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ORIGINAL ARTICLE

Evolutionary history of the heat shock protein 90 (Hsp90) family of 43 plants and characterization of Hsp90s in *Solanum tuberosum*

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Abstract

Heat shock protein 90 genes/proteins (Hsp90s) are related to the stress resistance found in various plant species. These proteins affect the growth and development of plants and have important effects on the plants under various stresses (cold, drought and salt) in the environment. In this study, we identified 334 Hsp90s from 43 plant species, and Hsp90s were found in all species. Phylogenetic tree and conserved domain database analysis of all Hsp90s showed three independent clades. The analysis of motifs, gene duplication events, and the expression data from PGSC website revealed the gene structures, evolution relationships, and expression patterns of the Hsp90s. In addition, analysis of the transcript levels of the 7 Hsp90s in potato (*Solanum tuberosum*) under low temperature and high temperature stresses showed that these genes were related to the temperature stresses. Especially StHsp90.2 and StHsp90.4, under high or low temperature conditions, the expression levels in leaves, stems, or roots were significantly up-regulated. Our findings revealed the evolution of the Hsp90s, which had guiding significance for further researching the precise functions of the Hsp90s.

Keywords Hsp90 · Heat resistance · Cold resistance · Potato (*Solanum tuberosum*) · Abiotic stress

Introduction

Plants are continuously affected by various environmental factors, including temperature variations, salinity, and drought during the growth process. These stresses can lead to many undesirable consequences such as hindered plant growth and reduced crop yields. Under various adverse environment conditions, plants have a complex set of physiological, biochemical and molecular regulatory mechanisms to prevent cell damage and maintain the normal growth and development of plants [1]. The Hsps in plants is a family that is very sensitive to temperature changes [2–4], and has an important influence on the heat resistance of plants [5].

Hsps are widely found in animals, plants and microorganisms [6], and according to the molecular weight, they can be divided into 6 types: smHsp (small Hsp, 15–39 kDa), Hsp60 (50–60 kDa), Hsp70 (66–78 kDa), Hsp90 (80–90 kDa), Hsp100 (90–100 kDa), Hsp110 (> 100 kDa) [7]. Among them, Hsp90s is a highly conserved molecular chaperone. Normally, Hsp90s in most cells of prokaryotic and eukaryotic account for 1–2% of all cellular proteins [8, 9]. However, under high temperature stress, the proportion of Hsp90s in total protein will rise to 4–6% [3].

Wan Li and Yue Chen have contributed equally to this work.

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The structures of Hsp90s are very conservative and consist of three regions: The N-terminal containing ATP binding and hydrolysis sites, the middle region (M) and the C-terminal domain containing the dimerization region [10]. According to the source and subcellular location, the Hsp90s can be divided into five subgroups: Hsp90A, Hsp90B, Hsp90C, TRAP (TNF receptor-associated protein) and HTPG (high temperature protein G) [11]. Hsp90A contains no signal peptide and is located in the cytoplasm. The main subtypes include Hsp90α (inducible form) and Hsp90β (constitutive form), which are the result of gene duplications about 500 million years ago [8, 12–14]. Hsp90B, Hsp90C and TRAP are located in the endoplasmic reticulum, chloroplast and mitochondria (Animalia) respectively because of containing signal peptides. HTPG refers to Hsp90 of prokaryotes and is distributed in most bacteria [11, 15]. Hsp90s are associated with protein folding, activation, and maturation, as well as the conformational transition and stability of proteins that related to signal transduction [16]. The signal transduction proteins, such as steroid hormone receptors [17], transcription factors and protein kinases [2, 3, 18], and some substrates that initiate stress signal transduction [19], many of which may be involved in controlling plant growth and development are the 'client' proteins of the Hsp90s [20].

Hsp90 family is a kind of protein with multiple functions, which is widely involved in important processes such as signal transduction and cell cycle [21]. Whether in animals or plants, the Hsp90 family has extremely important functions. Hsp90s are abundantly expressed in the cytoplasm in soluble form at normal temperatures in yeast, fruit flies, and vertebrate families, while they accumulate rapidly in the nucleus under heat shock conditions [22]. In potatoes, Hsp90s may be related to the color of potato tuber chips [23]. Hsp90s play vital roles in the growth of tumor cells. For example, geldanamycin can specifically interact with the ATPase active site of Hsp90, preventing the binding of Hsp90 and ATP, and finally achieve the purpose of inhibiting tumor [24]. Using interference technology, the expression level of Hsp90 was reduced, and it was found that the division rate of U937 cells was significantly reduced [25]. Hsp90s, as housekeeping proteins in plants, can be induced by various abiotic and biotic stresses [5, 26]. The expression level of UpHsp90 in *Ulva pertusa* is notably positively regulated by the change of temperature difference between day and night, but it was almost unaffected under long-term treatment with heavy metal stress [27]. Overexpression of rHsp90 gene which is from rice (*Oryza sativa*) in tobacco (*Nicotiana tabacum*) can significantly improve tobacco tolerance to salt and alkali [28]. In *Arabidopsis thaliana*, the overexpression of Hsp90.2 will inhibit the transcription of HsfA2, and HsfA2 expressed under the inhibition of Hsp90.2 contributes to the resistance to oxidative stress [29]. Similarly, an Hsp90 inhibitor produced by root-peripheral fungi can inhibit plant growth and development, but can increase the resistance of *Arabidopsis* to high temperatures [30]. The Hsp90 complex in *Arabidopsis* directly regulates the activity of resistance proteins and plays a key role in disease resistance [31]. The resistance of tobacco leaves to mosaic virus increases because of the interaction of Hsp90 with RAR1 and TIR-NB-LRR in tobacco leaves [32]. Overexpression of TaHsp90.2 and TaHsp90.3 can enhance the tolerance to stripe rust in wheat (*Triticum aestivum*) [33]. In tomato (*Solanum lycopersicum*), Hsp90 takes a critical part in the resistance to Pseudomonas [34].

At present, the research on plant Hsp90s lag significantly behind that of animal Hsp90s, and the understanding of its biological function is still quite lacking. Hsp90s have been identified from many plants (grape (*Vitis vinifera*), rice, tomato, *Brachypodium distachyon*) [15, 35–37]. There are seven Hsp90s appeared in *Arabidopsis* [15]. Among them, AtHsp90.1–AtHsp90.4 are located in the cytoplasm [38]; AtHsp90.5 and AtHsp90.6 are distributed in the chloroplast and mitochondria, respectively [39, 40]; AtHsp90.7 is the endoplasmic reticulum (ER) resident protein [41]. In this study, Hsp90s from the complete genomic sequences of 43 plants (covering the stages from lower plants to higher plants, including algae, moss, ferns, gymnosperms, angiosperms) were identified by using bioinformatics methods. Then we analyzed the phylogenetic relationships, motifs, gene duplications, gene characteristics and gene structure. In addition, we analyzed Gene Ontology (GO) annotations and transcriptional profiles of the Hsp90s in potato (*Solanum tuberosum*). Based on the results, the evolution of the Hsp90s can be better revealed, and some useful information can be provided for further research on potatoes.

Results and discussion

Identification of Hsp90s in 43 plant species

The Hsp90s sequences of *Arabidopsis* were used as a query to blast Hsp90s sequences from 42 other plant species. The 7 Hsp90s of *Arabidopsis* can be divided into three groups (Hsp90A, Hsp90B and Hsp90C) [15]. Based on the sequence structural feature and domain of the seven proteins, we analyzed candidate Hsp90s from 42 species, and removed incorrect and redundant sequences. In previous studies, Hsp90s have been identified in many species, such as grape, rice, tomato, *Brachypodium distachyon* [15, 35–37]. We integrated these results, and we searched and identified Hsp90 family again using the methods described in this article. For example, we screened with the latest version of the tomato database and found that Solyc04g081630 and Solyc05g010670 did not belong to the Hsp90 family [36], but a new one Solyc04g081570 was added. Finally,

we found a total of 334 Hsp90s in 43 plants and named and classified them (Table 1, Table S1a).

Forty-three plant species had 3–18 Hsp90s (Table 1). Only three Hsp90s were identified in each algae known as *Chlamydomonas reinhardtii* (Cr) and *Volvox carteri* (Vc), and wheat contained 18 Hsp90s. According to the general trend, the Hsp90s existed in both lower plants and higher plants. Then we gained the genome length of 43 species from the NCBI (Table 1) and found that there was a certain correlation between the genome length and the number of Hsp90s. But the correlation was not significant, which should be demonstrated with more evidences. Secondly, we found that the numbers of amino acids in most Hsp90s were between 600 and 900 (Table S1a), and that of several Hsp90s which only appeared in eudicots and monocots plant species were above 1000 or below 600. This showed that there was a relatively large difference between angiosperms and other plant species, which might confirm the evolution and functional differentiation of Hsp90 family.

