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Research Article
Genome-Wide Identification and Analysis of Genes Encoding Putative Heat Shock Protein 70 in Papaya (*Carica papaya*)

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Abstract

Background and Objective: In high plants, the 70 kDa heat stress proteins (Hsp70-s) have been regarded as one of the vital components of the cellular network of chaperones and folding catalysts that play important roles in numerous biological processes during growth and development. The Hsp70 families have been reported in many plant species, unfortunately, no information on this important protein family in papaya (*Carica papaya*). The objective of this study was to provide comprehensive information on the CpHsp70 family in papaya. **Materials and Methods:** The *CpHsp70* genes in the papaya genome were identified by a basic local alignment search tool against the papaya genome database by using well-known *Arabidopsis* Hsp70-s. Sequences were then analyzed by various bioinformatics tools to investigate the characteristics of the CpHsp70 family. **Results:** A total of 12 members of the CpHsp70 family has been identified and characterized in papaya. By using various computational tools, these results revealed that all general characteristics of the CpHsp70 family, like physic-chemical parameters, gene structure, phylogenetic tree and subcellular localization were provided. The transcriptome atlas was applied to re-analyze the expression patterns of genes encoding the CpHsp70 family in major tissues/organs during the growth and development of papaya plants. **Conclusion:** Results from this work exhibited the characteristics and expression analysis of the *CpHsp70* genes of this important tropical fruit crop. Taken together, this study could provide a solid foundation of the CpHsp70 family, which will be helpful in the construction of stress tolerance in papaya plants.

Key words: Papaya, 70 kDa heat stress proteins, genome-wide, identification, characterization, expression

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Papaya (Caricaceae) is one of the highest nutritional fruit crops that is widely cultivated in the world[1,2]. Containing a high concentration of nutrients and minerals, papaya fruits can be used as high-quality food, which has been fully reported to improve the cardiovascular system[3]. However, climate change, like heat stress is a great issue as it dramatically increases the risk of losing papaya production in many countries[4,5]. Thus, it would be significant to get insight into the molecular response of papaya to heat stress.

Principally, when subjected to heat stress, numerous physiological and molecular changes have occurred in crops[6-8], perhaps in papaya plants[5]. Among these molecular responses, heat stress proteins (Hsp-s) are triggered as the key transcription factors of the regulation of heat-responsive genes[9]. According to the specific structure, Hsp-s could be classified into three distinct families, including chaperonins (Hsp 60-s), small Hsp-s (Hsp100-s), 70 kDa Hsp-s (Hsp70-s) and Hsp90-s[10]. The Hsp70-s have been well-characterized to be responsible for a wide range of folding of proteins and play important roles in various cellular events, like involve in the developmental processes[11] and maintain cellular homeostasis[12]. Up till now, the Hsp70 family has been reported in numerous higher plant species, such as *Arabidopsis thaliana*[13,14], rice (*Oryza sativa*)[13,15], black cottonwood (*Populus trichocarpa*)[16], soybean (*Glycine max*)[17], quinoa (*Chenopodium quinoa*)[18], tea plant (*Camellia sinensis*)[19], pearl millet (*Pennisetum glaucum*)[20], tobacco (*Nicotiana tabacum*)[21], four cotton species, like *Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. raimondii*[22] and *Aquilaria sinensis*[23]. However, the understanding of the Hsp70 family in papaya has been still lacking, especially the genome assembly of this tropical fruit has been released recently[24].

The current study provided a genome-wide analysis of the CpHsp70 family in papaya by an *in silico* approach. By using various computational tools, the information of the CpHsp70 family, including annotations, gene organizations, protein features and phylogeny were performed. This study re-analyzed the RNA-Seq dataset to explore expression profiles of *CpHsp70*-s in major organs/tissues of papaya plants.

MATERIALS AND METHODS

Study area: The study was conducted from March to October, 2021. All *in silico* analyses were carried out at Hung Vuong University (Phu Tho, Vietnam), University of Engineering and Technology (Hanoi, Vietnam), Vietnam National University of Education (Hanoi, Vietnam), Thuyloi University (Hanoi, Vietnam) and Hanoi Pedagogical University 2 (Vinh Phuc, Vietnam).

