UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF HEAT TOLERANT GENOTYPES OF CHILI PEPPER BASED ON CELL MEMBRANE THERMOSTABILITY AND EXPRESSION OF HEAT SHOCK PROTEIN GENES

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By

MAGAJI USMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

October 2014

DEDICATION

This work is dedicated to my beloved parents; Hajiya Fatima Abubakar Kurfi and Alhaji Muhammad Aminu Abubakar Kurfi for their endless prayers and financial support.

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

IDENTIFICATION OF HEAT TOLERANT GENOTYPES OF CHILI PEPPER BASED ON CELL MEMBRANE THERMOSTABILITY AND EXPRESSION OF HEAT SHOCK PROTEIN GENES

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October 2014

Supervisor: **Professor Mohd Rafii Yusop, PhD**

Faculty: **Agriculture**

Terminal heat stress leads to change in the various parameters, such as heat shock protein genes and cell membrane thermostability, in chili pepper and is an important component of thermotolerance capacity. Experiments were carried out at Agrotechnology Unit, Agro Gene Bank and the Plant Physiology Laboratory, Institute of Tropical Agriculture, Universiti Putra Malaysia to study the mechanisms for heat tolerance in chili pepper genotypes. To assess these mechanisms, 36 genotypes were evaluated for morpho-physiological characters, cellular membrane thermostability (CMT) and for *HSP70* gene expression. Significant ($p < 0.05$) variation was observed among the genotypes for all the characters with genotypes AVPP9905 (1144.3 g) and Kulai (818.1 g) recording the highest yield per plant except chlorophyll content and days to flowering. The plants from selected chili pepper genotypes for CMT and *HSP70* gene experiments were grown in experimental plant growth chamber for 4-8 weeks after sowing. The mean value of CMT indicates in most of the genotypes that membrane integrity was not damaged by the high temperature treatment (50ºC), with a mean value of 64.64%. The genotypes were classified based on heat tolerant (>60%), moderately tolerant (30%-60%) and susceptible (<30%). Genotypes AVPP0702, AVPP0116 and AVPP9905 recorded the highest CMTs, at 89.27%, 89.27% and 85.10% respectively. AVPP9703 and AVPP0002 gave CMTs of 15.87% and 18.43%, respectively which might indicate their sensitivity to heat stress. *HSP70* gene was identified and found differentially expressed under heat stress condition. Under heat stress, significantly increased levels of the *HSP70* genes were detected after 2 hours temperature treatment at 42°C, which indicated these genes are quickly and sharply induced by heat shock. This was found especially 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. Variety Kulai was found to be significantly down regulated under heat stress. Genotypes AVPP0702, AVPP9905 and AVPP0116 could be considered as heat tolerant genotypes while Kulai and AVPP9703 as heat sensitive genotypes from this study. The result further proves that CMT and *HSP70* are key components of the defense mechanism against heat stress in chili pepper.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGENALPASTIAN GENOTIP CILI KETAHANAN HABA BERDASARKAN TERMOSTABILITI SEL MEMBRAN DAN PENGEKSPRESAN GEN PROTEIN RENJATAN HABA