Analysis of motifs and gene duplication events

In this study, we used the MEME online tool to identify the motifs of 334 Hsp90s (motifs = 20) (Fig. 1, Table S2). The number of motifs was between 10 and 20. Most proteins (more than 300 proteins) contained 17–20 motifs which had a basically same motif composition, and a few proteins just displayed 14, 15 or 16 motifs (Fig. 1). Additionally, PhHsp90.12 and BrHsp90.3 were the only protein with 10 and 13 motifs in *Physcomitrella patens* and *Brassica rapa*, respectively (Table S1a). From this, the motifs of Hsp90 family members are conserved, and these conserved motifs are involved in function and/or structure of the active proteins [42].

Gene duplication can explain the gene evolution process, and significantly affects the origin and evolution of species [43, 44]. In order to further understand the evolutionary relationship of Hsp90s, we analyzed the tandem and segmental duplications in 43 plant species based on the available information (In fact, due to the incomplete information of gene location and PGDD website, we can't identify the all gene duplication events) (Table 1, Table S1a, b). The gene duplication events were identified in 29 species. The tandem duplications were obviously less than segmental duplications. Tandem duplications didn't exist in soybean (*Glycine max*), but we found ten segmental duplications, which was the highest number in all species. *Physcomitrella patens* contained the most tandem duplication events, three pairs. With the evolution of species, the numbers of segmental duplications were significantly increased in most Angiosperms (especially in soybean), while the numbers of tandem duplications which were lower than that in *P. patens* were decreased in other higher plants (Fig. 2).

It is necessary and useful to calculating the ratio of non-synonymous nucleotides to synonymous nucleotide substitutions (Ka/Ks) for us to estimate selective pressure [45, 46], which is commonly used to analyze the molecular evolution rate [47]. In total, 75 gene pairs were analyzed using DnaSP6. Ka/Ks ratio values were much below 1.0 in all gene pairs (Table S1b), which indicated that these gene pairs were subject to purification selection [44]. The increase in the amount of Hsp90s may be related to the emergence of numerous duplication events which promote the evolution and differentiation of gene functions in plants, and the ratio of Ka/Ks can partially explain the evolutionary patterns of Hsp90s [43].

Analysis of the phylogenetic tree

The 334 Hsp90s from 43 plant species were used to construct the phylogenetic tree (Fig. 3). The 334 proteins can be roughly divided into three groups, which was consistent with the results of CDD analysis (Table 1). The Hsp90A family had more members than other groups, with more than 170 proteins. The Hsp90B family contained the fewest Hsp90 protein members, but there were also differences in details. If these proteins were classified by CDD according to the conserved domain of Hsp90s in *Arabidopsis*, CpHsp90.4, OsHsp90.2, PaHsp90.1 and TaHsp90.14 should belong to Hsp90B, Hsp90C, Hsp90B and Hsp90C subfamily, respectively. But in phylogenetic tree, CpHsp90.4, OsHsp90.2, PaHsp90.1 and TaHsp90.14 were distributed in Hsp90C, Hsp90B, Hsp90A and Hsp90B group, respectively. In addition, in algae, *Chlamydomonas reinhardtii* and *Volvox carteri* all didn't contain the proteins of Hsp90B subfamily. The classification results of Hsp90s of rice and *Chlamydomonas reinhardtii* were different from the previous research [15], which may be due to different classification criteria, or different genomic databases used.

Phylogenetic trees are a mature method for examining the structure and function of protein families and inferring functional relationships [52]. *Arabidopsis* is one of the most common model plants used to study the structure and function of the proteins [15]. We specifically marked the Hsp90s of *Arabidopsis* and potato that is our main research species. The function of Hsp90s in potato could be predicted based on functional performance of that in *Arabidopsis*. In *Arabidopsis*, increased expression level of AtHsp90.3 will cause a decrease in plant tolerance to Cd, a reduction in germination rate and in root length, an increase in MDA content, and a decrease in the activity and content of various enzymes (SOD, POD, CAT, etc.). The results show that Hsp90s play vital roles in plant tolerance to heavy metal stress and cellular responses [53]. However, the resistance to oxidative stress and the sensitivity to salt and drought stresses will decreased and increased, respectively, because of the

Table 1 The heat shock protein 90 (Hsp90) family members identified in 43 sequenced plant genomes

Lineage	Organism (Abbr.)	Genome total length (Mb)#	Numbers of Hsp90s	Numbers of motifs	Tandem duplication (pairs)	Segmental duplication (pairs)
Algae						
	Chlamydomonas reinhardtii (Cr)	120.405	3	15, 17, 19	0	0
	Volvox carteri (Vc)	137.684	3	16–17, 19	0	Not identified*
Mosses						
	Marchantia polymorpha (Mp)	225.761	5	17–20	0	Not identified
	Physcomitrella patens (Ph)	472.081	12	10, 16, 18, 20	3	1
Ferns						
	Selaginella moellendorffii (Sm)	212.315	6	17–18, 20	0	1
Gymnosperms						
	Picea abies (Pa)	11961.4	4	17, 19–20	0	Not identified
Angiosperms						
Amborellaceae	*Amborella trichopoda* (Ar)	706.495	4	19, 20	0	0
Eudicots						
Actinidiaceae	*Actinidia chinensis* (Ah)	653.926	13	17–20	0	5
Asteraceae	*Helianthus annuus* (Ha)	3027.84	9	17–20	0	Not identified
	Lactuca sativa (Ls)	2384.19	7	18–20	1	Not identified
Brassicaceae	*Arabidopsis thaliana* (At)	119.669	7	18, 20	2	0
	Brassica oleracea (Bo)	488.954	10	17–20	1	0
	Brassica rapa (Br)	401.927	9	13, 17–20	0	5
	Capsella rubella (Cb)	133.064	8	16–20	1	0
Caricaceae	*Carica papaya* (Cp)	370.419	5	16–17, 20	0	0
Chenopodiaceae	*Beta vulgaris* (Bv)	566.55	7	17–20	1	0
Cucurbitaceae	*Citrullus lanatus* (Cl)	365.45	5	18–20	1	0
	Cucumis sativus (Cs)	226.641	4	18, 20	0	0
Euphorbiaceae	*Ricinus communis* (Rc)	350.622	5	18–20	0	1
Leguminosae	*Glycine max* (Gm)	979.046	13	16–20	0	10
	Medicago truncatula (Mt)	412.924	7	18–20	1	0
	Phaseolus vulgaris (Pv)	521.077	6	18, 20	1	0
Malvaceae	*Eucalyptus grandis* (Eg)	691.43	10	14–15, 17–20	0	3
	Gossypium raimondii (Gr)	761.565	12	17–20	1	4
	Theobroma cacao (Tc)	324.88	6	18–20	0	1
Nelumbonaceae	*Nelumbo nucifera* (Nn)	804.648	11	18–20	Not identified	4
Rosaceae	*Malus domestica* (Md)	703.358	10	17–20	0	Not identified
	Prunus persica (Pp)	227.569	6	18–20	0	0
	Pyrus bretschneideri (Pb)	508.551	9	18–20	Not identified	6
Rutaceae	*Citrus sinensis* (Ci)	327.83	6	18–20	0	0
Salicaceae	*Populus trichocarpa* (Pt)	434.29	10	17, 19–20	0	4
Solanaceae	*Capsicum annuum* (Cu)	2935.88	7	16, 18–20	0	1
	Solanum lycopersicum (Sl)	828.349	6	18–20	0	2
	Solanum tuberosum (St)	705.934	7	17–20	0	2
Vitaceae	*Vitis vinifera* (Vv)	486.197	5	14–15, 18–19	0	0
Monocots						
Arecaceae	*Elaeis guineensis* (El)	1535.18	13	17–20	2	0
Musaceae	*Musa acuminate* (Ma)	472.231	11	14–19	0	5
Orchidaceae	*Phalaenopsis equestris* (Pe)	1064.2	6	18, 20	Not identified	0
Poaceae	*Brachypodium distachyon* (Bd)	271.299	8	18, 20	2	1
	Hordeum vulgare (Hv)	1779.49	5	18–20	0	Not identified
	Oryza sativa (Os)	374.423	8	18–20	1	2
	Triticum aestivum (Ta)	14547.3	18	14, 17–20	1	Not identified
	Zea mays (Zm)	2135.08	8	17–18, 20	0	4

*The gene mapping information is unclear, it is impossible to determine the tandem duplication pairs; or segmental duplication information cannot obtained from PGDD

#The data are from NCBI (www.ncbi.nlm.nih.gov/genome/)