Identification and annotation of the CpHsp70 family in papaya: To identify members of the CpHsp70 family, the full assembly of papaya (GCF_000150535.2)[24] was downloaded from the Phytozome v13 database[25] to perform a Basic Local Alignment Search Tool (BLAST) search. Particularly, well-characterized AtHsp70-s from *Arabidopsis*[13,14] was used as seed sequences for TBLASTN searches against the papaya's genome[24]. The conserved domain of the Hsp70 family was then validated by the Pfam tool[26]. Subsequently, the full-length protein, coding DNA (CDS) and genomic DNA (gDNA) sequences of each CpHsp70 were obtained for next *in silico* analyses.

Calculation of physic-chemical properties of the CpHsp70 family in papaya: To analyze the typical features of the CpHsp70-s, the full-length protein sequence of each member was used as a query for searching in the Expasy Protparam[27,28]. Particularly, the protein length (amino acid residues, aa-s), molecular mass (kilo Dalton, kDa), isoelectric point (pI), instability index (II), aliphatic index (AI) and grand average of hydropathy (GRAVY) were retrieved by the Expasy Protparam[27,28]. Among them, II values are more than 40 and less than 40 indicate stability and instability, respectively[29], while GRAVY values more than 0 and less than 0 indicate hydrophobic and hydrophilic characteristics, respectively[30].

Prediction of subcellular localization of the CpHsp70 family in papaya: To assess the subcellular distribution of CpHsp70-s in papaya, the full-length protein sequence of each member was used applied in the Target P tool[31,32] and WOLF PSORT[33]. Briefly, the organelle-specific peptides, like cTP (chloroplast transit peptide), mTP (mitochondrial targeting peptide) and SP (secretory pathway signal peptide) were predicted in the N-terminal pre-sequences and the reliability class of the prediction was from 1 (strongest) to 5 (weakest) as previously described[31,32].

Analysis of gene structure of the *CpHsp70* family in papaya: To investigate the exon/intron organization of genes encoding the *CpHsp70* family, the CDS and gDNA of each *CpHsp70* member were used to subject into the Gene Structure Display Server v2 (GSDS)[34]. The gene structure of each member was then arranged by the order from the MEGA-based phylogenetic tree. The results were described by Adobe Illustrator.

Construction of phylogenetic tree of *CpHsp70* family in papaya: To categorize the members of the CpHsp70-s, all full-length aa sequences were used to generate an unrooted phylogenetic tree by using the Molecular Evolutionary Genetics Analysis v7 (MEGA)[35] as previously described[36-38]. Additionally, to understand the relationship of the Hsp70-s between other plant species, we also constructed a phylogenetic tree of all members of the CpHsp70 family from papaya and well-characterized Hsp70-s from *Arabidopsis*[13,14] and rice[13,15]. The steps were carried out as following the previous studies[36-38].

Re-analysis of expression patterns of the *CpHsp70* gene family in papaya: To explore the expression patterns of the genes encoding CpHsp70-s, sought recent transcriptome datasets of papaya plants from the GEO NCBI[39,40]. Particularly, the dataset of organs/tissues during the fruit development (GSE116581)[41] was explored for the re-analysis. The expression profiles of the *CpHsp70* genes were described by the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) as previously mentioned[41].

RESULTS AND DISCUSSION

Genome-wide identification of the *CpHsp70* family in papaya: To identify all putative members of the *CpHsp70* family in papaya, well-characterized AtHsp70-s[13,14] were used as queries to perform BLAST searches against the recent proteome of papaya[24]. The occurrence of the conserved Hsp70 domain was then validated by Pfam[26]. As a result, a total of 12 putative members of the *CpHsp70* family has been defined from papaya. A list of gene names and their corresponding identifiers was then provided in Table 1.