Oleh

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Tekanan haba terminal membawa kepada perubahan dalam pelbagai parameter seperti gen protein renjatan haba dan termostabiliti sel membran ke atas pokok cili dan ianya merupakan satu komponen penting terhadap keupayaan ketahanan haba. Eksperimen ini telah dijalankan di Unit Agroteknologi, Agro-genebank dan Makmal Fisiologi, Institut Pertanian Tropika, Universiti Putra Malaysia untuk mengkaji mekanisma ketahanan haba ke atas genotip cili. Bagi mengukur mekanisma 36 genotip tersebut, ciri morfo-fisiologi, kestabilan termo membran sel (CMT) dan pengekspresan gen *HSP*70 telah dinilai. Kesemua ciri menunjukkan perbezaan yang ketara dikalangan genotip kecuali kandungan klorofil dan bilangan hari berbunga dengan genotip AVPP9905 (1144.3 g) dan Kulai (818.1 g) mencatatkan hasil tertinggi per pokok. Untuk kajian CMT dan *HSP*70, pokok dari genotip cili terpilih telah ditanam di dalam kebuk pertumbuhan selama 4-8 minggu selepas disemai. Berdasarkan nilai min CMT, kebanyakan genotip menunjukkan integriti membran tidak terjejas oleh suhu tinggi (50ºC), dengan nilai min 64.64%. Genotip dikelaskan kepada tahan haba (>60%), sederhana tahan (30%-60%) dan rentan haba (<30%). Genotip pokok cili yang mempunyai ketahanan haba dengan nilai CMT tertinggi, 89.27%, 89.27% dan 85.10% bagi genotip AVPP0702, AVPP0116 dan AVPP9905 masing-masing. Genotip AVPP9703 dan AVPP0002 memberikan bacaan 15.87% dan 18.43% masing-masing yang menunjukkan sensitif terhadap tekanan haba. Gen *HSP70* telah dikenalpasti dan didapati pengekspresannya di bawah keadaan tekanan haba. Di bawah tekanan haba, peningkatan ketara tahap kandungan gen *HSP70* telah dikenalpasti selepas 2 jam rawatan suhu pada 42°C, yang menunjukkan gen ini didorong oleh kejutan haba dengan cepat dan mendadak. Keputusan ini berdasarkan peningkatan pengawalan yang ketara oleh semua genotip iaitu peningkatan lebih daripada 36.9, 7.10, 3.87 dan 3 kali ganda, untuk AVPP0702, AVPP0116, AVPP0002 dan AVPP9703 masing-masing. Cili varieti Kulai menunjukkan penurunan yang ketara di bawah tekanan haba. Genotip AVPP0702, AVPP9905 dan AVPP0116 adalah genotip yang tahan haba sementara Kulai dan AVPP9703 sebagai genotip yang sensitif haba. Hasil keputusan kajian ini membuktikan bahawa CMT dan gen *HSP*70 adalah komponen penting untuk mekanisma ketahanan tekanan haba dalam cili.

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I certify that a Thesis Examination Committee has met on 23 October 2014 to conduct the final examination of Usman Magaji on his thesis entitled "Identification of Heat Tolerant Genotypes of Chili Pepper Based on Cell Membrane Thermostability and Expression of Heat Shock Protein Genes" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
AVRDC	Asian Vegetable Research and Development Centre
A_{664}	Absorbance at 664 wavelength
A_{647}	Absorbance at 647 wavelength
cm	Centimetre
cDNA	Complementary Deoxyribonucleic Acid
CMT	Cell Membrane Thermostability
ºC	Degree centigrade
DEPC	Diethylpyrocarbonate
EC	Electrical Conductivity
et al	et alia
GCV	Genotypic Coefficient of Variation
gm	Grams
HSC	Heat Shock Cognate
HSE	Heat Shock Element
HSF	Heat Shock Factor
HSP	Heat Shock Protein
kDa	Kilo Dalton
Kg	Kilogram
LSD	Least Significant Difference
µ	micro
µS	microSiemans
ml	millilitre
Mol	mole
MPa	Megapascal
%	Percentage
ppmH	Parts per million of heats
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
qRT-PCR	Quantitative Real Time PCR
RI	Relative Injury
RNA	Ribonucleic Acid
SAHN	Sequential Agglomerative Hierarchical Nonoverlapping
SE	Standard Error
S	Seconds
SHU	Scoville Heat Unit
spp	Species
UFLC	Ultra-Fast Liquid Chromatography
UPGMA	Unweighted Pair Group Method Average
σ^2	Variance

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Chili pepper (*Capsicum annuum* and *C. frutescence* L.) is widely cultivated mostly as a spice crop (Dahal, 2006). Optimum day temperatures for chili pepper (*C. annuum* L.) growth range from 20-30ºC (Supena *et al.,* 2006). Day time temperatures rise above 30ºC year round in Malaysia. Such conditions are the important factors limiting the production of chili. Though chili is an important spice crop, still Malaysian domestic production cannot meet the demand due to the poor performance of local varieties under high temperatures (Anonymous, 2013).