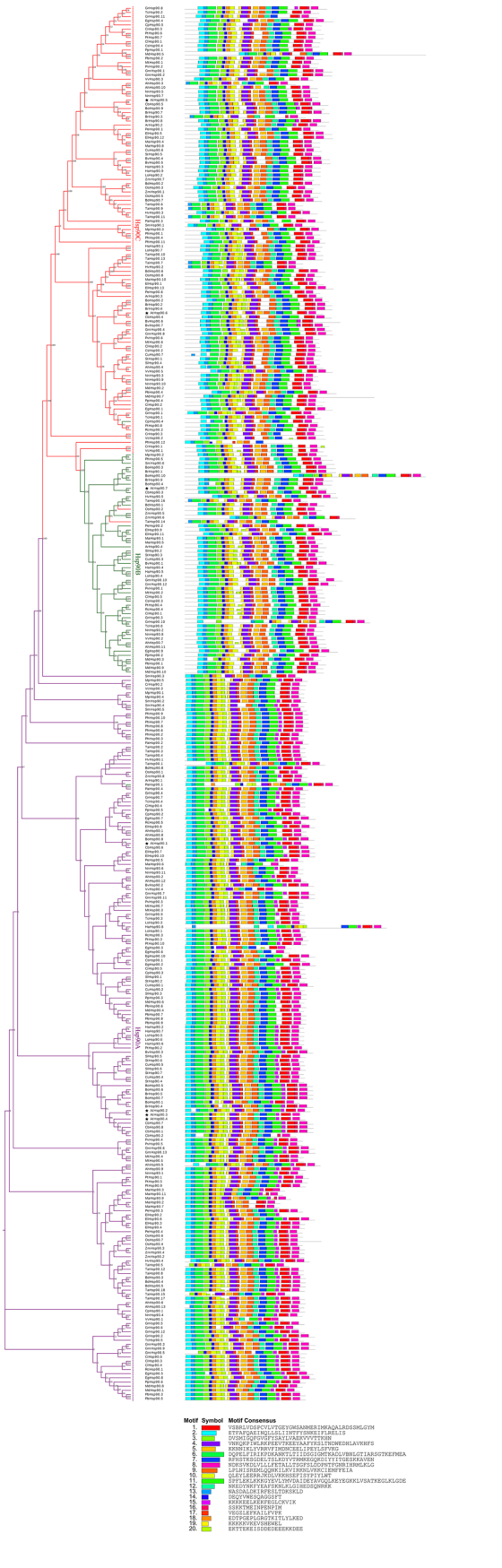

Fig. 1 Evolutionary relationships (left), and motifs prediction (right) ▶ of Hsp90s in 43 plant species. The evolutionary history was inferred using the Neighbor-Joining (N-J) method in MEGA7. Bootstrap values of 1000 replications were executed. The motifs, numbered 1–20, are displayed in different colored boxes

overexpression of AtHSP90.2, AtHSP90.5 and AtHSP90.7 [26, 54]. In addition, Hsp90s in *Arabidopsis* regulates the activity of resistance proteins and plays a key role in disease resistance [31, 32].

Moreover, OsHsp90 from rice enhances thermotolerance of *E. coli* (*Escherichia coli*) it functions as a chaperone, binding to a subset of substrates, and maintained *E. coli* growth well under high temperature stress [55]. ZmHsp90-1 may be involved in resistance to heat, high salt, ABA, cold and drought stress in maize (*Zea mays*) [56], and Hsp90s also play important roles in disease resistance of wheat and rice [28, 33]. In soybean, GmHsp90A2, GmHsp90A4, GmHsp90B1, GmHsp90C1.1 and GmHsp90C2.1 can reduce the damage of abiotic stress and may affect the synthesis and response system of proline [57]. Therefore, we also can speculate on the function of the Hsp90s in various species based on the existing results that can provide a clear idea and direction for the research.

The features and GO annotations analysis of StHsp90s

In order to better understand the sequence characteristics of the Hsp90 family, we performed sequence alignments on the Hsp90s of potato and *Arabidopsis* (Fig. 3), indicating that these sequences have significant sequence homology. According to previous studies, we divided the protein sequence into seven regions (conserved regions I, II, and III and variable regions A, B, C and D) [15, 48]. Among them, the variable regions reflect the sequence variation. The conserved region I contained histidine kinase-like ATPases sequences (HATPase-c family conserved signature sequence of Hsp90s), and Hsp90 family signature motif ''Yx-N/Q/H/D-K/H/R-D/E-I/V/A-F-L/M-R-E/D'' [37, 58]. In the conserved region III, we found that all members of the Hsp90A family in the cytoplasm had the C-terminal pentapeptide "MEEVD", which is a diagnostic sequence motif, and represents the functionality and characteristics of cytoplasmic Hsp90s in animals and plants [59, 60]. The C-terminus of the Hsp90B family members which were located on the ER had "KDEL" motifs, which marks proteins for ER retention [61]. The variable region B was rich in lysine (K) and glutamic acid (E). The functionally important residues experimentally identified were distributed in conserved regions I and II, and variable region B (Fig. 3).

To better understand the biological processes affected by Hsp90s, we selected potato Hsp90s for GO analysis

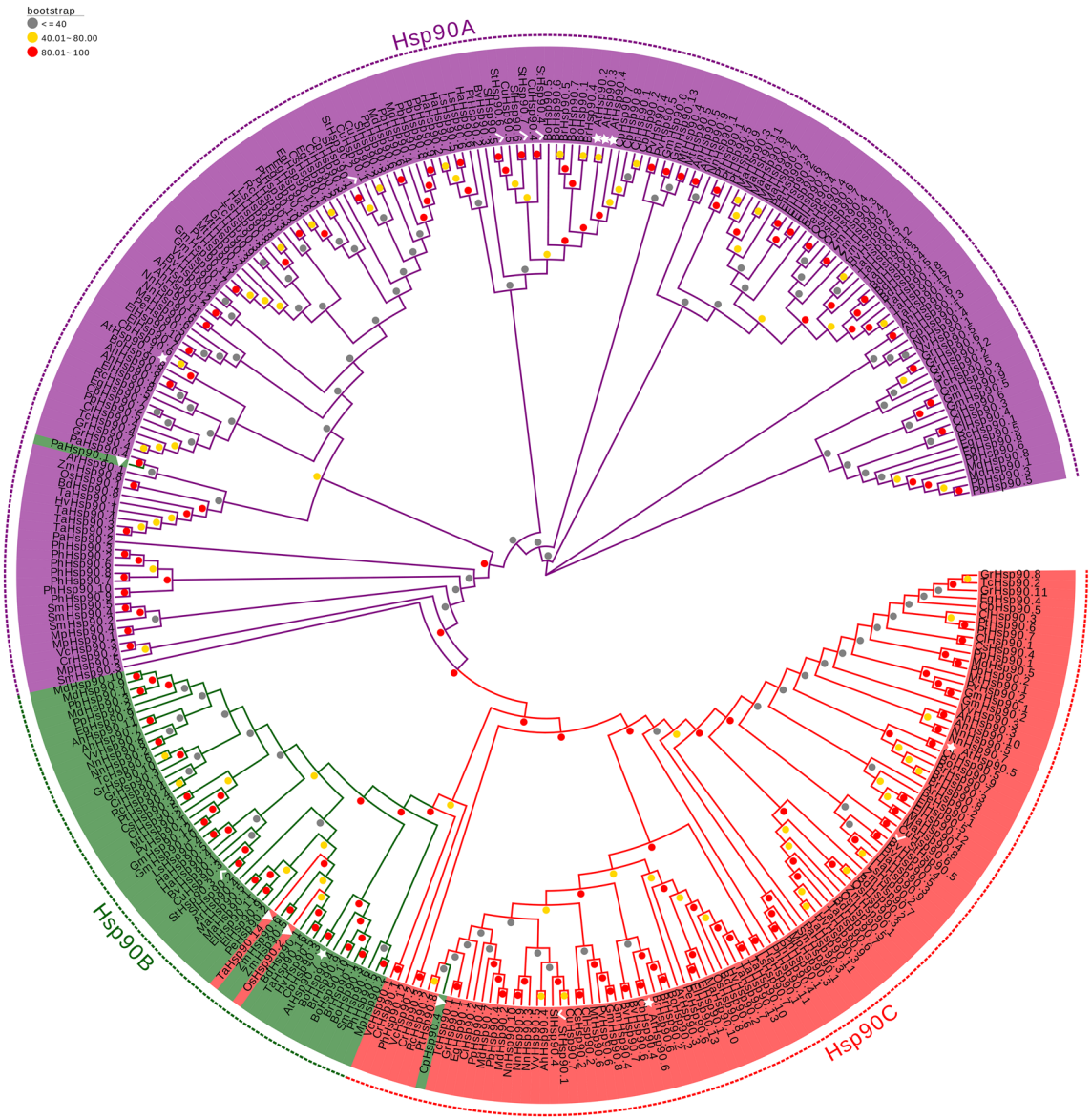

Fig. 2 Phylogenetic tree of Hsp90s. The unroot tree contains 334 proteins from 43 plant species. The protein distribution can easily divide into three main parts which were showed by different colors, respectively. Some Hsp90s are marked with different shapes. White star indicates *Arabidopsis*, white tick indicates potato (*Solanum tuberosum*), white triangle indicates the genes that differ from CDD classification