Previously, the *Hsp70* families were also reported in many higher plant species in Fig. 1. For example, a total of 18 members of the *Hsp70* family has been found in *Arabidopsis*[13,14]. Meanwhile, the *Hsp70* families, with 26, 18 and 16 members, were also reported in rice[13,15], pearl millet[20] and quinoa[18], respectively. In black cottonwood, 20 members of the *Hsp70* family have been comprehensively identified[16]. Moreover, the *Hsp70* families with the presences of 40, 30, 21 and 22 members were found in four cotton species, including *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. raimondii*, respectively[22]. More recently, a total of 15 putative *Hsp70*-s was identified in *A. sinensis* through genome-wide bioinformatics analysis[23]. Here, the number of members of the *CpHsp70* family from papaya (12) was noted to be less than that in other green plant species, like *A. sinensis* (15)[23], quinoa (16)[18], tea plant (18)[19], pearl millet (18)[20], *Arabidopsis* (18)[13,14], black cottonwood (20)[16], *G. arboreum* (21) and *G. raimondii* (22)[22], rice (26)[13,15], *G. barbadense* (30) and *G. hirsutum* (40)[22], tobacco (61)[21] and soybean (61)[17] (Fig. 1). The different amount of members between the *Hsp70* families in various plant species could be explained by the duplication events that occurred during the evolution process.

Analysis of protein features of the CpHsp70 family in papaya: To analyze the physic-chemical characteristics of the members of CpHsp70-s, their corresponding full-length protein sequence was queried in the Expasy Protparam[27,28]. Of our interest, six typical features, including protein length and mass, pI, II, AI and GRAVY values were then retrieved. The analysis of the physic-chemical properties of the CpHsp70-s was provided in Table 1. Particularly, our results revealed the number of aa-s of CpHsp70-s ranged from 572 (CpHsp70-06) to 837 (CpHsp70-07) residues, while the molecular mass of CpHsp70-s was varied from 61.79 (CpHsp70-06) to 92.82 kDa

Table 1: CpHsp70 family in papaya

Gene names	Locus names	Length (aa-s)	Mass (kDa)	pI	II	AI	GRAVY	SL
CpHsp70-01	evm.TU.supercontig_1.398	648	70.96	5.13	43.80	82.35	-0.41	C
CpHsp70-02	evm.TU.supercontig_15.10	665	73.27	5.13	34.14	89.44	-0.43	ER
CpHsp70-03	evm.TU.supercontig_20.39	680	73.09	5.59	32.35	85.53	-0.31	M
CpHsp70-04	evm.TU.supercontig_29.171	780	87.15	5.43	41.51	82.64	-0.52	C
CpHsp70-05	evm.TU.supercontig_46.176	651	71.59	5.19	31.62	84.06	-0.43	C
CpHsp70-06	evm.TU.supercontig_58.117	572	61.79	5.61	41.76	104.09	0.13	C
CpHsp70-07	evm.TU.supercontig_68.26	837	92.40	5.33	24.90	78.45	-0.44	C
CpHsp70-08	evm.TU.supercontig_113.35	712	76.24	5.36	23.06	85.29	-0.32	Cp
CpHsp70-09	evm.TU.supercontig_152.1	830	92.82	6.18	23.06	83.98	-0.32	C
CpHsp70-10	evm.TU.supercontig_260.3	654	71.46	5.13	30.40	82.35	-0.40	C
CpHsp70-11	evm.TU.supercontig_602.2	655	71.60	5.30	20.98	83.83	-0.42	C
CpHsp70-12	evm.TU.supercontig_755.2	681	73.26	6.03	32.77	88.56	-0.31	M

MW: Molecular weight, pI: Isoelectric point, AI: Aliphatic index, SL: Subcellular localization, C: Cytoplasm, Cp: Chloroplast, ER: Endoplasmic reticulum, M: Mitochondrion, aa-s: Amino acid residues and kDa: kilo dalton

