Expression of Target Gene *Hsp70* and Membrane Stability Determine Heat Tolerance in Chili Pepper

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ABSTRACT. Experiments were carried out to study the mechanisms for heat tolerance in chili pepper (*Capsicum annuum*). To assess these mechanisms, six genotypes were evaluated for cellular membrane thermostability (CMT) and for *HSP70* gene expression. The plants were grown in an experimental plant growth chamber. The mean value of CMT indicates that membrane integrity was not damaged by the high temperature treatment (50 °C) in most of the genotypes. The genotypes were classified as follows: heat-tolerant (greater than 60%), moderately tolerant (30% to 60%), and susceptible (less than 30%). The heat-tolerant plants recorded the highest CMTs at 89.27%, 88.03%, and 85.10% for AVPP0702, AVPP0116, and AVPP9905, respectively, which might be the reason for the change in their cell membrane thermostability. AVPP9703 and AVPP0002 showed CMTs of 15.87% and 18.43%, which might indicate their sensitivity to heat stress. Heat shock protein 70 kDa was identified and found to be differentially expressed under the heat stress. Under heat stress, significantly increased levels of the *HSP70* gene were detected after 2 h of temperature treatment at 42 °C, which indicated that this gene is quickly and sharply induced by heat shock. This was true for all genotypes tested, which were significantly up-regulated by more than 36.9-, 7.10-, 3.87-, and 3-fold for AVPP0702, AVPP0116, AVPP0002, and AVPP9703, respectively. The *HSP70* gene was found to be significantly down-regulated under heat stress in ‘Kulai’. AVPP0702, AVPP9905, and AVPP0116 could be considered as heat-tolerant genotypes, whereas ‘Kulai’ and AVPP9703 were found to be heat-sensitive genotypes in this investigation.

Chili pepper is widely cultivated, primarily as a spice crop (Dahal et al., 2006). It also is cultivated for fresh vegetable (Ajjaplavara et al., 2010) and postharvest processing markets (Acedo, 2010). In other words, some peppers are grown mainly for postharvest processing such as processing peppers into paste for commercial purposes. The optimum day temperatures for chili pepper growth ranges from 20 to 30 °C (Dahal et al., 2006), and temperature and other abiotic stresses are clearly limiting factors for the growth and development of this crop. Indeed, stresses resulting from high temperature can be harmful to all phases of plant development, and global climate change is thought to cause extreme environmental fluctuations in most agricultural regions (Sood et al., 2009). Temperature increase resulting from changing climatic condition is a serious threat

(Jones et al., 1999) that affects crop production. Heat stress occurs when temperatures are high enough to cause irreversible damage to plant function, affecting various physiological and metabolic processes in plants, and is detrimental in terms of growth and productivity (Ahmed and Hassan, 2011; Hall, 2010). Cell membrane thermostability is a phenotypic parameter used in measuring electrolyte leakage from leaves of plants at different temperatures. Several studies have indicated that CMT is effective in detecting genetic differences with regard to heat tolerance among several crops (Islam et al., 2014; Kumar et al., 2012; Yeh and Hsu, 2004). Heat stress leads to drastic changes in the cell membrane stability and ultimately influences the sensors present in the membrane (Suzuki and Mittler, 2006). Therefore, understanding how plants respond to stress is an important area of research.

Heat shock proteins (HSPs), or stress proteins, are highly conserved and present in all plants and animals. Previous results revealed that most HSPs serve as molecular chaperones (Bukau et al., 2006; Pratt et al., 2001). These proteins play an important role in protein stabilization such as assembling of multiprotein complexes, folding or unfolding of proteins, transport or sorting of proteins into correct compartments at the subcellular level,