using the NCBI database [43] (Fig. 4, Table S3). In terms of molecular functions and biological processes, the seven genes all played identical functions. These genes not only participated in more 20 molecular functions (such as protein binding, ATP binding), but also were involved in more 50 biological processes, including response to heat, response to stimulus, biological regulation, cellular process, etc. (Fig. 4a, b). In addition, predictions of the cellular components of the genes showed that, StHsp90s were involved in the component development of the intracellular, organelle, membrane-bounded organelle, and so on (Fig. 4c). This indicated that the StHsp90s played important roles in cell composition, potato growth and development.

RNA-sequence data analysis of StHsp90s

We analyzed the data that was related to the expression patterns of the 7 Hsp90s and was from PGSC website in different development stages with varied stresses in potato [62]. We processed the RNA-seq database and generated a heatmap (Figs. 5, 6). It can be seen from Fig. 5 that the expression levels of StHsp90s in all stages of potato development were different. StHsp90.3, StHsp90.4, StHsp90.5,

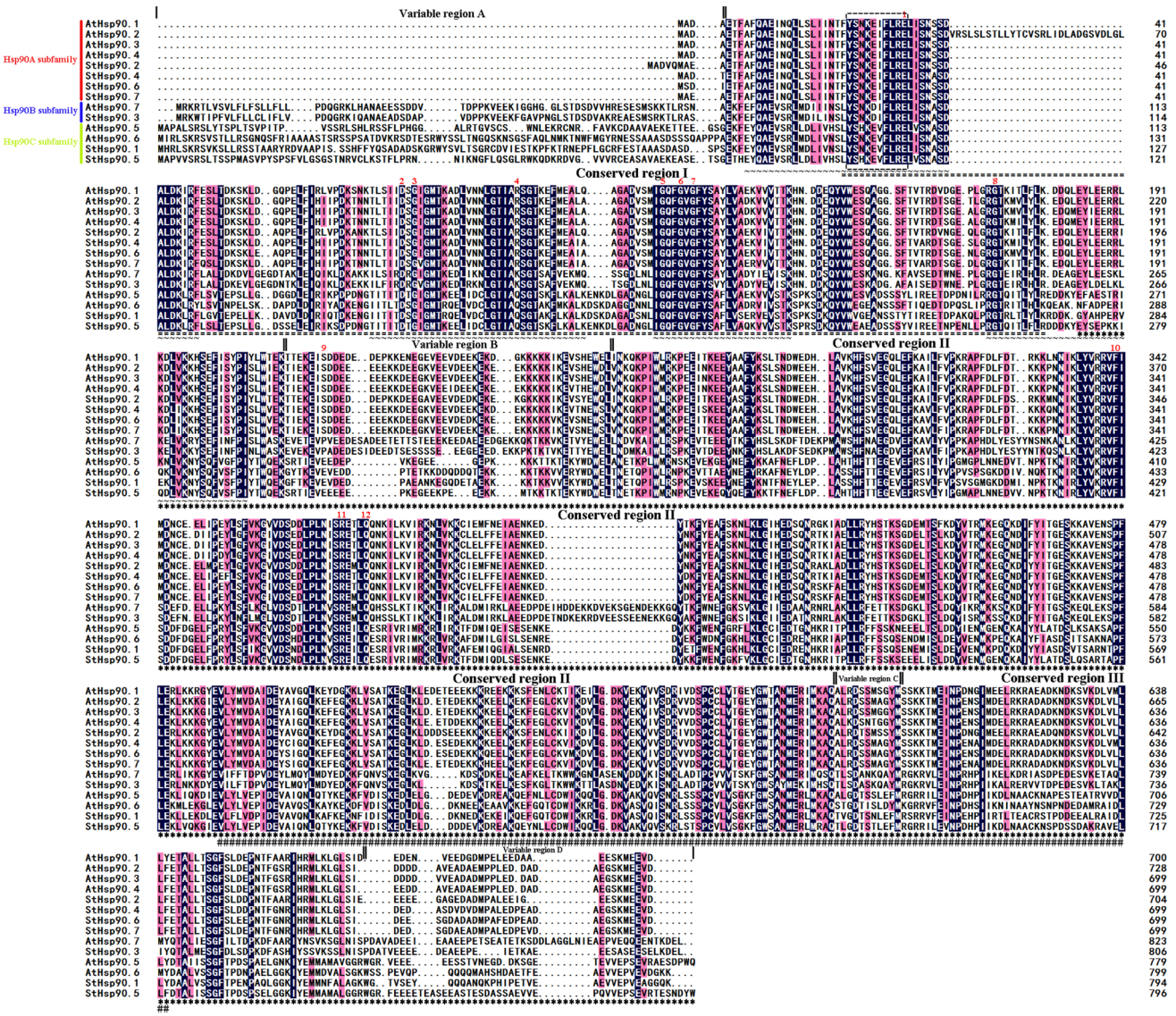

Fig. 3 Alignment of the Hsp90s sequences of potato and *Arabidopsis thaliana*. Identical or similar amino acids were colored blue or pink, respectively. Gaps were indicated with ".", the last residue was assigned a number at the end of each line. The rectangle drawn by dashed lines, close the N-terminal region, showed the Hsps90 family signature motif ''Yx-N/Q/H/D-K/H/R-D/E-I/V/A-F-L/M-R-E/D''. The conserved and functional domains are marked by: "=" for HATPase-c family; "~" for Hsp90 protein signature sequences; "*" for Hsp90 protein functional domain; "#" for fourhelical cytokine [15]. The red numbers above the alignment showed the functionally important residues: 1 (glutamic acid, E) for ATP hydrolysis; 2 (aspartic acid, D) for ATP binding [48]; 3, 5, 6, 7 and 8 (glycine, G) for both GA and p23 binding; 4 (lysine, K) for GA binding [49]; 9 (serine, S) for phosphorylation by casein kinase II [50]; 10 (phenylalanine, F) for interdomain interaction; 11 (arginine, R) and 12 (glutamine, Q) for ATPase activity [51]. (Color figure online)

StHsp90.6 and StHsp90.7 clearly expressed in all tissues. StHsp90.1 and StHsp90.2 were significantly expressed in most tissues. But in stamen, the expression of StHsp90.1 was very low, and StHsp90.2 was hardly expressed in flower and tuber (mature).

The plants were treated with ten different conditions, including phytohormone treatments (6-benzylaminopurine, BAP; indole-3-acetic acid, IAA; abscisic acid, ABA; gibberellic acid, GA3); abiotic stress (heat, drought (mannitol), and salinity (NaCl)); and biotic stress (pathogen; DL-β-amino-*n*-butyric acid, BABA; acibenzolar-S-methyl, BTH) [62, 63]. Under ten different treatments, most genes showed a down-regulated expression or little change (Fig. 6). Under phytohormone treatments, especially under BAP treatment, the expression levels of all genes was significantly decreased. But under the treatments of ABA and GA3, the expression of StHsp90.6 increased obviously. Under biotic stresses, BABA treatment induced increased expression of StHsp90.2 and StHsp90.3, and BTH treatment resulted in up-regulated expression of StHsp90.2,