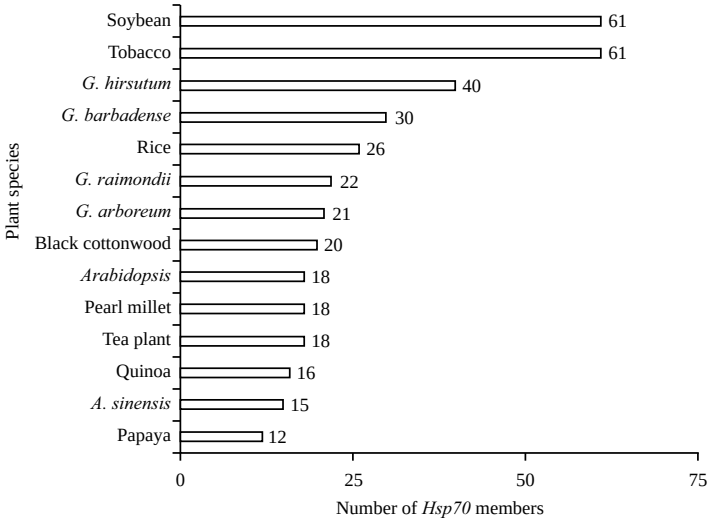

Fig. 1: Overview of *Hsp70* family in plant species

(CpHsp70-09) (Table 1). Meanwhile, pI values of whole members of CpHsp70-s were recorded to be acidic (Table 1). Next, a large number of members of CpHsp70-s (9 out of 12) were stable (II values <40.00), whereas, three members of CpHsp70-s, including CpHsp70-01, CpHsp70-04 and CpHsp70-06 were unstable (II values >40.00) (Table 1). The values of aliphatic side chains of the CpHsp70-s were varied from 78.45 (CpHsp70-07) to 104.09 (CpHsp70-06) (Table 1). Finally, the GRAVY values of a majority of members from CpHsp70-s (11 out of 12) were found to be less than 0 (Table 1), which indicates the hydrophilic nature of the proteins[27,28].

Previously, the general characteristics of the Hsp70-s in other plant species were also slightly variable. For example, the molecular mass of all Hsp70-s in *G. raimondii* ranged from 62.65-75.70 kDa[22]. The full-length protein sequences of the Hsp70-s in three other cotton species, including *G. arboreum*, *G. hirsutum* and *G. barbadense* were varied from 70.76-75.68, 65.56-75.70 and 52.03-106.45 kDa, respectively[22]. In soybean, the lengths and molecular weights of the Hsp70-s have been reported to range from 134-927 aa-s and 15.19-102.93 kDa, respectively[17]. Recently, the Hsp70-s from *A. sinensis* has been reported to range from 489-893 aa-s in length, while the predicted mass of these Hsp70-s was varied from 53.0-100.0 kDa[23]. Among them, only three members of the Hsp70 family from *A. sinensis* could be considered unstable proteins[23]. Interestingly, the Hsp70-s in all reported plant species, such as soybean, four cotton species and *A. sinensis* harbored a pI value of the alkaline range[17,22,23]. Taken together, the highly variable features of the Hsp70-s in plant species may indicate their divergent roles in numerous biological processes during the growth and developments of plants[11,12].

Investigation on the subcellular localization of the CpHsp70 family in papaya: As a part of this study, to investigate the subcellular localization of the CpHsp70-s, their corresponding full-length aa sequences were analyzed by the Target P tool[31,32] and WOLF PSORT[33]. As expected, the results from the Target P tool[31,32] and WOLF PSORT[33] revealed that the majority of CpHsp70-s (eight out of 12) were distributed in the cytoplasm (Table 1). Additionally, CpHsp70-02 could be localized on the endoplasmic reticulum, while CpHsp70-03 and CpHsp70-12 and CpHsp70-08 were predicted to be distributed in the mitochondrion and chloroplast, respectively (Table 1).

Previously, the Hsp70-s from four cotton species were reported to be localized in the cytoplasm, chloroplast and mitochondria by an *in silico* tool[22]. More specifically, 10, four, two, two, two and one members of the Hsp70 family from *G. arboreum* have been predicted to reside in the cytoplasm, mitochondria, endomembrane systems, extra-cellular space, chloroplast, organelle membrane, respectively[22]. The majority of members of the Hsp70 family from three other cotton species, like *G. barbadense* (24 out of 30), *G. hirsutum* (19 out of 40), *G. raimondii* (11 out of 22) also resided in the cytoplasm[22]. Recently, the Hsp70 family from *A. sinensis* has been reported to distribute in the cytosol (8 out of 15), mitochondria and chloroplast (3 out of 15), endoplasmic reticulum (2 out of 15), vacuole and nucleus (2 out of 15)[23]. Similar predictions were also reported in soybean in which a majority of the Hsp70-s resided in the cytosol[17]. Taken together, these findings strongly suggested that the Hsp70-s from papaya, perhaps from other plant species have been found in diverse subcellular compartments and the cytosol is their most likely subcellular localization.