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control of cell cycle, and signaling and cell protection against stress or apoptosis (Kuwabara and Imai, 2009). HSPs are known to be connected to the ability of the plant to respond to physiological stress, through biosynthesis, accumulation, or reduction (Wang et al., 2004). In plants, the expression pattern of a number of genes modulated in response to various acute environmental changes, resulting in an altered pattern result in altered biochemical and physiological activities of the cell and the developmental processes of the organism (Knight, 2010). When cells are exposed to various stress conditions such as heat stress, heat shock factors (HSFs) residing in the cytosol dissociate from HSPs (e.g., *HSP70*) are activated and undergo trimerization. These HSF trimers are phosphorylated and translocated to the nucleus where they bind to the heat shock elements, which are located in the promoter region of the HSP genes. HSP mRNA is then transcribed and translated, which leads to increased levels of HSPs in the cytosol. As such, these factors function as chaperones for denatured proteins and also assist in the translocation and/or degradation of damaged proteins (Young, 2010).

Within the diverse HSP family, *HSP70* is the most widely studied member and a highly conserved 70-kDa protein that plays a key role in the stress response in plants (Efeoglu, 2009). In addition to roles in heat stress, HSPs are essential components of cells and developmental processes under normal physiological conditions. All organisms express HSPs belonging to the *HSP70* family with molecular weights between 68 and 78 kDa (Lindquist, 1986). Of the HSP families, the *HSP70* family has a strong correlation with resistance to heat, either permanent or transient (Hightower, 1991). *HSP70* generally functions as a molecular chaperone; i.e., preventing the accumulation of and assisting in the re-folding of proteins under the conditions of stress. The role of *HSP70* in relation to heat tolerance has been reported in several crop plants such as soybean [*Glycine max* (Ortiz and Cardemil, 2001)], wheat [*Triticum aestivum* (Duan et al., 2011)], tomato [*Solanum lycopersicum* (Fender and O'Connell, 1989)], and creeping bentgrass [*Agrostis palustris* (Ye et al., 2012)]. *HSP70* also plays a regulatory role in the stress-associated expression of other genes (Lee and Schöffl, 1996). However, research on HSPs in chili pepper is quite insufficient. The topic of enhancing protection mechanisms (including HSPs and CMT) to induce heat resistance is very interesting, and research in this area has many repercussions for the understanding of heat stress responses. This study therefore aimed at analyzing the changes in CMT and expression of HSP genes in chili pepper under heat stress. The response mechanism of chili pepper to elevated temperature would aid in the development of genotypes that perform better under heat stress. However, the mechanism for heat tolerance using the electrolyte leakage and the expression of HSPs in chili pepper needs to be further exploited to identify heat-tolerant lines for the development of high-yielding heat-tolerant hybrid cultivars.

Materials and Methods

PLANTING MATERIALS. Chili pepper genotypes (AVPP0702, AVPP0116, AVPP9905, AVPP9703, AVPP0002, and 'Kulai') were grown under controlled conditions [28 °C, 16/8 h (light/dark)] to study the membrane stability and expression of *HSP70* gene under heat stress. Seeds were collected from AVRDC—The World Vegetable Center [Tainan, Taiwan (Table 1)].

AVPP0702, AVPP0116, and AVPP9905 were selected because of their thermo tolerance (AVRDC, unpublished data; Usman et al., 2014a). 'Kulai' is a local cultivar cultivated in Malaysia. It was included in the trial as a control and its performance was compared with the AVRDC-introduced cultivars. The chili pepper genotypes were first evaluated to study their morphological, physiological, and biochemical characteristics at the Agrotechnology site, Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor. Characteristics assessed are represented in Table 2.

