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Comprehensive identification and expression analysis of *Hsp90s* gene family in *Solanum lycopersicum*

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ABSTRACT. Heat shock protein 90 (*Hsp90*) is a protein produced by plants in response to adverse environmental stresses. In this study, we identified and analyzed *Hsp90* gene family members using a bioinformatic method based on genomic data from tomato (*Solanum lycopersicum* L.). The results illustrated that tomato contains at least 7 *Hsp90* genes distributed on 6 chromosomes; protein lengths ranged from 267-794 amino acids. Intron numbers ranged from 2-19 in the genes. The phylogenetic tree revealed that *Hsp90* genes in tomato (*Solanum lycopersicum* L.), rice (*Oryza sativa* L.), and *Arabidopsis* (*Arabidopsis thaliana* L.) could be divided into 5 groups, which included 3 pairs of orthologous genes and 4 pairs of paralogous genes. Expression analysis of RNA-sequence data showed that the *Hsp90-1* gene was specifically expressed in mature fruits, while *Hsp90-5* and *Hsp90-6* showed

opposite expression patterns in various tissues of cultivated and wild tomatoes. The expression levels of the *Hsp90-1*, *Hsp90-2*, and *Hsp90-3* genes in various tissues of cultivated tomatoes were high, while both the expression levels of genes *Hsp90-3* and *Hsp90-4* were low. Additionally, quantitative real-time polymerase chain reaction showed that these genes were involved in the responses to yellow leaf curl virus in tomato plant leaves. Our results provide a foundation for identifying the function of the *Hsp90* gene in tomato.

Key words: Expression analysis; Gene duplication; Heat shock protein; Tomato

INTRODUCTION

Growth and development in plants are typically affected by various adverse environmental conditions, such as abiotic stresses including high and low temperatures, drought, and salt, as well as biotic stresses including fungi, bacteria, viruses, and nematodes. Plants have developed regulatory mechanisms against adverse environmental conditions through evolution. Previous studies have found that the synthesis of heat shock proteins (Hsp) in plant cells was enhanced significantly under high temperature stress (Heckathorn et al., 2002; Young et al., 2001; Wegele et al., 2004), and it was confirmed that these proteins play important roles in plants' resistance to high temperatures (Pareek et al., 1995). Heat shock proteins widely exist in animals, plants, and microorganisms (Lindquist, 1986). These proteins are divided into different families, mainly including Hsp60, Hsp70/Hsp80, Hsp90, Hsp110, and a low-molecular weight Hsp (smHsp) family (Al-Whaibi et al., 2011). The *Hsp90* gene family contains highly conserved structures and is a molecular chaperone family that exists widely in the eukaryotic cytoplasm (Prasinos et al., 2005). Hsps are involved in protein folding, activation, and maturation, as well as the conformational transition and stability of proteins involved in signal transduction (Prasinos et al., 2005).

Previous studies revealed that the *Hsp90* gene not only is induced by abiotic stresses such as saline-alkaline, high temperatures, low temperatures, and heavy metals (Pareek et al., 1995; Song et al., 2009), but also is involved in the plants' resistance to pathogens. Wang et al. (2011) found that wheat plants over-expressing the *Hsp90* gene family members *TaHsp90.2* and *TaHsp90.3* showed significant resistance to stripe rust. The resistance to angular leaf spot-mediated by the *R* gene *Pto* in tobacco, resistance to tobacco mosaic virus-mediated by genes *X* and *N*, and the Rx-mediated resistance of tomato virus are all dependent on the *Hsp90* gene (Sangster and Queitsch, 2005). Virus-induced gene silencing technology plays an important role in the study of plant resistance. The heat shock protein *Hsp90* was required for *Pto*-mediated resistance in a study of the tomato disease-resistance gene *Pto* to *Pseudomonas syringae* (Lu et al., 2003). Scofield et al. (2005) explored the functions of the suppressor of G2 allele of suppressor of *kinetochore protein1*, required for MLA12 resistance 1, and *Hsp90* in wheat using barley stripe mosaic virus-virus-induced gene silencing. The results demonstrated that these genes were indispensable in the resistance mediated by the leaf rust resistance gene *Lr21*. Hein et al. (2005) demonstrated that these genes were very important in the powdery mildew resistance gene of barley *Mla13*-mediated resistance using barley stripe mosaic virus-virus-induced gene silencing.