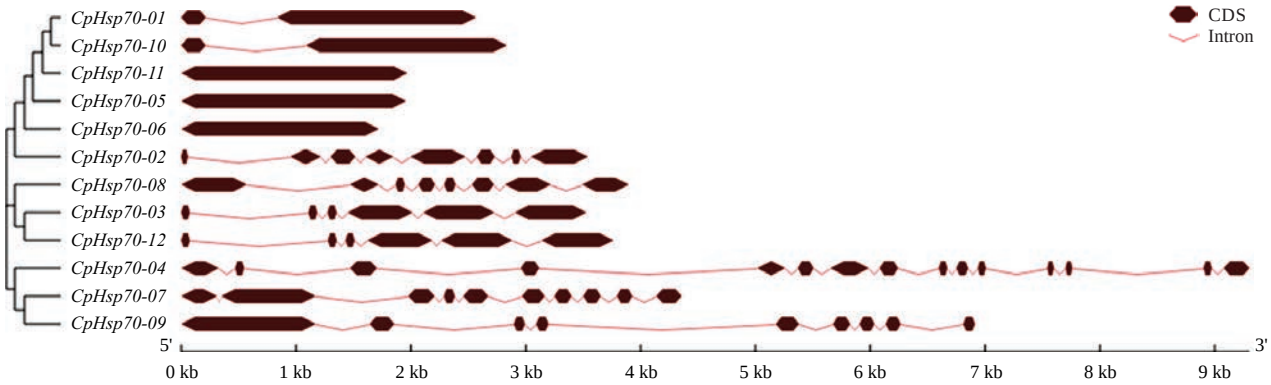

Fig. 2: Gene organizations of the *CpHsp70* family in papaya

Gene structure and categorization of the *CpHsp70* family in papaya: To get insights into the structure of genes encoding CpHsp70-s in papaya, we analyzed the exon/intron organization in the coding sequences of the *CpHsp70* family by the GSDS tool[34]. As a result, the exon/intron organizations of genes encoding CpHsp70-s were highly variable in Fig. 2. The amounts of exons of the *CpHsp70* genes were varied from 1-15 (Fig. 2). Particularly, three genes, like *CpHsp70-05*, *CpHsp70-06* and *CpHsp70-11* were intronless, while two genes, like *CpHsp70-01* and *CpHsp70-10*, had two exons (Fig. 2). Next, two (*CpHsp70-03* and *CpHsp70-12*) and two (*CpHsp70-02* and *CpHsp70-08*) genes harboured six and eight exons, respectively (Fig. 2). Three remaining genes, including *CpHsp70-04*, *CpHsp70-07* and *CpHsp70-09* contained 15, 10 and nine exons (Fig. 2).

Previously, the exon/intron structures of *Hsp70* gene families from other plant species were also reported. For example, the number of exons of *Hsp70* genes from *A. sinensis* ranged from 1 (intronless) to 15[23]. Among these genes, the majority of genes encoding Hsp70-s contained 6-10 exons, whereas, two *Hsp70* genes were intronless[23]. In cotton species, most of the *Hsp70* genes contained introns, whereas, only three (out of 21), nine (out of 30), three (out of 22) and four (out of 40) genes from *G. arboreum*, *G. barbadense*, *G. raimondii* and *G. hirsutum* were reported to be intronless, respectively[23]. This phenomenon was also recorded in earlier reports of *Hsp70* gene families from other plant species, like tobacco[21] and tea plant[19].

Next, generated a MEGA-based phylogenetic tree of the CpHsp70 family from papaya and two well-characterized Hsp70-s from *Arabidopsis*[13,14] and rice[13,15]. The phylogenetic tree diagram of Hsp70 families from papaya, *Arabidopsis*[13,14] and rice[13,15] was then provided in Fig. 3. As described in the phylogenetic tree, Hsp70 families from papaya, *Arabidopsis*[13,14] and rice[13,15] could be classified into six sub-groups (Fig. 3). Among them, except sub-group 2, all the other sub-groups included Hsp70-s from three plant species (Fig. 3). Sub-group 2 and 3 were observed to be the smallest clusters, containing only three Hsp70-s, whereas, sub-group 6 was the largest cluster, containing 23 Hsp70-s (Fig. 3). Interestingly, Hsp70-s in the same branch contained similar characteristics, like physic-chemical features (Table 1) or gene structure (Fig. 2), suggesting that they may share similar functions. The categorization of CpHsp70-s in papaya was also similar to the classification of Hsp70-s families from other plant species, like soybean[17], quinoa[18], tea plant[19], pearl millet[20], tobacco[21], *Gossypium* spp.[22] and *A. sinensis*[23].