Plant growth and development is the product of the interaction between the genotype (genetic potential) and the environment in which the plant grows. Plant growth and development depends on biochemical processes (e.g. photosynthesis) that in turn depends on factors in the environment in order to proceed optimally (Acquaah, 2009). When the environmental condition is less than the optimum requirement, plant experiences stress which adversely affects its growth and development and ultimately, its productivity and economic value (Howarth, 2005). The common stresses that plants may be exposed to include; heat, drought, salinity, and mineral toxicity (Zinn *et al*., 2010). Heat stress occurs when temperatures are high enough to cause irreversible damage to plant function (Hall, 2010). Heat stress affects various physiological and metabolic processes in plants and is detrimental in terms of growth and productivity (Chinnusamy *et al.,* 2007; Larkindale and Vierling, 2008; Frank *et al.,* 2009; Snider *et al*., 2009; Saha *et al*., 2010; Ahmed and Hassan, 2011). Among the abiotic stresses, temperature increment due to changing climatic conditions is a serious threat which affects crop production (Jones *et al*., 1999).

Along with conditions such as light and humidity, temperature is an important environmental factor for plants (Went, 1953). Temperature, with its daily fluctuations and seasonal changes, regulates plant growth, distribution, and survival (Long and Woodward, 1988). Most physiological processes of plants remain normal at temperatures ranging from approximately 0 to 40ºC, and plants respond to various temperatures in this range. Plants show responses to two distinct temperature regions: non-extreme temperatures and extreme temperatures (Viswanathan and Zhu, 2002). Well-known responses of plants to extreme temperatures are cold acclimatization and vernalization (Sheldon *et al*., 2000). The molecular mechanisms of these responses have recently been determined through intensive studies (Sharma *et al.,* 2005; Chinnusamy *et al*., 2006; Kuwabara and Imai, 2009). Under high temperature, flowering asynchrony, decreased pollen fertility and, abscission of flower buds, flowers and fruits are more common in chili (Barnabas *et al.,* 2008; Hedhly *et al.,* 2009; Craufurd and Wheeler, 2009; Thakur *et al*., 2010). In Malaysia, among the stresses, high temperature is one of the major problems for chili cultivation (Anonymous, 2013).

Cell membrane thermostability (CMT) is a very sensitive and rapid method to identify heat tolerance in plants (Wu and Wallner, 1983). As a phenotypic parameter, CMT is used for measuring electrolyte leakage from leaves of plants at different temperatures (Sullivan, 1972). Several studies have indicated that CMT is effective in detecting genetic difference with regard to heat tolerance among several crops (Saadalla *et al.,* 1990; Yeh and Hsu, 2004; Islam *et al.,* 2014). Stress resistance is an inherent part of all cultivars development programs. However, understanding the way plants respond to heat stress is the number one step in the development of thermo tolerant crops. In general, hybrid varieties have improved yield characteristics and suffer less from different stresses than the traditional ones. Therefore, research is needed to develop thermo tolerant varieties of chili to increase yield and production. In Malaysia, researches on heat shock proteins are insufficient for the development of heat tolerant chili pepper varieties. A better knowledge on the identification and characterization of *HSPs* as well as measure of the electrolyte leakage is necessary in order to identify heat tolerant chili pepper lines for the development of high yielding heat tolerant hybrid varieties, which will contribute to achieve self-sufficiency in chili pepper in Malaysia.