Some members of the *Hsp90* gene family in plants have been identified, including 7 *Hsp90* genes in *Arabidopsis thaliana* (Krishna and Gloor, 2001) and 9 in rice (*Oryza sative* L.) (Hu et al., 2009). Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the world. However, its production is often impacted by biotic and abiotic stresses. Sequencing of the entire tomato plant genome has enabled in-depth investigation of the *Hsp90* gene (Sato et al., 2012). In the present study, members of the Hsp90 gene family in tomato were identified. The number of *Hsp90* genes, structural features, chromosomal locations, phylogenetic relationships, and expression patterns were further analyzed in order to lay a foundation for the functional identification of *Hsp90* genes in tomato plants.

MATERIAL AND METHODS

Identification of Hsp90 gene family in tomato

Information regarding *Hsp90* gene family members in tomato plants was primarily obtained from 2 genome databases (http://solgenomics.net/ and http://mips.helmholtz-muenchen.de/plant/tomato/searchjsp/index.jsp). The *Hsp90* gene family in *Arabidopsis* was obtained from the NCBI website in accordance with previous studies (Krishna and Gloor, 2001). *Hsp90* gene family members in rice were obtained from the rice genome website: (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml) based on the results of Hu et al. (2009). We obtained information about *Hsp90* genes in tomato plants using 2 methods: 1) the key word of "heat shock protein 90" was input into the above databases and searched in order to obtain relevant information about *Hsp90* genes in tomatoes. 2) A Blastp search was carried out in the database of tomato plants using the amino acid sequence of the *Hsp90* gene in *A. thaliana*. Redundant genes were removed from the search results to obtain candidate genes, followed by identification of candidate genes using the Pfam website (http://pfam.janelia.org/). The isoelectric point of the *Hsp90* proteins in tomatoes, as well as the molecular weight, were obtained using the pI/MW calculation tool on the website (http://web.expasy.org/compute_pi/).

Chromosome location of *Hsp90* gene in tomatoes

Location information for the *Hsp90* gene was obtained from the genome database of tomato plants. Chromosome localization was determined using the MapDraw V2.1 software. Duplication of the *SlHsp90* genes was analyzed using the website http://chibba.agtec.uga.edu/duplication/index/locus.

Comparative analysis of *Hsp90* gene families in tomato, *Arabidopsis*, and rice

The amino acid sequences obtained of the *Hsp90* proteins in *Arabidopsis*, rice, and tomatoes were saved in FASTA format, and alignment of the amino acid sequences was carried out using the ClustalX software (Chenna et al., 2003). A phylogenetic tree was constructed for the *Hsp90* proteins from *Arabidopsis*, rice, and tomatoes using the neighbor-joining method of the MEGA 5.0 software (Tamura et al., 2011). The bootstrap value was 1000, and the nodes showing less than a 60% bootstrap support rate were excluded.

Expression analysis based on RNA-Seq data

RNA-Seq sequencing data for various tissues in tomatoes were downloaded from a functional genomics database (http://ted.bti.cornell.edu/cgi-bin/TFGD/digital/home.cgi), and expression data of the *Hsp90* gene in tomato were searched. Next, the expression pattern of the *Hsp90* gene was analyzed by using the MeV software (Mohr and Iliadis, 2012).

