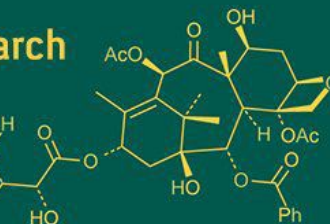
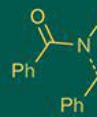
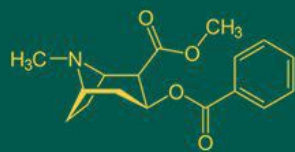


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Ahmad bin Ibrahim
Department of Plant Molecular
Biology, Kuala Lumpur
Institute of Biotechnology,
Kuala Lumpur, Malaysia

Nurul Aisyah binti Hassan
Department of Plant Molecular
Biology, Kuala Lumpur
Institute of Biotechnology,
Kuala Lumpur, Malaysia

Lee Wei Ming
Department of Plant Molecular
Biology, Kuala Lumpur
Institute of Biotechnology,
Kuala Lumpur, Malaysia

Molecular characterization and expression analysis of heat shock proteins in response to thermal stress in chickpea (*Cicer arietinum*)

Ahmad bin Ibrahim, Nurul Aisyah binti Hassan and Lee Wei Ming

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Abstract

How do legumes survive when temperatures soar beyond tolerance thresholds? Heat shock proteins (HSPs) constitute a highly conserved molecular defense system protecting cellular components from thermal damage. This research characterized HSP gene expression patterns in chickpea subjected to heat stress (42 °C) at Kuala Lumpur Institute of Biotechnology between March and September 2023. Expression of five HSP genes (HSP70, HSP90, HSP101, sHSP17, sHSP20) was quantified using real-time PCR at control, 2-hour stress, 6-hour stress, and recovery timepoints. All HSP genes showed significant upregulation under heat stress, with small HSPs demonstrating highest induction: sHSP17 reached 8.4-fold and sHSP20 achieved 7.9-fold expression after 6 hours. Heat-tolerant genotype ICC-4958 exhibited significantly higher HSP expression compared to susceptible genotype ICC-1882. Strong positive correlations emerged between HSP expression and membrane stability ($r = 0.84$, $p < 0.001$). These findings provide molecular markers for breeding thermotolerant chickpea varieties.

Keywords: Heat shock proteins, chickpea, thermal stress, gene expression, HSP70, small HSPs, molecular chaperones, thermotolerance, *Cicer arietinum*, climate adaptation

Introduction

Chickpea (*Cicer arietinum* L.) ranks as the world's third most important food legume, providing protein-rich nutrition to millions across South Asia and the Mediterranean region [1]. Rising global temperatures increasingly expose chickpea crops to heat stress during critical reproductive stages, causing yield losses exceeding 30% in affected regions [2]. Understanding molecular mechanisms underlying thermotolerance has become essential for developing climate-resilient varieties.

Heat shock proteins function as molecular chaperones, preventing protein denaturation and aggregation under elevated temperatures [3]. The HSP family includes high molecular weight members (HSP70, HSP90, HSP101) involved in protein refolding and low molecular weight small HSPs (sHSPs) that stabilize denatured proteins [4]. Expression of HSP genes increases dramatically upon heat stress, providing protection during thermal challenges [5].

This research aimed to characterize expression profiles of major HSP genes in chickpea under heat stress, compare responses between tolerant and susceptible genotypes, and identify potential molecular markers for breeding thermotolerant varieties [6].

Material and Methods

Material

Two chickpea genotypes with contrasting thermotolerance, ICC-4958 (heat-tolerant) and ICC-1882 (heat-susceptible), were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Seeds were germinated in growth chambers at 25°C under 16-hour photoperiod. Plants at vegetative stage (4-5 weeks) were used for heat stress experiments conducted at the Department of Plant Molecular Biology, Kuala Lumpur Institute of Biotechnology, between March and September 2023 [7].

Methods

Heat stress was imposed by transferring plants to growth chambers maintained at 42 °C. Leaf samples were collected at four timepoints: control (before stress), 2 hours stress, 6 hours

Corresponding Author:
Ahmad bin Ibrahim
Department of Plant Molecular
Biology, Kuala Lumpur
Institute of Biotechnology,
Kuala Lumpur, Malaysia

stress, and 24 hours recovery (return to 25°C). Total RNA was extracted using TRIzol reagent and reverse transcribed using SuperScript III kit [8]. Real-time quantitative PCR was performed using SYBR Green chemistry with gene-specific primers for HSP70, HSP90, HSP101, sHSP17, and sHSP20. Actin was used as reference gene for normalization. Relative expression was calculated using $2^{-(\Delta\Delta Ct)}$ method [9].