EVALUATION OF CELL MEMBRANE THERMOSTABILITY. Samples of similar leaf sizes were selected 60 d after sowing. Each sample for assay consisted of a paired set [control (C) and treatment (T)] of six leaf disks, each 1.3 cm², using a leaf puncher 13 mm in diameter. The disks were cut from five fully expanded third or fourth leaves from the top of the stem axis from each genotype. Samples were replicated three times each. Before the assay, the paired set of leaf disks were placed in two separate test tubes (50 mL) and washed thoroughly with four exchanges of deionized water, 10 mL each time, to remove electrolytes adhering to the cut surface of the leaf discs. After the final wash, both sets of test tubes were filled with 10 mL deionized water and sealed with aluminum foil to avoid evaporation. The T set of the test tubes was incubated for 20 min at 50 °C in a temperature-controlled water bath (Oil-baths Model ONE 7; Memmert, Frankfurt, Germany); the C set of test tubes was kept at room temperature (≈25 °C). Both sets of test tubes were then incubated at 4 °C (kept in a refrigerator) for 24 h. Initial conductance readings of both sets (CEC 1 and TEC 1) were performed using a bench electrical conductivity meter (Starter 3000C; Ohaus Corp., Parsippany, NJ) after bringing the test tubes to room temperature. The tubes were then sealed again with aluminum foil and autoclaved at 121 °C and 0.15 MPa for 20 min to completely kill the leaf tissue. The autoclaved tubes were cooled to room temperature, the contents were mixed thoroughly, and a final conductance (CEC 2 and TEC 2) reading was taken. The CMT was calculated using the following equation, where TEC and CEC are a measure of conductance in the treated and control test tubes, respectively, for the initial (CEC 1 and TEC 1) and final (CEC 2 and TEC 2) conductance measurements (Gajanayake et al., 2011).

$$\text{CMT}(\%) = \frac{1 - (\text{TEC1}/\text{TEC2})}{1 - (\text{CEC1}/\text{CEC2})} \times 100$$

HEAT STRESS TREATMENT. Seedlings were randomly divided into two groups, treated (42 °C) and control (30 °C), at 4 weeks after sowing. The plants were exposed to a gradual temperature increase (10 min for every 1° increase) from 25 to 30 °C using an experimental plant growth chamber (GC-101C; Daeyang^{ETS}, Hwasung-si, Kyunggi-do, South Korea). After the temperature reached 30 °C, the plants were kept at 30 °C for 2 h, and the same method of stress treatment was applied for extreme heat stress for 2 h. After each stress treatment, leaf samples were harvested and immediately suspended in liquid nitrogen for subsequent analyses.

RNA EXTRACTION AND QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION. Total RNA was extracted from each test sample using the Trizol method (Chomczynski and Sacchi, 1987). The RNA integrity was verified on a 1.5% agarose gel and using a spectrophotometer (Nanodrop 2000c; Thermo

Fisher Scientific, Wilmington, DE). RNA with a 260/280 ratio of 1.8 to 2.0 was used for quantitative real-time polymerase chain reaction (PCR). The primers used for the reactions were designed from the deduced sequence corresponding to *HSP70* of tomato from the NCBI GenBank (accession no. NM 001247562.1) (Table 3). UBI-3 was used as a reference gene for normalization of the cDNA template quantity with previously published specific primers (Wan et al., 2011).

For each stress condition and the controls, expression measurements were performed using triplicate biological replications. Quantitative PCR was performed in 20- μ L reactions using gene-specific primers, 3 μ L of RNA template, and the

SYBR FAST One-Step qPCR Kit (Kapa Biosystems, Wilmington, MA). The reactions were performed using the CFX96 Real-Time PCR system (Bio-Rad, Mississauga, Ontario, Canada). The thermal profile for quantitative PCR was 5 min at 42 °C for cDNA synthesis, 3 min at 95 °C followed by 40 cycles each consisting of 95 °C for 3 s and 60 °C for 30 s. The primer specificity and the formation of primer-dimers were monitored by a dissociation curve analysis and agarose gel electrophoresis through a 1% agarose gel. The expression level of the pepper UBI-3 gene was used as an internal standard for the normalization of cDNA template quantity using specific primers (Table 3). Controls with no RNA template were also included

Table 1. List of *Capsicum annuum* genotypes used in this study of heat tolerance, including the source, pedigree, and heat tolerance.

No.	Genotype ^z	Source	Pedigree	Heat tolerance ^y
1	AVPP9703	CCA 1410A	HDA210/Szechwan10/MC4	—
2	AVPP9905	Susan's Joy	Susan's Joy	Excellent
3	AVPP0702	CCA6023-9-1-1-2-1	Jin's Joy/4/Bangchang-selex/HDA210/Szechwan10/MC4	Excellent
4	AVPP0116	CCA 3743	PSP-11/Jin's Delight/Kulai	Excellent
5	AVPP0002	CCA 3331	Arunalu/Tumpang	Fair
6	Kulai	Local	Cultivar cultivated in Malaysia	—

^zAV = AVRDC (The World Vegetable Center, Tainan, Taiwan); PP = pepper.