Plant material, virus inoculation, specific primers, and quantitative real-time-polymerase chain reaction (PCR) analysis

The tomato accession breeding line, 0054D was used in this experiment. For virus inoculation, plants in the 3-leaf growth stage were inoculated with the TYLCD-associated infectious clones using *A. tumefaciens* with the stem puncture method as described previously (Monci et al., 2005). After inoculation, leaves were selected as research materials at 0, 7, 14, 21, 28, and 35 days. Three biological replicates were used. RNA was extracted according to the instructions of the total RNA Extraction kit (Tiangen, Beijing, China). RNA purity was detected by agarose gel electrophoresis. The RNA samples were then stored at -70°C. First-strand cDNA was synthesized using 2 μL total RNA with the first cDNA strand synthesis kit (Tiangen) according to the kit instructions. Specific primers for the *Hsp90* gene were designed using the BioXM 2.6 software. Primer information is shown in Table 1. The amplification volume of the fluorescence quantitative PCR was 20 μLm including: 10 μL 2X TransStart™ Eco Green qPCR SuperMix, 0.4 μL Passive Reference Dye, 0.4 μL forward primer, and 0.4 μL 10 μM reverse primer, 1 μL cDNA, and 7.8 μL ddH_2O. PCR amplification conditions were as follows: 95°C for 30 s; 95°C for 5 s; 55°C for 15 s; and 72°C for 30 s for 40 cycles. The experiment was repeated 3 times. Data analysis was conducted using the $2^{-\Delta\Delta Ct}$ method.

Table 1. Specific primers of *Hsp90* genes in tomato.

Gene	Primers		Tm	Size (bp)
Hsp90-1	F1: ACTGGAGAGAGCAAGAAGG	R1: GACTTCTTCTTCTCCTCCT	58/56	221
Hsp90-2	F2: GAGTGAGAACAAGGAGGATT	R2: TCCTGGCACTCTGTAAGCT	58/58	230
Hsp90-3	F3: TACTACATCACTGGAGAGAG	R3: TTCTTCGCTCTCGTCATCG	58/58	198
Hsp90-4	F4: TCCCACGCTACCTAAGTTT	R4: AACGCTTTCCGCACCAAC	56/56	130
Hsp90-5	F5: ATGTGACCAGAATGAAGGAG	R5: TTCTCATCTTCACTCTCATC	58/56	235
Hsp90-6	F6: TGGCTAATGTGTCAAAGGTG	R6: GACTCCACCACAACACAAG	58/58	238
Hsp90-7	F7: GCTGAAGAAGAAGGGTTATG	R7: CTCCTTTAGTTCTTCTTGCT	58/56	169

RESULTS

Identification of *Hsp90* gene family in tomatoes

A total of 7 *Hsp90* genes were identified from the genome database of the tomato, including *Hsp90-1* through *Hsp90-7* as shown in Table 2. The length of the encoded amino acid sequence was 267-794 amino acids. The Hsp90 protein with the largest number of amino acids was Hsp90-4 (Solyc07g047790.2.1), which contained 794 amino acids, followed by Hsp90-2 (Solyc05g010670.2.1) which contained 787 amino acids. Furthermore, the Hsp90 protein with the lowest number of amino acids was Hsp90-6 (Solyc10g078930.1.1), which contained only 267 amino acids. The molecular weight sizes of Hsp90 family proteins ranged from

31.30-90.43 kDa, of which the protein with the largest molecular weight was Hsp90-4 with a molecular weight of 90.43 kDa. The protein with the smallest molecular weight was Hsp90-6 with a molecular weight of only 31.30 kDa. The isoelectric points of the Hsp90 proteins in tomato were 4.94-8.18. The protein with the highest isoelectric point was Hsp90-6, while the protein with the lowest isoelectric point was Hsp90-2. Except for the Hsp90-6 protein, isoelectric points were all were below 6, indicating that most tomato Hsp90 proteins are acidic.

Table 2. Hsp90 gene family in tomato.