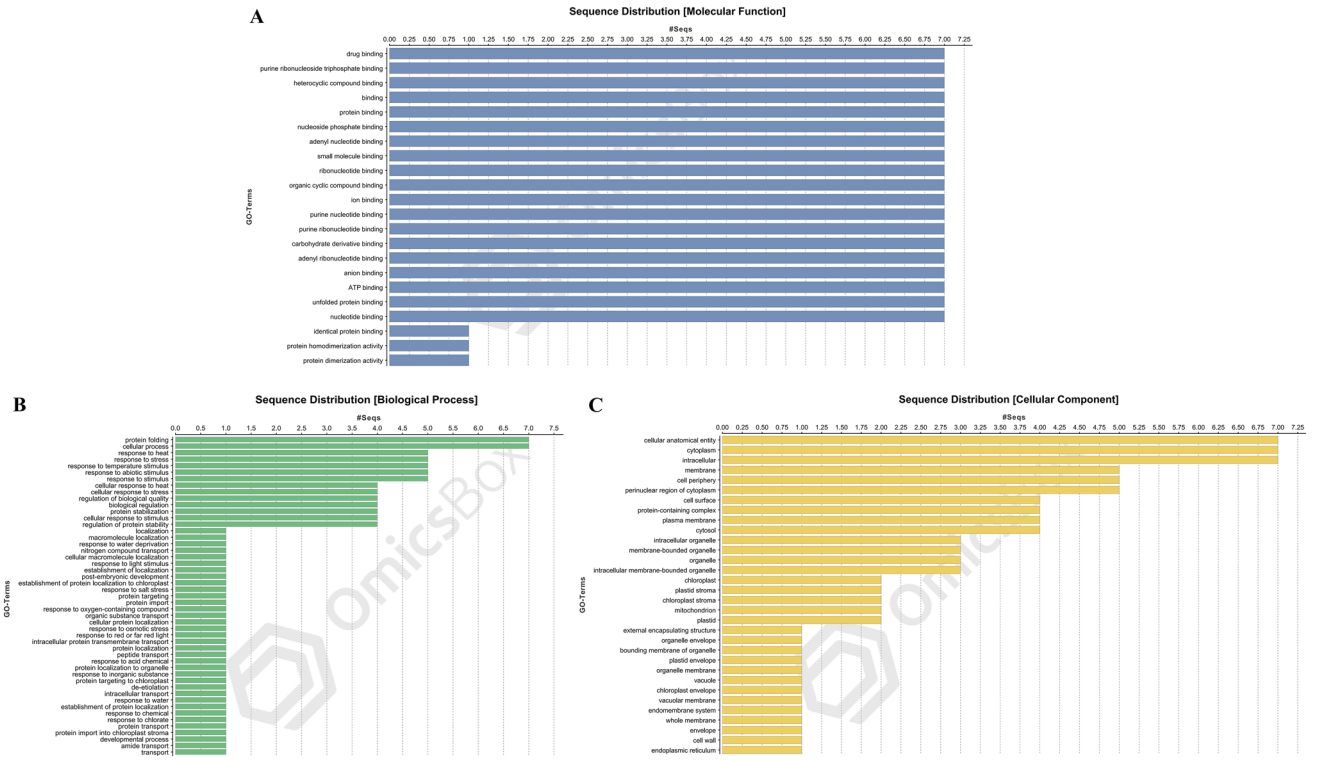

Fig. 4 The GO annotation information of whole Hsps. The X-axis indicates the protein numbers, and the Y-axis indicates the types of GO terms. Different types of gene annotation are shown in different colors

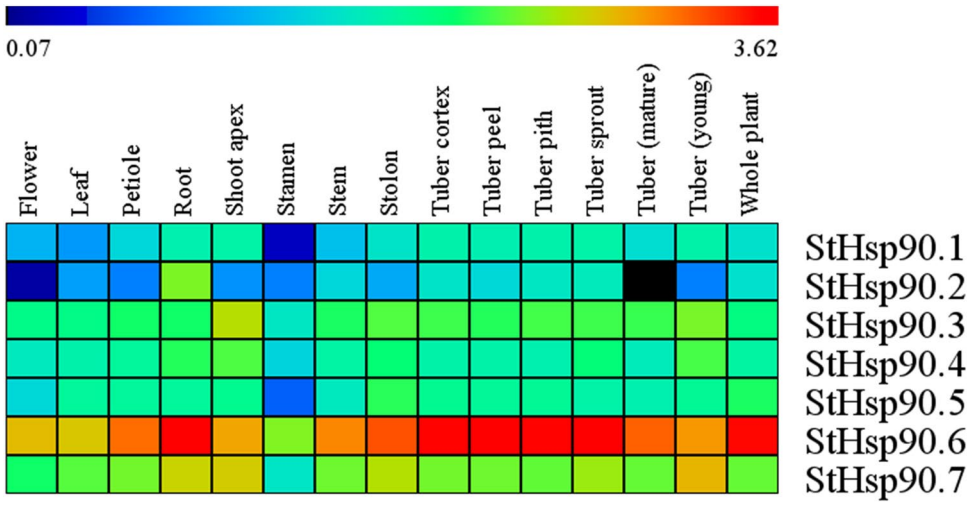

Fig. 5 The heatmap based on the RNA-seq database in 14 different tissues of potato. The heatmap showed Hsp90s expressions across 14 tissues throughout the entire potato life cycle, including flower, leaf, stolon, petiole, root, young tuber, mature tuber, shoot apex, tuber peel, tuber cortex, tuber sprout, tuber pith, and stamen. In the heat map, high expression is in red and low expression is in black. The data used in the Figure was the base-10 logarithm of raw data from the PGSC database

StHsp90.3, StHsp90.4 and StHsp90.7. When the plants were treated with abiotic stresses, StHsp90.1 and StHsp90.2 were up-regulated expressed under salinity and drought stresses. The expression of StHsp90.3 was increased under salinity stresses, and StHsp90.5 and StHsp90.6 were increased slightly under heat, salinity and drought stresses.

There are many studies confirming that the Hsp90 family can improve the stress resistance of plants [5, 26, 30–33, 56]. But in potatoes, there is still little research on the Hsp90 family. And in the ten treatments, there was no cold stress. Therefore, we analyzed the expression patterns of the seven genes under cold (4 °C) and heat (35 °C) stresses using the tetraploid cultivar "Diseree".

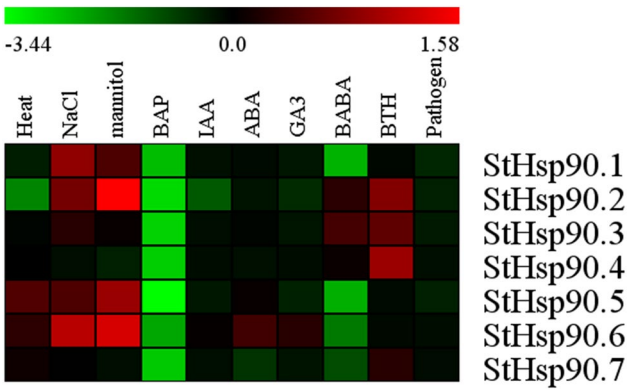

Fig. 6 Expression profiles of 7 Hsp90s in potato based on the RNA-seq database under 10 different treatments. Abiotic stress, biotic stress, and phytohormone treatment data and control data were obtained from the PGSC database. Transcripts were measured via RNA-Seq technology. The data used in the Figure = log2 (experimental value/control value). In the heat map, upregulated expression is shown in red and downregulated expression is shown in green. (Color figure online)

Hsp90s expression profiles in response to temperature variations

To demonstrate the change of Hsp90s in different tissues (leaf, stem, and root) in potato under low-temperature (4 °C) and high-temperature (35 °C) treatments, qRT-PCR analysis was used to conduct expression analysis (Fig. 7, Table S5). We mainly focused on the Hsp90s of potato.

In leaves (Fig. 7a), under low temperature stress, the expression levels of StHsp90.1, StHsp90.3, StHsp90.5, and StHsp90.7 did not change much, and StHsp90.2, StHsp90.4, and StHsp90.6 were up-regulated expressed. The expression levels of all StHsp90s were increased under high temperature condition, especially StHsp90.2, StHsp90.4, StHsp90.6, and StHsp90.7 were significantly up-regulated expressed. In stems (Fig. 7b), the transcript levels of StHsp90.4 and StHsp90.7 were slightly increased, while the expression of other genes almost unchanged at 4 °C. Under high temperature condition, four genes (StHsp90.2, StHsp90.4, StHsp90.6, and StHsp90.7) were up-regulated expressed, and the expression levels of StHsp90.2 and StHsp90.4 were notably increased. However, StHsp90.5 showed a down-regulation expression at 4 °C and 35 °C. In roots (Fig. 7c), the expression of StHsp90.2 was increased significantly at high temperature,