^yAVRDC, unpublished data.

Table 2. Morphological and physiological characteristics of *Capsicum annuum* measured in this study of heat tolerance.

Characters	Description
Plant height (cm)	Taking from the surface of planting medium to the top of the plant
Days to flowering (d)	Number of days required to first flowering after transplanting
Disease incidence (%)	Percentage of infected leaves by mosaic virus to total number of leaves in three random branches selected from each plant
Fruit length (cm)	The length of fruit from pedicel to tip of the fruit
Fruit weight (g)	The weight of individual fruit
Number of fruit (no.)	Total number of fruits harvested from each plant
Yield (g/plant)	Total weight of fresh fruits harvested from each plant
Photosynthesis rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Net photosynthetic rate for the 36 genotypes was measured from the leaves of 90-d-old seedlings using an LI-6400XT portable photosynthesis system (LI-COR, Lincoln, NE)
Chlorophyll content ($\mu\text{g}\cdot\text{mL}^{-1}$)	Measurement of chlorophyll content was done following Coombs et al. (1985) procedure
Cell membrane thermostability	The membrane stability was measured using the procedure described by Gajanayake et al. (2011)
Pungency (SHU scale)	The extractions of capsaicin from the chili pepper samples was done using the method described by Collins et al. (1995) and were analyzed using ultra-fast liquid chromatography (UFLC) as described by Usman et al. (2014b); Scoville heat units (SHU) were used to calculate the heat units for all samples and classified them according to their degree of pungency as described by Weiss (2002)

Table 3. Primer sequence (5' - 3'), amplicon length, efficiency (E), coefficient of determination (R^2), and annealing temperature (T_m) of the target gene *HSP70* and housekeeping gene *UBI-3* analyzed in five AVRDC (The World Vegetable Center, Tainan, Taiwan) *Capsicum annuum* genotypes and one local Malaysian cultivar (Kulai) used for quantitative real-time polymerase chain reaction analysis.

Gene	Gene symbol	Primer sequence (5' - 3') ^z	Amplicon length (bp)	E	R^2	T_m (°C)
Ubiquitin-conjugating protein	UBI-3	For TGTCCATCTGCTCTCTGTTG Rev CACCCCAAGCACAAATAAGAC	204	1.05	0.9989	54.4 53.8
Heat shock protein-70	HSP-70	For CTGATCGACGGCATCCTCAA Rev ACATGAGAAACGCAGTGAAGG	198	0.975	0.995	57.0 55.4

^zForward (For) and reverse (Rev) primers.

in the quantitative PCR analysis. The quantitative real-time PCR product was analyzed for molecular weight (MW) and *HSP70* gene specificity using Kapa™ Universal Ladder (Kapa Biosystems) as a standard (Fig. 1) with a gel doc XR system (Bio-Rad). The MW was estimated using Image Laboratory software Version 5.0 provided by BioRad and ranged from 68 to 70 kDa.

STATISTICAL ANALYSIS. The data for membrane stability were subjected to a statistical analysis of variance, and the means were separated using Duncan’s multiple range test with SAS (Version 9.2; SAS Institute, Cary, NC). The data analysis for *HSP70* was performed using the software provided by Bio-Rad.

Fig. 1. Gel electrophoresis showing the target *HSP70* gene identified in *Capsicum annuum*. UBI-3 was used as the reference gene for normalization. The Kapa™ Universal Ladder [Ladder (Kapa Biosystems, Wilmington, MA)] was used as standard.

The comparative Ct ($2^{-\Delta\Delta C_t}$) method was used to calculate the changes in gene expression as a relative fold difference between an experimental and calibrator sample. The genes that were up- or down-regulated by more than 2-fold ($P < 0.05$) were considered to be differentially expressed.