Expression patterns of the *CpHsp70* genes in papaya during the growth and development: The expression patterns of genes encoding the CpHsp70 family may suggest their roles during the growth and development of papaya plants. Of our interest, the expression levels of the *CpHsp70* genes were generated based on the FPKM values as previously described[41]. As a result, this study found that the *CpHsp70* gene family showed a difference in expression during the growth and development of papaya plants (Fig. 4). Particularly, the majority of *CpHsp70* genes (7 out of 12) were reported as highly expressed genes in at least one organ/tissue in Fig. 4. Among them, three genes, including *CpHsp70-01*, *CpHsp70-08* and *CpHsp70-10* were noted to exclusively express in all tissues, like leaves, fruit flesh tissues at four stages and fruit tissues at two stages (Fig. 4). Two genes, like *CpHsp70-02* and *CpHsp70-11*, were highly expressed in leaf tissues and fruit flesh tissues at four stages, respectively, while *CpHsp70-03* and *CpHsp70-07* genes shared a similar expression like strongly expressed in fruit flesh and fruit tissues (Fig. 4).

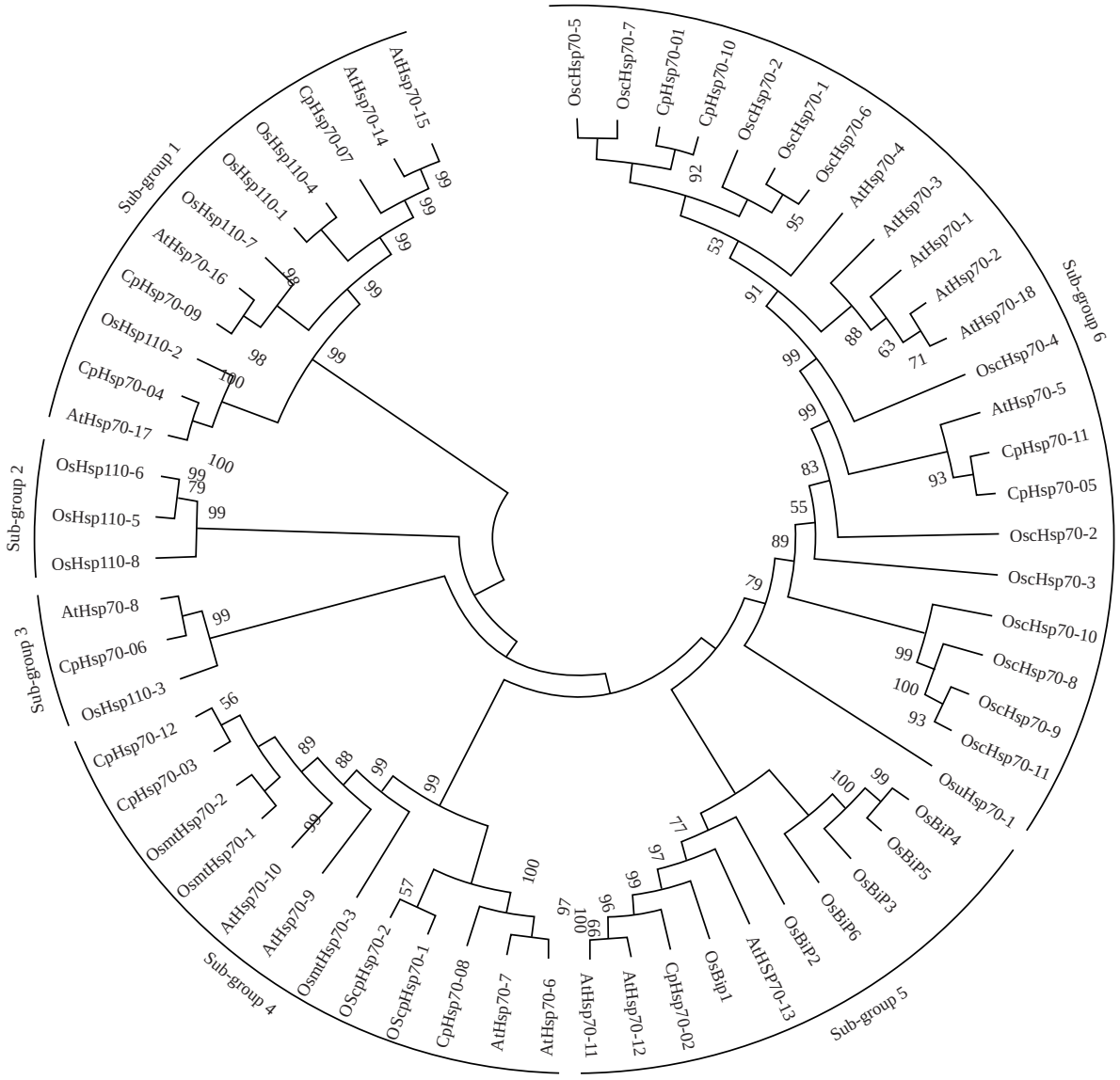

Fig. 3: Phylogenetic tree diagram of Hsp70 families from papaya, *Arabidopsis* and rice

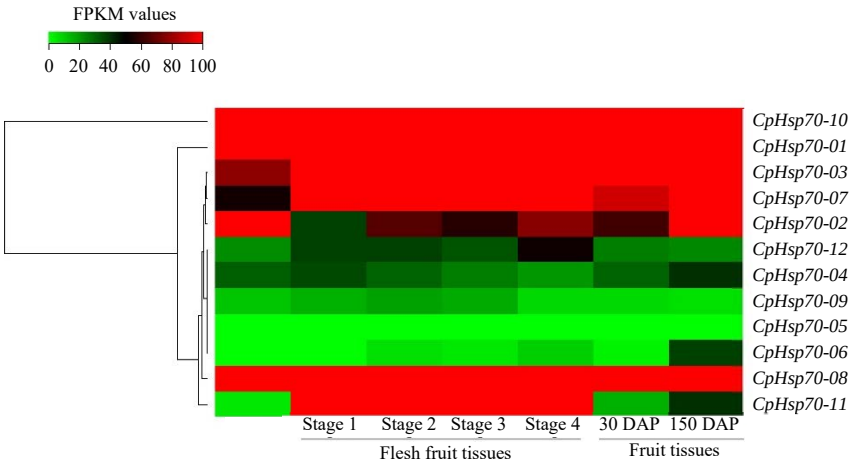

Fig. 4: Expression patterns of the *CpHsp70* gene family during the growth and development of papaya plants

Previously, all genes encoding Hsp70-s showed broad expression levels across numerous tissues and organs in plant species. For example, seven (out of 15) *Hsp70* genes from *A. sinensis* were highly expressed in flower and bud, revealing that they might function in these vegetative organs[23]. The *Hsp70*-s have been