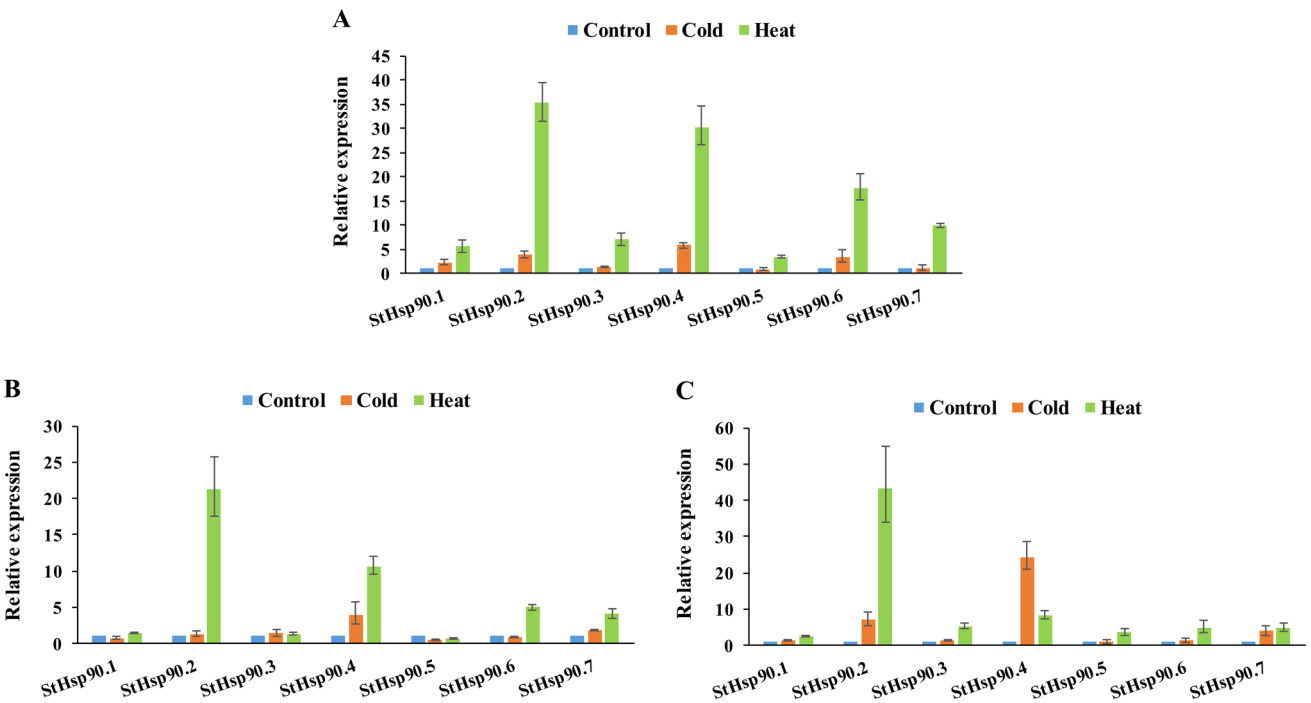

Fig. 7 Expression of 7 Hsp90s in potato leaves, stems, and roots at 35 °C (heat) and 4 °C (cold). **a** Leaf, **b** Stem, **c** Root. X-axes showed 7 representative Hsp90s, and the y-axes showed scales of relative expression levels. The Ef1a gene was used as a reference transcript. Leaf, stem, and root tissues were sampled from the same parts of control and experimental plants. The quantitative data were measured by taking three biological replicates and two technical replicates, and the relative expression level of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method

and the expression of StHsp90.4 was increased significantly at low temperature. Interestingly, the expression levels of StHsp90.2 and StHsp90.4 were extremely significantly increased in leaves, stems, or roots at 4 °C or 35 °C, which indicated that StHsp90.2 and StHsp90.4, which were sensitive to the temperatures, is worthy of further study.

Studies have shown that Hsp90s will not only be induced under high temperature, but also respond to other abiotic stresses. Under high temperature conditions, Hsp90 can help the substrate protein fold correctly, thereby playing a key role in protecting cells from thermal damage, and respond to cold and heat stresses with a temperature-dependent expression and exposure time effect [64, 65]. The Hsp90s in soybean all show high transcription levels in the leaves, and can be strongly induced under high temperature, osmotic pressure, and salt stress, but cannot be induced under cold stress [57]. In *Arabidopsis thaliana*, Hsp81-1, Hsp81-2 and Hsp81-3 are HSP90-family members. Under high temperature (35 °C), HSP81-1 is tissue-special expressed in large quantities, and under ABA, GA3, kinetin, IAA, NaCl or mannitol stresses, the transcripts levels of HSP81-2 and -3 are significantly increased [66]. In *Brassica napus*, Hsp90s play important roles under low temperature stress [67]. In *Porphyra yezoensis Ueda* (*Bangiales*, *Rhodophyta*), PyHsp90 is essential for maintaining the protein configuration under temperature and salinity stress, and is necessary under drought stress [68]. In *Lilium formolongi*, LfHsp90 can be induced by high and low temperatures, and by drought and salt stresses, indicating their likely association with tolerance to these stress conditions [69]. But so far, the response of Hsp90s to temperature has not been studied in potatoes. This paper analyzed the gene expression pattern of Hsp90 family in potatoes at 4 °C and 35 °C, and provided a greater theoretical basis and data in plants to further explore the heat and cold tolerance of plants.

Materials and methods

Identifcation of Hsp90s in various species

The genes, proteins, and coding sequences of 42 species were download from the Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html), the PGSC (https://solanaceae.plantbiology.msu.edu/pgsc_download.shtml), and the NCBI (https://www.ncbi.nlm.nih.gov) database. Seven *Arabidopsis* Hsp90 protein sequences that were obtained from the TAIR database (https://www.Arabidopsis.org/) were used as queries to perform a protein search against the database of 42 species proteins with a strict E value ($< 1e^{-10}$) requirement by using Blast 2.6.0 [70]. All candidate Hsp90 protein sequences were screened by using the Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/cdd/), and were aligned using Molecular Evolutionary Genetics Analysis (MEGA7) software.

Sequence analysis of Hsp90s/proteins

The information of the length of protein sequences and chromosome location was obtained from the Phytozome, the PGSC, the NCBI, and other database (Table S1). Multiple Em for Motif Elicitation (MEME; https://meme-suite.org/tools/meme) was used to calculate the motifs of the protein sequences, with the parameter of the number of motifs = 20.

Construction of the phylogenetic tree and gene duplication analyses

Phylogenetic analysis of the Hsp90 family from 43 species was constructed using MEGA7 software. Phylogenetic trees were produced using the Neighbor-Joining (NJ) method with the parameters of the Jones–Taylor–Thornton (JTT) model and 1000 replicates for bootstrap analysis [71]. The EvolView online tool (https://www.evolgenius.info/evolview/#login) was employed to draw and manage the phylogenetic trees.

Gene tandem duplication events of Hsp90s were analyzed following the methods of Gu et al. (2002) [72], the major criteria was that the length of alignable sequence covers > 75% of longer gene, and similarity of aligned regions > 75%. Two genes were regarded as tandem pairs if they were located on the same chromosome and were separated by no more than ten unrelated genes [73]. The segmental duplication pairs were analyzed by using the Plant Genome Duplication Database (PGDD; https://chibba.agtec.uga.edu/duplication). Ka and Ks values were calculated by DnaSP software.

GO annotation and expression pattern analysis

Blast2GO software was used to analyze the gene ontology (GO) [74]. The full-length amino acid sequences were uploaded into the program, and the NCBI database was chosen as the reference to analyze the molecular function, cellular components, and biological processes.

The expression data for the responses of Hsp90s to different stresses was obtained from the PGSC database, and the transcript abundance was represented by using FPKM (fragments per kilobase million) values [75].

Plant materials and growth conditions

Potato (*Solanum tuberosum* L.) tetraploid cultivar "Diseree" was used in this study. Potato plantlets were cultured in MS medium with 10 g/L agar and 20 g/L sucrose (pH 5.8)

and were kept under 25 ± 1 °C under 10,000 lx in light for 16 h and 20 ± 1 °C under 0 lx for 8 h. After two months, we selected seedlings of the same size for experimentation and divided them into three groups. The first was subjected to temperatures of 35 °C for 24 h, the second was subjected to temperatures of 4 °C for 24 h and the third was kept at normal condition as the control. We subsequently sampled the root, stem and leaf tissues from the same parts of the control plants and experimental plants with a weight of 100 mg. All of the samples were flash frozen in liquid nitrogen and were stored at − 80 °C prior to utilization.

RNA extraction and real-time qRT–PCR analysis of Hsp90 gene expression

The RNA simple Total RNA Kit (Code No. RP3301, BioTeke, Beijing, China) was used to extract RNA. The Elongation factor 1-a (Ef1a) gene was chosen as the reference gene [76]. Specifc primers were designed using Primer Premier 5 software. First-strand cDNAs were synthesized from 1 μg RNA with the PrimeScript™ RT reagent Kit (Code No. RR047A, TaKaRa, Dalian, China) in 20 μL reaction volume, including gDNA Eraser. Real-time PCR was set up on the basis of a 2 × Plus SYBR real-time PCR mixture (Code No. PR7702, BioTeke, Beijing, China) and was performed on a QuantStudio™ 7 Flex Real-time PCR System (Applied Biosystems Inc., U.S.A.) in a 10 μL reaction volume. The relative expression level of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method [77].