Gene	SGN locus	Group	Genome position	ORF length	Deduced polyeotide		
					Length (aa)	Mol wt (kDa)	pI
SlHsp90-1	Solyc03g007890.2.1	II	Chr03:2414110-2410871	2100	699	5.03	80.27
SlHsp90-2	Solyc05g010670.2.1	IV	Chr05:4891847-4883167	2364	787	4.94	89.73
SlHsp90-3	Solyc06g036290.2.1	II	Chr06:22492761-22495996	1824	607	5.20	70.20
SlHsp90-4	Solyc07g047790.2.1	IV	Chr07:56334029-56327803	2385	794	5.23	90.43
SlHsp90-5	Solyc07g065840.2.1	I	Chr07:64662396-64658676	2100	699	4.95	80.14
SlHsp90-6	Solyc04g081630.1.1	V	Chr04:63191338-63189193	804	267	8.18	31.30
SlHsp90-7	Solyc12g015880.1.1	I	Chr12:5871038-5868004	2100	699	4.97	80.16

Localization of the *Hsp90* gene in tomatoes

The 7 *Hsp90* genes identified of the tomato were located on 6 chromosomes based on the genome database information of the tomato using the MapDraw V2.1 software (Figure 1A). Except for the *SlHsp90-4* and *SlHsp90-5* genes, the remaining 5 genes were located on different chromosomes in tomato. The *SlHsp90-1*, *SlHsp90-2*, and *SlHsp90-7* genes were located on the upper parts of the chromosomes, while the *SlHsp90-4*, *SlHsp90-5*, and *SlHsp90-6* genes were located on the lower end parts of chromosomes. An online website (http://chibba.agtec.uga.edu/duplication/index/locus) revealed a collinear relationship in the region including the 2 pairs of genes (*SlHsp90-1* and *SlHsp90-3*; *SlHsp90-5* and *SlHsp90-7*), of which the former showed an inversion phenomenon, and the gene sequence of the latter was the same (Figure 1B). Therefore, segments were duplicated during the evolution of these 2 pairs of genes.

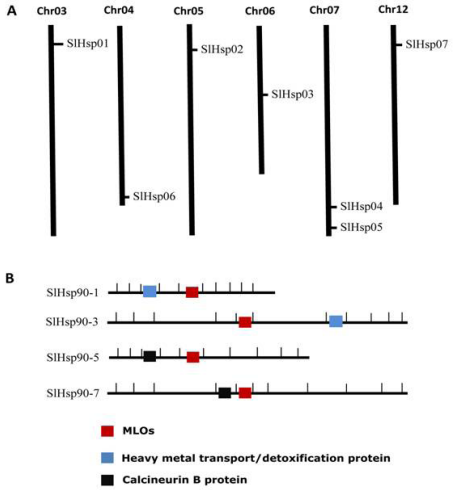

Figure 1. Chromosomal location of *Hsp90* gene in tomato.

Comparative analysis of *Hsp90* gene families of tomato, *Arabidopsis*, and rice

To further analyze the evolutionary relationships between *Hsp90* genes in tomato plants, the phylogenetic relationships of the *Hsp90* genes of *Arabidopsis*, rice, and tomato were analyzed. The results showed that a total of 23 *Hsp90* genes were included in the phylogenetic tree, of which 7 genes were from tomato, 7 genes from *Arabidopsis*, and 9 genes from rice. As shown in Figure 2, these genes were divided into 5 sub-branches from I-V. With the exception of the III and V sub-branches, the other clades contained *Hsp90* genes from 3 plant species. The sub-branch with the largest number of genes was the I sub-branch, containing 7 *Hsp90* genes. The sub-branch with the smallest number of genes was the V sub-branch, containing only 2 *Hsp90* genes. The present investigation revealed that 14 *Hsp90* genes had kinship, which accounted for approximately 60.86% (14/23) of the total number of genes. There were 3 pairs of orthologous genes between the 3 species (*AtHsp90-7* and *Os06g50300*; *AtHsp90-6* and *Hsp90-4*; *Hsp90-6* and *Os09g30438*). There were 4 pairs of paralogs within the species, of which 2 pairs (*Hsp90-5* and *Hsp90-7*, *Hsp90-1* and *Hsp90-3*) were from tomato and 2 pairs (*Os08g39140* and *Os09g30418*, *Os08g38086* and *Os09g29840*) were from rice.