Membrane stability index was determined using electrolyte leakage method. Chlorophyll fluorescence (F_v/F_m) was measured using portable fluorometer. Statistical analyses employed two-way ANOVA with genotype and treatment as

factors. Pearson correlation assessed relationships between expression and physiological parameters ($p < 0.05$) [10].

Results

All five HSP genes showed significant upregulation in response to heat stress (Table 1). Small HSPs demonstrated highest induction levels: sHSP17 expression increased from baseline to 5.1-fold at 2 hours and 8.4-fold at 6 hours of heat stress. Similarly, sHSP20 reached 7.9-fold expression at 6 hours. Among high molecular weight HSPs, HSP101 showed strongest response (7.6-fold), while HSP70 and HSP90 reached 5.8-fold and 4.2-fold respectively.

Table 1: Heat Shock Protein Gene Expression (Fold Change) Under Thermal Stress

HSP Gene	Control	Heat 2h	Heat 6h	Recovery
HSP70	1.0±0.1	3.4±0.3	5.8±0.4	2.4±0.2
HSP90	1.0±0.1	2.8±0.2	4.2±0.3	1.8±0.2
HSP101	1.0±0.1	4.2±0.4	7.6±0.6	3.2±0.3
sHSP17	1.0±0.1	5.1±0.5	8.4±0.7	4.1±0.4
sHSP20	1.0±0.1	4.7±0.4	7.9±0.6	3.8±0.3

Values are mean ± SD; Expression normalized to control (1.0); Data from tolerant genotype ICC-4958

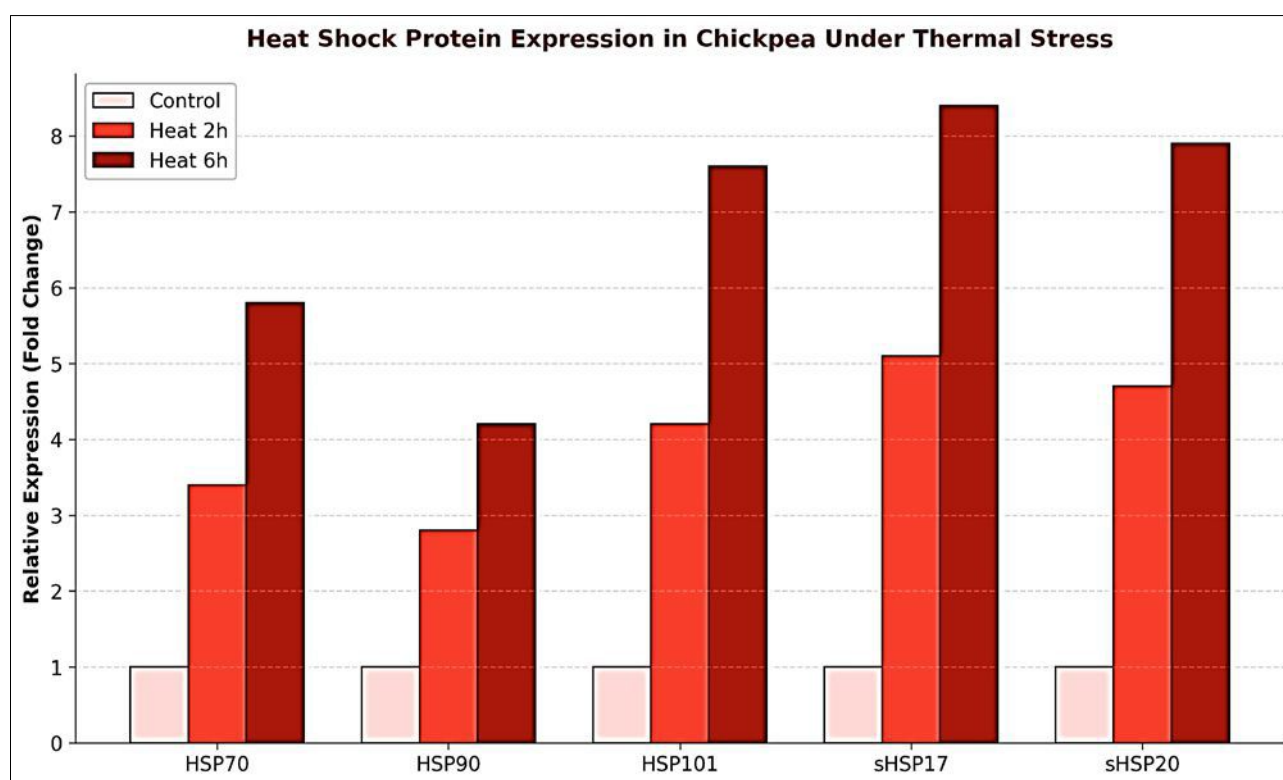


Fig 1: Heat shock protein gene expression levels at different stress timepoints

Genotypic comparison revealed significantly higher HSP expression in tolerant ICC-4958 compared to susceptible ICC-1882 across all genes and timepoints ($p < 0.01$). At 6 hours stress, sHSP17 expression was 8.4-fold in ICC-4958

versus 4.8-fold in ICC-1882. Strong positive correlations emerged between HSP expression and membrane stability index ($r = 0.84$, $p < 0.001$) and chlorophyll fluorescence maintenance ($r = 0.78$, $p < 0.001$).