1.2 Statement of the Problem

In Malaysia, among the factors responsible for low production of chili pepper is the non-availability of high yielding varieties suitable for cultivation under heat stress such as in glass-house and rain shelter and increasing high temperature. The production is seasonal due to lack of appropriate cultivars and techniques. Early summer to early rainy season is the lean period of production. Prevailing high temperature, blowing of hot wind and shortage of soil moisture during early summer, and high temperature and excessive moisture during rainy summer are the major factors limiting its cultivation during summer and rainy months. Such condition induces the abscission of flower buds, flowers and young fruits which is the most important factors limiting the production and consequently reduce yield of chili (Ipgri, 1995). Current speculation about global climate change is that most agricultural regions will experience more extreme environmental fluctuations (Solomon *et al*., 2009). For plants, the possibilities to escape from these stresses are limited because plants are immobile (Kuiper, 1998). Temperature and other abiotic stresses are clearly limiting factors for the growth and development of crop. Stresses due to high temperature can be detrimental to all phases of plant development. During the short time surrounding fertilization, even a single hot day can be fatal to reproductive success for many plant species (Zinn *et al.,* 2010). So understanding how the plants respond to stress is a challenging area of research (Suzuki and Mittler, 2006). Impending global climate change with predicted 1.5 to 5.8ºC increases in temperature by 2100, pose threat to agricultural production (Rosenzweig *et al*., 2001). Crop yields are predicted to decrease approximately 10% for every one degree increase in temperature (Mittler, 2006). Climate change especially high temperature is projected to negatively impact future agricultural production worldwide (Wahid *et al.,* 2007; Battisti and Naylor, 2009). Membranes and proteins are among the most affected cell components during stress (Palta, 1990). Changes in these components can alter several processes such as uptake of water and ions, translocation of solutes, photosynthesis and respiration, and produce inactivation of enzymes, accumulation of unprocessed peptides, and proteolysis. Thus, metabolic damage is produced and growth is, therefore, reduced (Chen *et al.,* 1982).

Chili pepper cultivars are among the high-value important vegetable crop in the world being used as spice. Therefore, understanding the effect and mechanism of high temperature on chili pepper are the important issues for the improvement of the quality of chili pepper in Malaysia. As domestic requirement is not fulfilled with native production, Malaysia depends on import of different food items along with chili pepper (MoA, 2013). Under this context, to feed the increasing population along with decreasing cultivable land, more innovative research and technological advancement is essential to increase food production. Therefore, to increase production there is the need to identify genotypes having high yield potentials as well as tolerance against high temperature to be suitable for lowland cultivation. Presently, the commercial vegetable growers are quite aware about the importance of new varieties due to having high yield potential with uniformity in maturity, having tolerance to abiotic and biotic stresses, and better quality as compared to the standard open pollinated varieties. The commercial production of hybrid chili pepper has been found to be successful, though it is not so many as compared to the other vegetables like tomato and eggplant under the same family Solanaceae. The experience on the possibility of exploiting the hybrid vigour and heterosis in chili has shown considerable promise. Under this context, it is imperative to identify and characterise the *HSPs* for heat tolerance in chili pepper and to identify the heat tolerant lines that can be used to develop hybrid chili varieties.

In general, typical response to environmental stress conditions is established by the induction of a set of stress proteins that protects the organism from cellular damage. A basic response of plants to heat stress that is very common is the *HSPs* expression, which is known as a mechanism in plant tolerance to heat stress (Howarth, 1991; Vierling, 1991; Feder and Hoffman, 1999). Protein metabolism involving protein synthesis and degradation is one of the most sensitive processes to heat stress. Changes in the level and expression pattern of some proteins may play an important role in plant adaptation to abiotic stress. Several tools and resources have been used in the identification of stress-responsive proteins and their pathways, including two-dimensional electrophoresis and mass spectrometry, and the rapidly expanding nucleotide and amino acid sequence databases. Heat stress may induce or enhance protein expression or cause protein degradation. The induction of heat-responsive proteins, particularly *HSPs*, plays a key role in plant tolerance to heat stress. In regulating response of plants to stress, particularly heat, protein degradation involving several proteases is also significant. Research is therefore needed with respect to identification of heat-responsive proteins associated with heat tolerance, heat induction and characteristics of *HSPs* and protein degradation in relation to plant responses to heat stress. The *HSPs* have either high (80-100 kDa, HMW *HSPs*), intermediate (68-73 kDa, IMW-*HSPs*), or small molecular masses (15- 20kDa, *sHSPs*). The latter are classified as gene products of six gene families based on DNA sequence similarity, immunological cross-reactivity and intracellular localization (Waters *et al*., 1996; Sun *et al*., 2002). Heat Shock Proteins (*HSP*s), produced under high temperature for plant adaptations, have been identified by several researchers (Howarth, 1991; Vierling, 1991; Feder and Hoffman, 1999).

1.3 Objectives of the Study

1. To study the genetic difference among the genotypes based on morpho-physiological traits
2. To identify heat tolerant chili pepper genotypes and
3. To identify Heat Shock Proteins (*HSPs*) and its expression among chili pepper genotypes against heat stress.

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