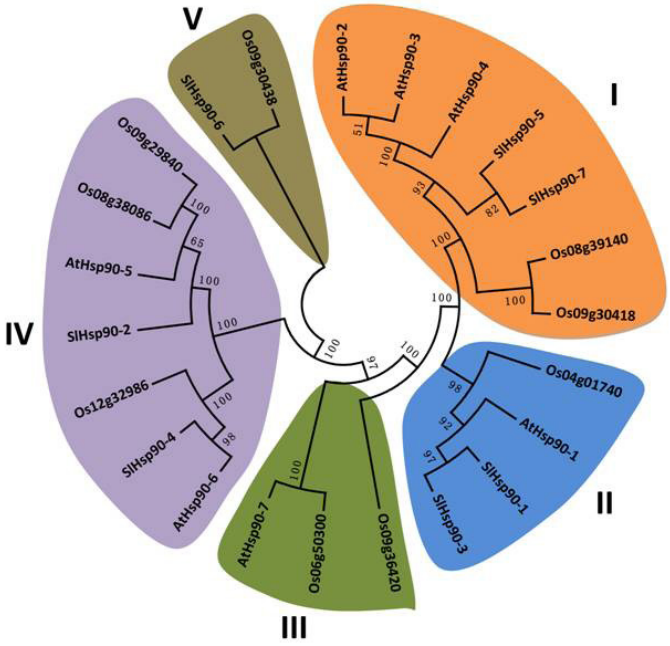

Figure 2. Phylogenetic relationships of *Hsp90* genes of tomato, *Arabidopsis*, and rice.

Gene expression analysis of *Hsp90* gene in tomato

To determine the expression patterns of *Hsp90* genes in different tissues of tomato and in the developmental processes of the fruit, we analyzed the expression patterns of the *SlHsp90* genes (Figure 3) using the RNA-Seq sequencing database on the website of the func-

tional genomics database of the tomato plant. The results showed that expression levels of the *SlHsp90-5* genes in different developmental stages of the root, leaf, flower, blossom bud, and fruit of the cultivated and currant tomato plants were high, while the expression levels of the *SlHsp90-6* genes in various tissues of the cultivated and currant tomato plants were low. The expression level of the *SlHsp90-7* gene in the leaves was relatively low, but high in the remaining tissues. In addition, the expression levels of the *SlHsp90-3* and *SlHsp90-4* genes were similar and highly expressed during the developmental processes of blossom buds, young leaves, and fruit of the currant tomato. The expression levels of *SlHsp90-1*, *SlHsp90-2*, and *SlHsp90-3* in the various tissues of the cultivated tomato were similar. Additionally, expression of the *SlHsp90-1* gene in the mature fruit was high in both the cultivated and currant tomatoes, while expression of the *SlHsp90-1* gene in other tissues was relatively low.