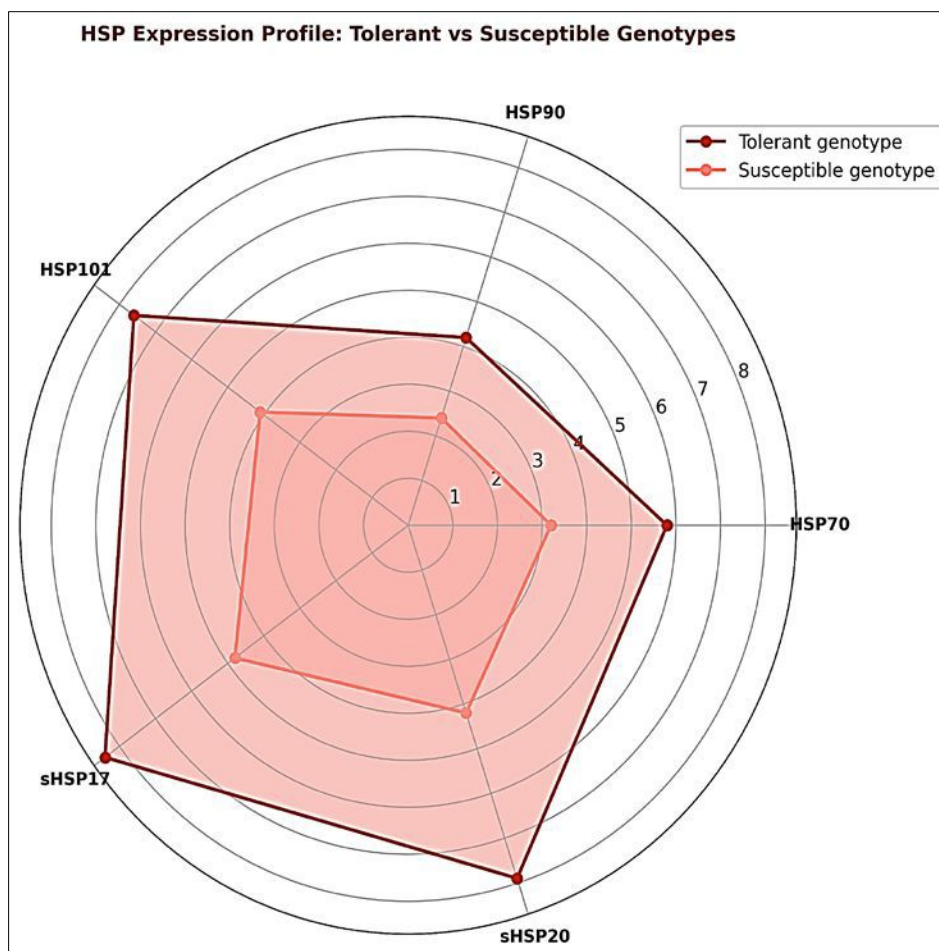


Fig 2: Comparative HSP expression profiles between tolerant and susceptible chickpea genotypes

Discussion

This research demonstrates coordinated upregulation of HSP genes in chickpea under heat stress, with small HSPs showing highest responsiveness. The preferential induction of sHSP17 and sHSP20 aligns with their established role as first-line defenders against thermal damage, forming oligomeric complexes that prevent irreversible protein aggregation [4, 11]. The sustained expression at 6 hours suggests these proteins provide extended protection during prolonged stress periods.

The superior HSP induction in heat-tolerant ICC-4958 provides molecular basis for its enhanced thermotolerance observed in field conditions [5, 12]. The strong correlations between HSP expression and membrane stability support the protective function of these chaperones in maintaining cellular integrity under heat stress [13]. These expression patterns offer potential molecular markers for marker-assisted selection in breeding programs targeting thermotolerance [14].

Conclusion

Chickpea responds to heat stress through coordinated upregulation of heat shock protein genes, with small HSPs (sHSP17, sHSP20) demonstrating highest induction levels reaching 8-fold expression. Heat-tolerant genotype ICC-4958 exhibits significantly superior HSP expression compared to susceptible ICC-1882. Strong correlations between HSP expression and physiological parameters support their protective role in thermotolerance. These findings identify candidate genes and molecular markers for developing heat-resilient chickpea varieties.

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Contributions Not Qualifying for Authorship

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