Conclusions

We identified 334 Hsp90s from 43 plant species, which could be divided into three groups based on CDD results and phylogenetic tree. We analyzed the gene mapping, motifs, gene duplication events, phylogenetic tree, GO annotations and the expression data from PGSC website. These results are important for understanding the properties and functions of Hsp90s, and also provide the basis for studying the evolutionary relationship of Hsp90s in plants. Although the motif composition of Hsp90s was conservative, there were also quantitative changes in several other proteins. In addition, in angiosperms, there were some Hsp90s with too large or too small number of amino acids, which showed that Hsp90s evolved with the evolution of plants. The view could be also confirmed through the notable increase in the number of gene segmental duplication events. Our analysis also showed that all 7 Hsp90s in leaves, stems, and roots of potato were related to temperature variations. Especially StHsp90.2 and StHsp90.4, the expression levels in leaves, stems, or roots were upregulated significantly under high or low temperature conditions, which suggested that the two genes may have important effects on heat- or cold-resistance in potato. In general, our findings enhance the knowledge about the evolutionary relationship and lay a foundation for further functional analysis of the Hsp90s in potato. In subsequent researches, we will try to study the functions and roles of Hsp90s in biological processes that affect crop resistance, and use molecular biology techniques to improve potato response to certain stresses.

Author contributions WL, DW and QC designed the experiments; WL, QC, DW, MY and YC carried out the experiments; WL and MY analyzed the experimental results. WL, MY, and DW analyzed the data and developed the analysis tools. WL and QC wrote the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274
2. Wegele H, Müller L, Buchner J (2004) Hsp70 and Hsp90-a relay team for protein folding. Rev Physiol Biochem Pharmacol 151:1–44
3. Young JC, Moarefi I, Hartl FU (2001) Hsp90: a specialized but essential protein-folding tool. J Cell Biol 154:267–274
4. Heckathorn S, Ryan S, Baylis J, Wang D, Hamilton EW, CundiffB L, LutheC DS (2002) In vivo evidence from an *Agrostis stolonifera* selection genotype that chloroplast small heat-shock proteins can protect photosystem II during heat stress. Funct Plant Biol 29:935–946
5. Pareek A, Singla SL, Grover A (1995) Immunological evidence for accumulation of two high-molecular-weight (104 and 90 kDa) HSPs in response to different stresses in rice and in response to high temperature stress in diverse plant genera. Plant Mol Biol 29:293–301
6. Lindquist S (1986) The heat-shock response. Annu Rev Biochem 55:1151–1191
7. Schirmer EC, Glover JR, Singer MA, Lindquist S (1996) HSP100/Clp proteins: a common mechanism explains diverse functions. Annu Rev Biochem 21:289–296
8. Csermely P, Schnaider T, Soti C, Prohászka Z, Nardai G (1998) The 90-kDa molecular chaperone family: structure, function, and

clinical applications. A comprehensive review. Pharmacol Ther 79:129–168
9. Frydman J (2001) Folding of newly translated proteins in vivo: the role of molecular chaperones. Annu Rev Biochem 70:603–647
10. Rizzolo K, Wong P, Tillier ERM, Houry WA (2014) The interaction network of the Hsp90 molecular chaperone[M]//the molecular chaperones interaction networks in protein folding and degradation. Springer, New York
11. Yamano T, Mizukami S, Murata S, Chiba T, Tanaka K, Udono H (2008) Hsp90-mediated assembly of the 26 S proteasome is involved in major histocompatibility complex class I antigen processing. J Biol Chem 283:28060–28065
12. Hoffmann T, Hovemann B (1988) Heat-shock proteins, Hsp84 and Hsp86, of mice and men: two related genes encode formerly identified tumour-specific transplantation antigens. Gene 74:491–501
13. Rebbe NF, Ware J, Bertina RM, Modrich P, Stafford DW (1987) Nucleotide sequence of a cDNA for a member of the human 90-kDa heat-shock protein family. Gene 53:235–245
14. Krone PH, Sass JB (1994) Hsp 90α and Hsp 90β genes are present in the zebrafish and are differentially regulated in developing embryos. Biochem Biophys Res Commun 204:746–752
15. Chen B, Zhong D, Monteiro A (2006) Comparative genomics and evolution of the HSP90 family of genes across all kingdoms of organisms. BMC Genomics 7:156
16. Prasinos C, Krampis K, Samakovli D, Hatzopoulos P (2005) Tight regulation of expression of two Arabidopsis cytosolic Hsp90s during embryo development. J Exp Bot 56:633–644
17. Jackson SE, Queitsch C, Toft D (2004) Hsp90: from structure to phenotype. Nat Struct Mol Biol 11:1152–1155
18. Richter K, Buchner J (2001) Hsp90: chaperoning signal transduction. J Cell Physiol 188:281–290
19. Zuehlke A, Johnson JL (2010) Hsp90 and co-chaperones twist the functions of diverse client proteins. Biopolymers 93:211–217
20. Chory J, Wu D (2001) Weaving the complex web of signal transduction. Plant Physiol 125:77–80
21. Pearl LH, Prodromou C (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu Rev Biochem 75:271–294
22. Tapia H, Morano K (2010) Hsp90 nuclear accumulation in quiescence is linked to chaperone function and spore development in yeast. Mol Biol Cell 21:63–72
23. Sołtys-Kalina D, Szajko K, Sierocka I, Śliwka J, Strzelczyk-Żyta D, Wasilewicz-Flis I, Jakuczun H, Szweykowska-Kulinska Z, Marczewski W (2015) Novel candidate genes AuxRP and Hsp90 influence the chip color of potato tubers. Mol Breed 35:224
24. Manchado M, Salas-Leiton E, Infante C, Ponce M, Asensio E, Crespo A, Zuasti E, Cañavate JP (2008) Molecular characterization, gene expression and transcriptional regulation of cytosolic Hsp90s in the flatfish Senegalese sole (*Solea senegalensis* Kaup). Gene 416:77–84
25. Galea-Lauri J, Latchman DS, Katz DR (1996) The role of the 90-kDa heat shock protein in cell cycle control and differentiation of the monoblastoid cell line U937. Exp Cell Res 226:243–254
26. Song H, Fan P, Li Y (2009) Overexpression of organellar and cytosolic AtHSP90 in *Arabidopsis thaliana* impairs plant tolerance to oxidative stress. Plant Mol Biol Rep 27:342–349
27. Tominaga H, Coury DA, Amano H, Miki W, Kakinuma M (2012) cDNA cloning and expression analysis of two heat shock protein genes, Hsp90 and Hsp60, from a sterile *Ulva pertusa* (Ulvales, Chlorophyta). Fish Sci 78:415–429
28. Liu D, Zhang X, Cheng Y, Takano T, Liu S (2006) Cloning and characterization of the rHsp90 gene in rice (*Oryza sativa* L.) under environmental stress. Mol Plant Breed 4:317–322
29. Yamada K, Fukao Y, Hayashi M, Fukazawa M, Nishimura M (2007) Cytosolic HSP90 regulates the heat shock response that is responsible for heat acclimation in *Arabidopsis thaliana*. J Biol Chem 282:37794–37804
30. McLellan CA, Turbyville TJ, Wijeratne EMK, Kerschen A, Vierling E, Queitsch C, Whitesell L, Gunatilaka AAL (2007) A rhizosphere fungus enhances Arabidopsis thermotolerance through production of an HSP90 inhibitor. Plant Physiol 145:174–182
31. Takahashi A, Casais C, Ichimura K, Shirasu K (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. Proc Natl Acad Sci USA 100:11777–11782
32. Liu Y, Burch-Smith T, Schiff M, Feng S, Dinesh-Kumar SP (2004) Molecular chaperone Hsp90 associates with resistance protein N and its signaling proteins SGT1 and Rar1 to modulate an innate immune response in plants. J Biol Chem 279:2101–2108
33. Wang G-F, Wei X, Fan R, Zhou H, Wang X, Yu C, Dong L, Dong Z, Wang X, Kang Z et al (2011) Molecular analysis of common wheat genes encoding three types of cytosolic heat shock protein 90 (Hsp90): functional involvement of cytosolic Hsp90s in the control of wheat seedling growth and disease resistance. New Phytol 191:418–431
34. Lu R, Malcuit I, Moffett P, Ruiz MT, Peart J, Wu A-J, Rathjen JP, Bendahmane A, Day L, Baulcombe DC (2003) High throughput virus-induced gene silencing implicates heat shock protein 90 in plant disease resistance. Embo J 22:5690–5699
35. Zhang M, Shen Z, Meng G, Lu Y, Wang Y (2017) Genome-wide analysis of the *Brachypodium distachyon* (L.) P. Beauv. Hsp90 gene family reveals molecular evolution and expression profiling under drought and salt stresses. PLoS ONE 12:e0189187
36. Zai WS, Miao LX, Xiong ZL, Zhang HL, Ma YR, Li YL, Chen YB, Ye SG (2015) Comprehensive identification and expression analysis of Hsp90s gene family in *Solanum lycopersicum*. Genet Mol Res 14:7811–7820
37. Banilas G, Korkas E, Englezos V, Nisiotou AA, Hatzopoulos P (2012) Genome-wide analysis of the heat shock protein 90 gene family in grapevine (*Vitis vinifera* L.). Aust J Grape Wine Res 18:29–38
38. Milioni D, Hatzopoulos P (1997) Genomic organization of hsp90 gene family in Arabidopsis. Plant Mol Biol 35:955–961
39. Cao D, Froehlich JE, Zhang H, Cheng CL (2003) The chlorate-resistant and photomorphogenesis-defective mutant cr88 encodes a chloroplast-targeted HSP90. Plant J 33:107–118
40. Prassinos C, Haralampidis K, Milioni D, Samakovli D, Krambis K, Hatzopoulos P (2008) Complexity of Hsp90 in organelle targeting. Plant Mol Biol 67:323–334
41. Ishiguro S, Watanabe Y, Ito N, Nonaka H, Takeda N, Sakai T, Kanaya H, Okada K (2002) SHEPHERD is the Arabidopsis GRP94 responsible for the formation of functional CLAVATA proteins. Embo J 21:898–908
42. Yamasaki K, Kigawa T, Seki M, Shinozaki K, Yokoyama S (2013) DNA-binding domains of plant-specific transcription factors: structure, function, and evolution. Trends Plant Sci 18:267–276
43. Zhang C, Zhang L, Wang D, Ma H, Liu B, Shi Z, Ma X, Chen Y, Chen Q (2018) Evolutionary history of the glycoside hydrolase 3 (GH3) family based on the sequenced genomes of 48 plants and identification of jasmonic acid-related GH3 proteins in *Solanum tuberosum*. Int J Mol Sci 19:1850–1865
44. Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. Science 290:1151–1155
45. Zhang Z, Li J, Zhao XQ, Wang J, Wong KS, Yu J (2006) KaKs_ Calculator: calculating Ka and Ks through model selection and model averaging. Genom Proteom Bioinf 4:259–263
46. Yang C, DongdongWang ZC, Kong N, Ma H, Chen Q (2019) Comparative analysis of the PIN auxin transporter gene family in different plant species: a focus on structural and expression profiling of PINs in *Solanum tuberosum*. Int J Mol Sci 20:3270