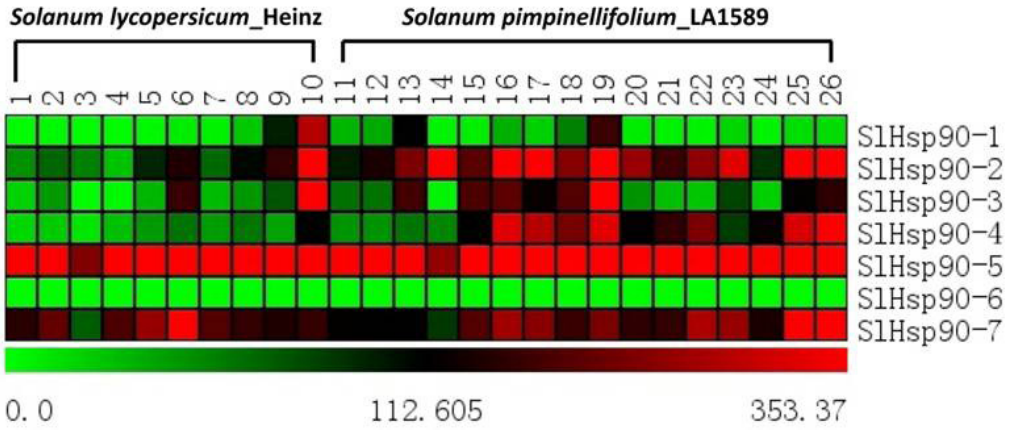

Figure 3. Expression analysis of Hsp90 gene in tomato during growth and development. 1: Heinz_bud, 2: Heinz_flower, 3: Heinz_leaf, 4: Heinz_root, 5: Heinz_1cm_fruit, 6: Heinz_2cm_fruit, 7: Heinz_3cm_fruit, 8: Heinz_MG, 9: Heinz_B, 10: Heinz_B10, 11: Pimp_IM, 12: Pimp_B, 13: Pimp_B5, 14: Pimp_leaf, 15: anthesis flowers (0 DPA), 16: 10 days post-anthesis fruit (10 DPA1), 17: 10 days post-anthesis fruit 2 (10 DPA2), 18: 20 days post-anthesis fruit (20 DPA), 19: ripening fruit (33 DPA), 20: cotyledons (COTYL), 21: hypocotyl (HYPO), 22: vegetative meristems (MERI), 23: mature leaves (ML), 24: whole root (ROOT), 25: young flower buds (YFB), and 26: young leaves (YL).

Expression of *Hsp90* gene in tomato plants under biotic stress

To further clarify whether the *SlHsp90* gene in tomato responds to biotic stress, quantitative real-time polymerase chain reaction was used to analyze gene expression in tomato leaves following treatment with tomato yellow leaf curl virus (Figure 4). Expression of all *Hsp90* genes was detected to different degrees over the various treatment times with yellow leaf curl virus. The expression levels of the *SlHsp90-1*, *SlHsp90-2*, *SlHsp90-3*, and *SlHsp90-4* genes in the leaves were highest after treatment for 14 days, after which the levels decreased. The decrease in the expression levels of *SlHsp90-1* and *SlHsp90-3* was rapid, while the decrease in the expression levels of *SlHsp90-2* and *SlHsp90-4* was slower. Their expression trends were similar.

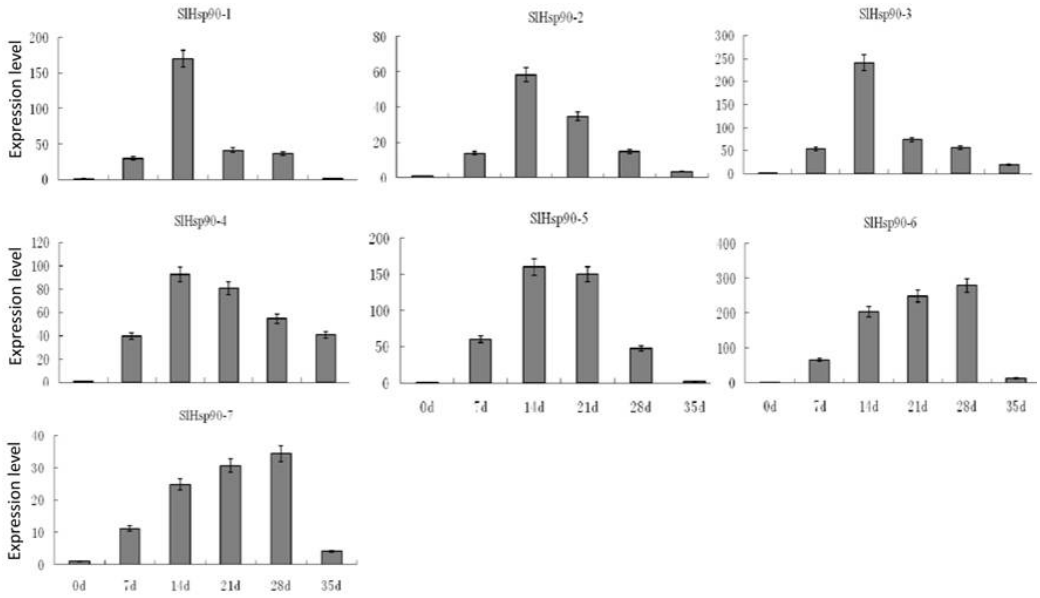

Figure 4. Expression pattern of *Hsp90* gene in tomato under high temperature stress.

