGC	TAGCAGAGGGGGAACAAGGT
PtHsp100-51	TGGTGATTCTTCCTCTGCGG	CATCGCCAAACCACTTGCTC
PtHsp100-71	GTGGTAGGACAGGACCAAGC	TCCCACATACCCAGGAGGAG
18S rRNA	TCAACTTTCGATGGTAGGATAGTG	CCGTGTCAGGATTGGGTAATTT

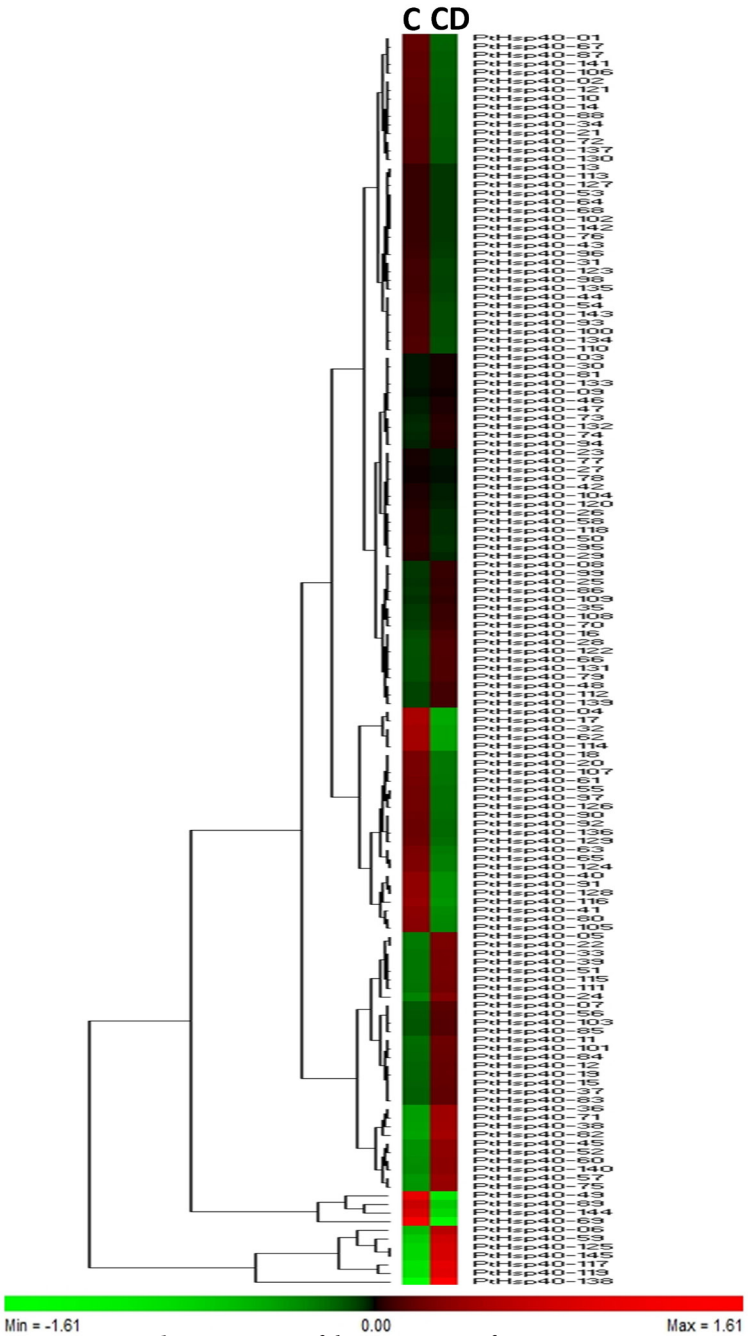
Supplementary material 1: List of primer sequences used for gene expression analysis inqRT-PCR.