Results and Discussion

FIELD EVALUATION. The morphological, physiological, and biochemical characteristics of the studied genotypes were assessed and presented in Table 4.

CELL MEMBRANE THERMOSTABILITY. Electrical conductivity has been used as an index of membrane stability to identify heat-tolerant genotypes in chili (Gajanayake et al., 2011) and for screening heat-tolerant genotypes in different crops (Wahid et al., 2007). A highly significant ($P < 0.05$) difference was observed among the genotypes for CMT (Fig. 2). The heat-tolerant plants showed the highest CMT at 89.27%, 88.03%, and 85.10% for AVPP0702, AVPP0116, and AVPP9905, respectively, which might explain the change in CMT. AVPP9703 exhibited 15.87%, which might indicate its sensitivity to heat stress. According to Craufurd et al. (2003), CMT above 60% is heat-tolerant, 30% to 60% moderate, and less than 30% sensitive to heat stress with slight changes. The present investigation is in agreement with the works of Gajanayake et al. (2011), in which CMT was measured in 12 ornamental pepper genotypes, with most being thermotolerant, with a mean of 59.50%. Similar results reported in wheat cultivars by

Table 4. Mean values of plant height at harvest, days to flowering (DF), disease incidence (DI), chlorophyll content (CPL), photosynthesis rate, fruit length (FL), fruit weight (FW), number of fruit, yield, and pungency level characteristics measured in the six studied genotypes of *Capsicum annuum* (n = 3).

Genotype	Plant ht (cm)	DF (d)	DI (%)	CPL ($\mu\text{g}\cdot\text{mL}^{-1}$)	Photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	FL (cm)	FW (g)	Fruit (no.)	Yield (g/plant)	Pungency (SHU scale) ^z
AVPP9703	60.60	63.00	31.25	5.64	14.28	10.79	9.08	49.33	338.08	Non-pungent
AVPP9905	68.27	62.67	41.24	5.57	12.74	16.43	22.94	63.00	1144.30	Highly pungent
AVPP0702	56.47	63.67	39.14	6.72	10.04	12.66	8.61	73.00	398.99	Moderately pungent
AVPP0002	60.23	62.83	40.79	6.30	10.92	9.09	3.29	208.33	490.44	Very highly pungent
AVPP0116	92.13	64.50	28.14	5.60	17.62	10.55	11.65	46.33	411.35	Moderately pungent
Kulai	72.17	59.00	39.96	6.44	19.32	17.23	17.68	64.00	818.10	Moderately pungent

^zScoville heat units.

Fig. 2. Cell membrane stability index of *Capsicum annuum* genotypes, each bar showing the stability of the membrane on exposure to heat stress; bars followed by the same letters are not statistically significant ($P < 0.05$) using Duncan’s multiple range test. Error bars indicate SE (n = 3).

Kumar et al. (2012) with indications of changes in membrane stability against heat stress.

The mean value of CMT of the genotypes from this investigation indicates that the membrane integrity of the heat-tolerant genotypes was not damaged by the high-temperature treatment of 50 °C for 20 min, whereas AVPP9703 and AVPP0002 were sensitive and damaged at 50 °C. The membrane is the first line of defense with many heat-responsive sensors that help plants to activate defense mechanisms early in heat shock (Singh et al., 2007). The unification and roles of cell membranes are sensitive to high-temperature stress. Indeed, heat stress alters the post-secondary structures of membrane proteins, and the permeability of electrolytes increases as a result of such changes. Increased electrolyte leakage indicates decreased CMT, which has long been used as an indirect mechanism of heat stress tolerance in several crop plant species, including tomato (Islam et al., 2014), potato [*Solanum tuberosum* (Nagarajan and Bansal, 1986)], and wheat (Kumar et al., 2012).