DISCUSSION

Recent studies have shown that the *Hsp90* gene is not only related to stress signal transduction in plants, the folding of steroid kinase receptor, kinase, and transcription factors, as well as other physiological and biochemical processes (Wegele et al., 2004; Jackson et al., 2004; Shinozaki et al., 2006; Zuehlke and Johnson, 2010), but also participates in the response to biological stress (Lu et al., 2003; Scofield et al., 2005; Hein et al., 2005; Sangster and Queitsch, 2005; Wang et al., 2011). Therefore, the *Hsp90* genes exhibit diverse functions. Identification of the tomato *Hsp90* gene is important for revealing the potential function of the gene as well as for providing a basis for research analysis regarding the evolution of *Hsp90* genes in plants. The tomato genome sequencing was completed in 2012 (Sato et al., 2012), which enabled identification of members of the *Hsp90* gene family of tomato plants on the whole-genome level. In the present study, a total of 7 *SlHsp90* genes were identified in tomato (Figure 2). The lengths of the encoded sequence was 276-794 amino acids, and the isoelectric points were mainly 4.00-8.18. With the exception of *Sl*Hsp90-6, most *Hsp90* proteins in tomato were acidic. The *SlHsp90* genes were heterogeneously distributed in the chromosomes of the tomatoes, and were mainly concentrated at both ends of the chromosomes (Figure 2), which is similar to the distribution of *Hsp90* genes in rice (Hu et al., 2009). Comparative studies of rice and *Arabidopsis* have shown that gene duplication was an important mechanism in the evolutionary process of gene families (Young et al., 2001; Yang et al., 2008a,b). In the current investigation, 2 pairs of *SlHsp90* genes in tomatoes (*Hsp90-1* and *Hsp90-3*; *Hsp90-5* and *Hsp90-7*) were located on repeat segments of chromosome fragments, indicating that gene duplication occurred during the evolution of *Hsp90* genes of tomato plants.

Analysis of the phylogenetic relationships between the *SlHsp90* genes in *Arabidopsis*,

rice, and tomato revealed 3 pairs of orthologous genes and 4 pairs of paralogs. This number accounted for 60.86% of the total number of genes (14/23), suggesting that these genes were duplicated in the genomes of tomato, *Arabidopsis*, and rice. This phenomenon has also been observed in studies of gene families in other plants (Zhang et al., 2005; Jain et al., 2006).

Based on RNA-Seq analysis, there were significant differences in the expression levels of the *SlHsp90* genes in various tomato tissues. The expression levels of the *SlHsp90-5* genes were high in different developmental stages, including root, leaf, flower, flower bud, and fruit of the cultivated and wild tomato plants, indicating that the gene was closely involved in the normal physiological and biochemical activities of the tomato plant. In addition, the expression levels of the *SlHsp90-3* and *SlHsp90-4* genes were similar, and their expression levels during the developmental stages of the flower bud, young leaves, and fruit were high, suggesting that these genes are involved in the vegetative growth and reproduction of the tomato. In addition, the expression levels of *SlHsp90-1* genes in the mature fruit of the cultivated and currant tomatoes were high, while expression levels in other tissues were low. This suggests that the gene may be involved in the maturation process of the tomato fruit. Quantitative real-time polymerase chain reaction analysis showed that the expression levels of the *SlHsp90* gene were increased to some degree in the leaves of the tomatoes following treatment with yellow leaf curl virus. However, there were some differences in the trends, suggesting that the *Hsp90* protein may be involved in the tomato leaf response to biological stress, and the protein plays important roles in tomato plant resistance to infection with yellow leaf curl virus.

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