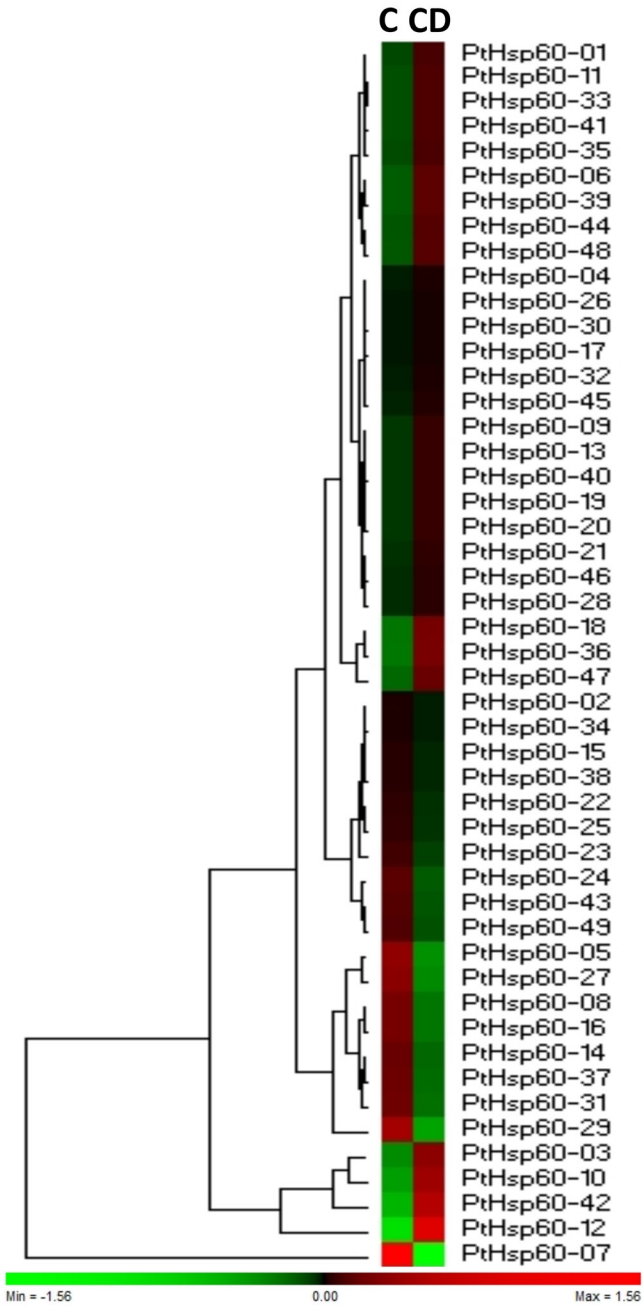
Supplementary material 2: Heat maps of the *sHsps* genes for SRP018922 transcriptome data.



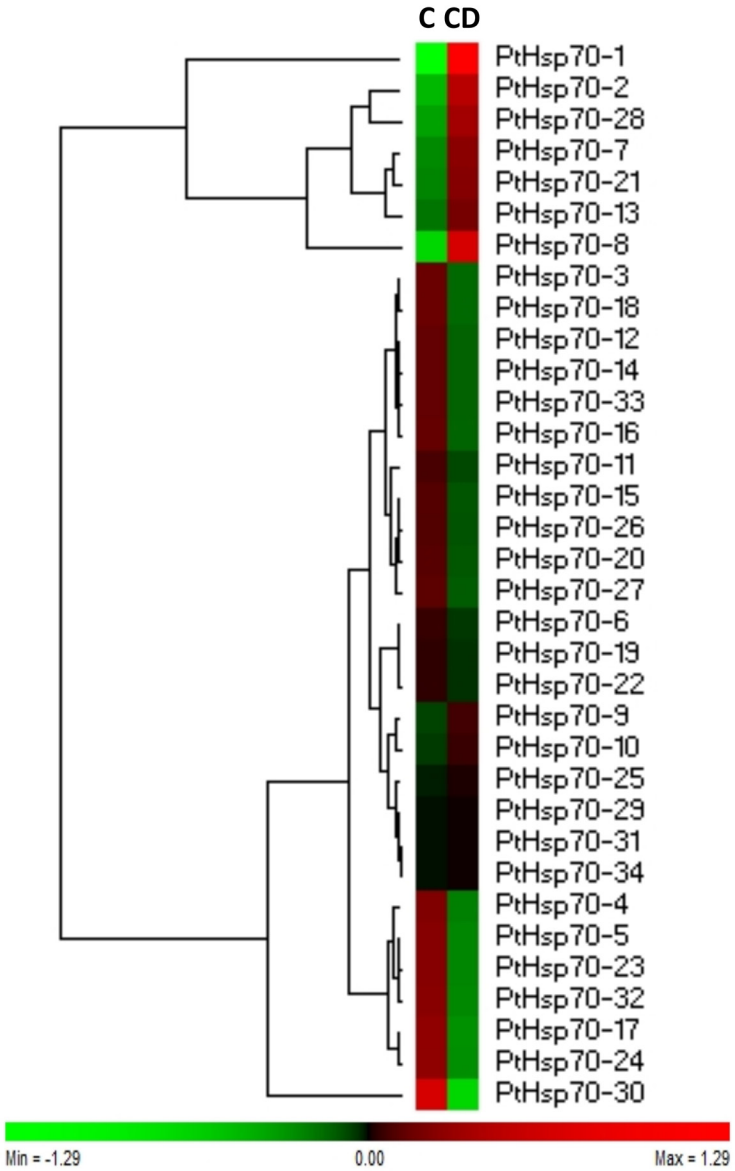
Supplementary material 3: Heat maps of the *Hsp40* genes for ArrayExpress E-MEXP-3741 data.