demonstrated to participate in various biological processes under normal conditions, like the development of flower and fruit tissues[41]. Taken together, re-analysis strongly believed that the *CpHsp70* family may function on the growth and development of papaya plants.

CONCLUSION

This study provided a first genome-wide analysis of the *CpHsp70* family in papaya, resulting in a total of 12 members that are categorized into six distinct sub-groups. Various *in silico* analyses, like gene organization, phylogenetic tree, protein features of the CpHsp70 family were also comprehensively analyzed and discussed. Re-analysis indicated that the transcript levels of the *CpHsp70* genes are highly variable over different tissues. Taken together, current findings would provide a solid foundation for further functional characterizations in papaya plants and species beyond.

SIGNIFICANCE STATEMENT

This study firstly displayed the characteristics and expression analysis of the *CpHsp70* genes of papaya. Results assigned the foundation for further research of functionality, particularly those involving *CpHsp70* genes of this important tropical fruit crop.

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It's a great pleasure for us to inform you that below mentioned manuscript has been accepted for publication in Pakistan Journal of Biological Sciences as Research Article on the recommendation of the reviewers.

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Author's Name:	Thi Man Le, Thi Thanh Huyen Tran, Ha Duc Chu, Xuan Quyen Vu, Thuy Chau Pham, Hien Thi Le, Quynh Thi Ngoc Le, Viet Hong La and Phi Bang Cao
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Regards

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