AMPLIFICATION AND IDENTIFICATION OF THE *HSP70* TARGET GENE. A known-specific *HSP70* primer of tomato was used as a template to examine its presence in the candidate chili peppers. Strong positive threshold cycle (Cq less than 29) values were observed in all genotypes, indicative of abundant target nucleic acid in the samples, except for 'Kulai', which showed a weak reaction (Cq of 38 to 40) indicative of a minimal amount of the target nucleic acid in the sample (Table 5). Heat stress is known to trigger changes in the transcription of HSP genes. Therefore, we assessed the expression profile of the *HSP70* gene under heat stress using quantitative real-time PCR. The Cq is defined as the number of cycles required for the fluorescent signal to exceed the background level, and threshold cycles are inversely proportional to the quantity of target nucleic acid in the sample: the lower the Cq level, the greater the quantity of target nucleic acid in the sample. The real-time assays used 40 cycles of amplification, and all genotypes showed abundant target nucleic acid in leaf tissues under heat stress, except for 'Kulai', which showed a minimal amount of the target gene. Under heat stress, significantly increased levels of the *HSP70* gene were detected after 2 h of temperature treatment at 42 °C, which indicated that this gene is quickly and sharply induced by heat shock. This was true for all genotypes,

which were significantly up-regulated by more than 36.9-, 7.10-, 3.87-, and 3-fold for AVPP0702, AVPP0116, AVPP0002, and AVPP9703, respectively (Table 5). In contrast, 'Kulai' showed significant *HSP70* down-regulation under heat stress (Table 5).

ANALYSIS OF FOLD DIFFERENCES IN *HSP70* GENE EXPRESSION UNDER HEAT STRESS. The genes that were up- or down-regulated by more than 2-fold and with $P < 0.05$ were considered to be differentially expressed. AVPP0702, AVPP0116, and AVPP9703 in this study showed differential expression: *HSP70* was up-regulated by 36.8-, 7.1-, and 2.58-fold in AVPP0702, AVPP0116, and AVPP9703 with $P < 0.05$, respectively (Fig. 3). The up-regulation of *HSP70* in AVPP0702, AVPP9905, AVPP0116, and AVPP9703 indicates that *HSP70* increases in the cell when exposed to heat stress; the down-regulation of *HSP70* in 'Kulai' indicates the opposite.

From this present investigation, genotypes having a high CMT appear to have high expression of *HSP70*, which might indicate thermotolerance. AVPP0702, AVPP9905, and AVPP0116 could be considered as heat-tolerant genotypes, whereas 'Kulai' and AVPP9703 can be considered as heat-sensitive genotypes. A deficiency in the expression of chaperones often results in increased thermosensitivity or death, even under normal growth conditions (Chen et al., 1982). The expression of *HSP70* in thermotolerant genotypes is higher than thermosensitive genotypes, indicating better protection against heat shock stress. A significant positive correlation between *HSP70* gene expression and the acquisition of thermotolerance has been studied by Lee et al. (2009), resulting in increased tolerance to water and high-temperature stress in plants (Alvim et al., 2001; Ono et al., 2001; Sung and Guy, 2003). Thus, AVPP0702, AVPP9905, and AVPP0116 have better protection against heat stress than AVPP9703 and 'Kulai'. The works of Ferguson et al. (1990) showed that membrane stability and *HSP70* accumulation is higher in heat-tolerant Chilean mesquite (*Prosopis chilensis*) than heat-susceptible soybean (*Glycine max*) at 20 min of treatment at 40 °C as well as after 60 and 90 min of heat shock.

FUNCTION OF *HSP70* GENE UNDER HEAT STRESS. Heat stress is one of the abiotic factors that cause the most severe damage to crop plants. In nature, individual stresses such as drought and high temperature usually do not occur separately. Two major

Table 5. Descriptive information of the target gene *HSP70* for each of the six *Capsicum annuum* genotypes as revealed by quantitative real-time polymerase chain reaction analysis.^z

Genotype	Mean Cq ^y	Mean efficiency corrected Cq	Normalized expression ^x	Relative normalized expression ^w	Regulation	Compared with regulation threshold	P value
AVPP0702	23.38	22.89	3.93	36.86	36.86	Up-regulated	0.000039
AVPP9905	26.38	25.83	0.41	15.10	15.10	Up-regulated	—
AVPP0116	26.05	26.05	0.46	7.10	7.10	Up-regulated	0.000103
AVPP0002	25.5	24.96	0.30	3.87	3.87	Up-regulated	0.129202
AVPP9703	24.02	23.52	6.33	2.58	2.58	Up-regulated	0.001436
Kulai	38.03	37.23	0.00028	0.06	-17.38	Down-regulated	0.000404

^zAnalysis of the *HSP70* under heat stress (42 °C) includes amplification efficiency (Cq), normalized and relative normalized expression, gene regulation, and probability value.