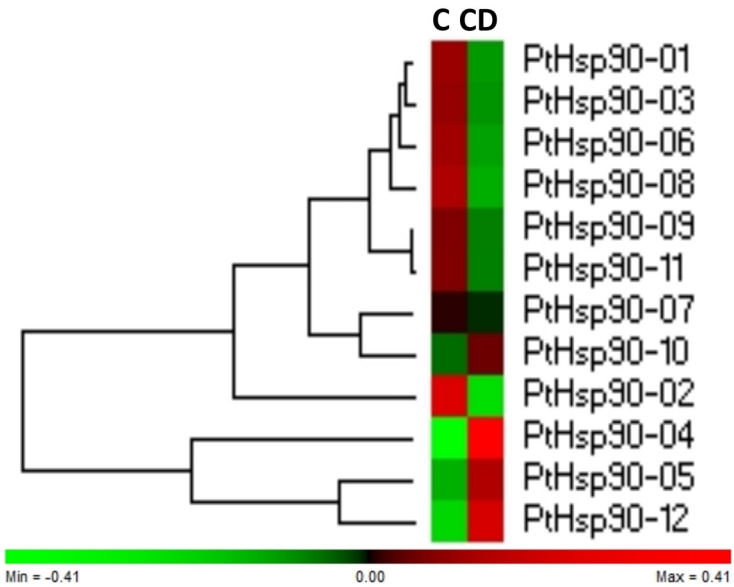
Supplementary material 4: Heat maps of the *Hsp40* genes for SRP018922 transcriptome data.



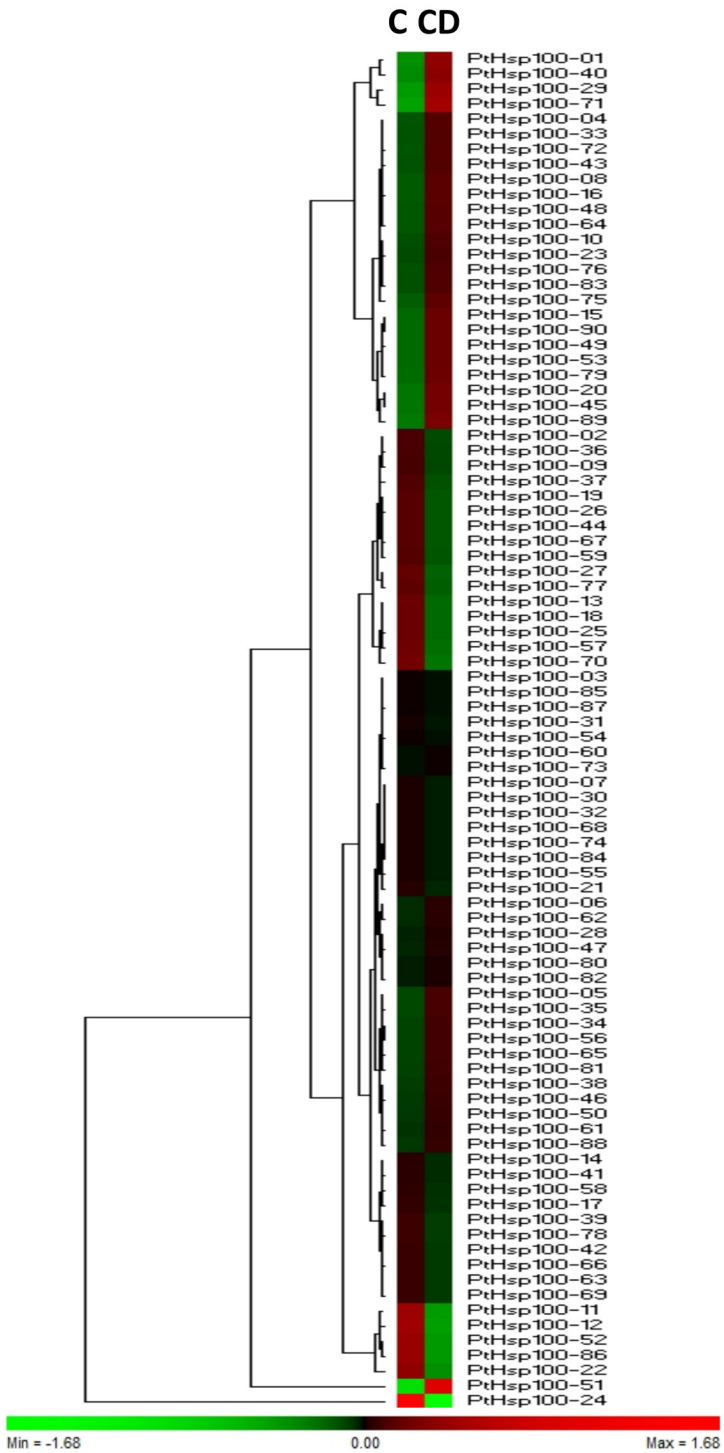
Supplementary material 5: Heat maps of the *Hsp60* genes for SRP018922 transcriptome data.



Supplementary material 6: Heat maps of the *Hsp70* genes for SRP018922 transcriptome data.



Supplementary material 7: Heat maps of the *Hsp90* genes for SRP018922 transcriptome data.



Supplementary material 8: Heat maps of the *Hsp100* genes for SRP018922 transcriptome data.