47. Li J, Zhang Z, Vang S, Yu J, Wong KS, Wang J (2009) Correlation between Ka/Ks and Ks is related to substitution model and evolutionary lineage. J Mol Evol 68:414–423
48. Obermann W, Sondermann H, Russo A, Pavletich N, Hartl F (1998) In vivo function of Hsp90 is dependent on ATP binding and ATP hydrolysis. J Cell Biol 143:901–910
49. Grenert JP, Sullivan WP, Fadden P, Haystead TA, Clark J, Mimnaugh E, Krutzsch H, Ochel HJ, Schulte TW, Sausville E et al (1997) The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. J Biol Chem 272:23843–23850
50. Lees-Miller SP, Anderson CW (1989) Two human 90-kDa heat shock proteins are phosphorylated in vivo at conserved serines that are phosphorylated in vitro by casein kinase II. J Biol Chem 264:2431–2437
51. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98
52. Sze H, Geisler M, Murphy AS (2014) Linking the evolution of plant transporters to their functions. Front Plant Sci 4:547–548
53. Song HM, Wang HZ, Xu XB (2012) Overexpression of AtHsp90.3 in *Arabidopsis thaliana* impairs plant tolerance to heavy metal stress. Biol Plant 56:197–199
54. Song H, Zhao R, Fan P, Wang X, Chen X, Li Y (2009) Overexpression of AtHsp90.2, AtHsp90.5 and AtHsp90.7 in *Arabidopsis thaliana* enhances plant sensitivity to salt and drought stresses. Planta 229:955–964
55. Liu D, Lu Z, Mao Z, Liu S (2009) Enhanced thermotolerance of *E. coli* by expressed OsHsp90 from rice (*Oryza sativa* L.). Curr Microbiol 58:129–133
56. Liu LL, Liu SS, Weng JF, Wang CT, Hao ZF (2013) Cloning and expression analysis of heat shock protein gene ZmHsp90-1 in maize. Acta Agron Sin 38:1839–1846
57. Xu J, Xue C, Xue D, Zhao J, Gai J, Guo N, Xing H (2013) Overexpression of GmHsp90s, a heat shock protein 90 (Hsp90) gene family cloning from soybean, decrease damage of abiotic stresses in *Arabidopsis thaliana*. PLoS ONE 8:e69810
58. Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet 22:631–677
59. Krishna P, Gloor G (2001) The Hsp90 family of proteins in *Arabidopsis thaliana*. Cell Stress Chaperones 6:238–246
60. Gupta RS (1995) Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. Mol Biol Evol 12:1063–1073
61. Munro S, Pelham HRB (1987) A C-terminal signal prevents secretion of luminal ER proteins. Cell 48:899–907
62. Li W, Dong J, Cao M, Gao X, Wang D, Liu B, Chen Q (2019) Genome-wide identifcation and characterization of HD-ZIP genes in potato. Gene 697:103–117
63. Liu H, Cao M, Chen X, Ye M, Zhao P, Nan Y, Li W, Zhang C, Kong L, Kong N et al (2019) Genome-wide analysis of the lateral organ boundaries domain (LBD) gene family in *Solanum tuberosum*. Int J Mol Sci 20:5360
64. Yonehara M, Minami Y, Kawata Y, Nagai J, Yahara I (1996) Heat-induced chaperone activity of HSP90. J Biol Chem 271:2641–2645
65. Zhang L, Fan Y, Shi F, Qin S, Liu B (2012) Molecular cloning, characterization, and expression analysis of a cytosolic HSP90 gene from *Haematococcus pluvialis*. J Appl Phycol 24:1601–1612
66. Yabe N, Takahashi T, Komeda Y (1994) Analysis of tissue-specific expression of *Arabidopsis thaliana* HSP90-family gene HSP81. Plant Cell Physiol 35:1207–1219
67. Krishna P, Sacco M, Cherutti JF, Hill S (1995) Cold-induced accumulation of hsp90 transcripts in *Brassica napus*. Plant Physiol 107:915–923
68. Zhou XH, Li X-S, Wang P, Yan BL, Teng YJ, Yi L-F (2010) Molecular cloning and expression analysis of HSP90 gene from *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta). J Fish China 34:1844–1852
69. Howlader J, Park J-I, Robin A, Sumi K, Nou I-S (2017) Identification, characterization and expression profiling of stress-related genes in easter lily (*Lilium formolongi*). Genes (Basel) 8:172
70. Wang Y, Deng D, Bian Y, Lv Y, Xie Q (2010) Genome-wide analysis of primary auxin-responsive Aux/IAA gene family in maize (*Zea mays* L.). Mol Biol Rep 37:3991–4001
71. Liu B, Zhao S, Wu X, Wang X, Nan Y, Wang D, Chen Q (2017) Characterization of phosphate transporter genes in potato. J Biotechnol 264:17–28
72. Gu Z, Cavalcanti A, Chen FC, Bouman P, Li WH (2002) Extent of gene duplication in the genomes of Drosophila, nematode, and yeast. Mol Biol Evol 19:256–262
73. Jiang SY, González JM, Ramachandran S (2013) Comparative genomic and transcriptomic analysis of tandemly and segmentally duplicated genes in rice. PLoS ONE 8:e63551
74. Zhang C, Kong N, Cao M, Wang D, Chen Y, Chen Q (2020) Evolutionary significance of amino acid permease transporters in 17 plants from Chlorophyta to Angiospermae. BMC Genomics 21:391
75. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L (2010) Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 28:511–515
76. Nicot N, Hausman JF, Hoffmann L, Evers D (2005) Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J Exp Bot 56:2907–2914
77. Liu F, Xu Y, Jiang H, Jiang C, Du Y, Gong C, Wang W, Zhu S, Han G, Cheng B (2016) Systematic identification, evolution and expression analysis of the Zea mays PHT1 gene family reveals several new members involved in root colonization by Arbuscular mycorrhizal fungi. Int J Mol Sci 17:930–947