^yCycle threshold (Cq) indicates the abundance of the target nucleic acid; the lower the Cq level, the higher the nucleic acid (in this case *HSP70*).

^xNormalization of the expression level of *HSP70* target genes to that of UBI-3 housekeeping gene in each sample so that the level of *HSP70* target gene in one sample can be compared with another. This normalization process controls for unequal RNA loading, differences introduced during RT reaction, etc.

^wRelative normalized expression is the threshold set of the expression level for *HSP70* target gene in the leaf tissues for all samples that can be used to know whether the gene expresses higher in the stressed plants than in the normal plants.

Fig. 3. Change in the expression level of *HSP70* gene in AVPP0702, AVPP0116, and AVPP9905 (tolerant) and AVPP9703 and 'Kulai' (sensitive) *Capsicum annuum* genotypes under heat shock treatment of 30 and 42 °C for 2 h. UBI-3 was used as endogenous control. Error bars indicate SE (n = 3).

factors that lead to an increased respiration rate, the closure of the stomata, reduced photosynthesis rates, and increased leaf temperature in plants are heat shock and drought (Ye et al., 2012). To counter the effects of such stress, plants express a variety of defense genes such as HSPs (Ye et al., 2012). The transcription level of all *HSP70* genes in this study was significantly enhanced under heat stress in the heat-tolerant genotypes, which showed that this gene may play a role in heat stress responses.

The synthesis and accumulation of specific proteins are established during rapid heat stress, and these proteins are described as HSPs. Many studies maintain that HSPs are molecular chaperones protecting the native arrangement of cellular proteins under heat stress. Among these, *HSP70* is one of the most abundant HSPs in eukaryotic cells. *HSP70* binds in an ATP-dependent manner to hydrophobic patches of partially unfolded proteins and prevents protein aggregation (Mayer and Bukau, 2005). Although *HSP70s* accumulate during heat shock, their constitutively expressed cognates (*HSC70*) are essential for general cellular functions as a result of their involvement in the control of protein homeostasis. They assist in the folding of nascent polypeptides released from the ribosome (Hartl and Hayer-Hartl, 2002), sorting of proteins to cellular organelles by interaction with mitochondrial and chloroplast protein import complexes (Mirus and Schleiff, 2009; Zhang and Glaser, 2002), and form a link with the ubiquitin-mediated proteasomal degradation pathway (Ballinge et al., 1991; Lüders et al., 2000).

Conclusion

Terminal heat stress leads to changes in the various parameters such as HSP genes and CMT in chili pepper and is an important component of thermotolerance capacity. Altered CMT values were also observed with differential heat shock. An abundance of *HSP70* gene transcript was observed in leaf tissues in the heat-tolerant genotypes compared with the heat-susceptible genotype; genotypes having a high CMT appear to have high *HSP70* expression, which might indicate

thermotolerance. Despite the up-regulation of *HSP70* gene in AVPP0002 and AVPP9703, the expression levels were found to be very low and hence might indicate sensitivity to heat stress. The reason behind down-regulation/up-regulation is that the cellular components decrease or increase in response to external variables such as heat stress. The extent of differences observed in *HSP70* and membrane stability can be used to improve thermotolerance capacity among the studied genotypes. For further chili breeding improvement program, this might also help chili breeders breeding for abiotic stress. The further analysis of *HSP70* promoter elements may lead to a better understanding of the regulatory mechanisms controlling the tissue-

specific expression of the *HSP70* gene for use in enhancing the thermotolerance capacity of plants.

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