GENETIC DIVERSITY AND SELECTION OF HIGH-YIELD CHILLI (*Capsicum annuum* L.) GENOTYPES UNDER SOILLESS CULTIVATION

By

SITI SAHMSIAH BINTI SAHMAT

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

This thesis is dedicated to my family: my parents for their love, my husband for his support, and my lovely children for their endless joy.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chilli (*Capsicum annuum* L.) is a crop of immense value, celebrated for its widespread consumption, versatile applications, and integral role in culinary traditions around the globe. Despite its importance, enhancing yield production faces significant hurdles, particularly with traditional genotype analysis methods that often overlook essential effects and interactions critical for crop yield and selection. This study seeks to address these challenges by adopting innovative strategies for yield enhancement by employing hydrogel-amended soilless cultivation systems, marking a significant leap forward in agricultural practices.

Furthermore, the study evaluates the genetic diversity of chilli genotypes via morpho-physiological traits and ISSR markers, establishing a solid foundation for future breeding programs and advancing our understanding of effective chilli cultivation. This comprehensive approach involved analyzing 30 chilli genotypes, including 19 advanced mutant breeding lines from the

Chilli Bangi variety (M_6 generation) and 11 commercial genotypes, to explore their productivity under soilless culture conditions. Morphological characteristics revealed a significant heterogeneity among the 30 chilli genotypes grown under soilless condition. The genotypes were classified into five distinct genetic diversity clusters, revealing a substantial level of polymorphism at 74.39% by ISSR markers.

The experiment on irrigation intervals revealed that adopting an alternate-day irrigation interval yields the most beneficial outcomes for chilli cultivation when adding hydrogel in the planting medium. This approach balances moisture retention and nutrient delivery optimally. Subsequently, the experiment on soilless cultivation techniques illustrated those soilless cultures, such as hydroponics and hydrogels (BioHydrogel and HydroStock), significantly enhance nutrient absorption, resulting in healthier and more productive chilli plants. Hydroponics optimized the control of nutrients (such as N, P, and K), while hydrogels ensured consistent moisture for improved hydration and nutrient uptake. In evaluating yield and yield-related attributes, the study carried out the genotype by soilless culture interaction analysis. This assessment revealed the hydrogel-amended planting mediums, namely HydroStock, and BioHydrogel, as favorable cultivation mediums. Genotype stability analysis identified G30, G27, G26, and G7 as highly stable, with both high mean yields and consistent performance across diverse soilless cultures.

The study recommends using soilless culture techniques, particularly those incorporating hydrogels like BioHydrogel and HydroStock, to enhance nutrient absorption and improve chilli plant yield. These genotypes (G30, G27, G26, and G7) demonstrated remarkable yield stability, suggesting their potential for future productive and sustainable chilli production.

Keyword: Chilli, genetic diversity, soilless culture, urban farming

SDG: GOAL 1: No Poverty, GOAL 2: Zero Hunger

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KEPELBAGAIAN GENETIK DAN PEMILIHAN GENOTIP CILI (*Capsicum annuum* L.) BERHASIL TINGGI SECARA PENANAMAN TANPA TANAH

Oleh

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Cili (*Capsicum annuum* L.) adalah tanaman bernilai tinggi kerana penggunaannya yang pelbagai dan meluas, sekaligus mempunyai peranan yang penting di dalam tradisi kulinari di seluruh dunia. Namun, cabaran utama dalam menganalisis prestasi genotip adalah penggunaan kaedah penanaman secara konvensional yang sering mengabaikan kesan dan interaksi penting yang mempengaruhi faktor hasil tanaman dan pemilihan bagi meningkatkan pengeluaran hasil.

Oleh itu, penilaian genotip untuk mengenal pasti genotip yang berhasil tinggi dan stabil adalah penting untuk memastikan kelestarian jangka panjang pengeluaran tanaman cili. Objektif utama kajian termasuklah menentukan selang penyiraman yang optimum dalam media penanaman yang diperkayakan dengan hidrogel dan membandingkan pelbagai sistem penanaman tanpa tanah serta menilai kepelbagaian genetik genotip cili menggunakan ciri-ciri morfo-fisiologi dan penanda ulangan jujukan mudah

berselang (ISSR). Bagi mencapai objektif, kajian ini menggunakan 30 genotip cili, terdiri daripada 19 genotip pembiakbakaan maju mutan (generasi M_6) yang berasal dari varieti Cili Bangi dan 11 genotip komersial. Pendekatan ini digunakan untuk memahami dengan lebih baik potensi produktiviti genotip tersebut dalam penanaman tanpa tanah. Ciri-ciri morfologi menunjukkan kepelbagaian yang signifikan di antara 30 genotip cili tersebut yang ditanam mengunakan sistem tanpa tanah. Genotip-genotip ini telah diklasifikasikan ke dalam lima kumpulan kepelbagaian genetik yang berbeza, yang menunjukkan tahap polimorfisma yang luas pada 74.39% oleh penanda ISSR.

Eksperimen berkaitan selang penyiraman telah menunjukkan bahawa selang penyiraman selang sehari memberikan hasil yang paling tinggi bagi tanaman cili apabila menggunakan hidrogel sebagai media tanaman. Pendekatan ini menyeimbangkan pengekalan kelembapan dan penghantaran nutrien secara optimal. Selanjutnya, perbandingan di antara teknik penanaman tanpa tanah menunjukkan bahawa sistem tanaman tanpa tanah iaitu hidroponik dan hidrogel (BioHydrogel dan HydroStock) secara signifikan meningkatkan penyerapan nutrient yang dapat menghasilkan tanaman cili yang lebih sihat dan produktif. Hidroponik mengoptimumkan kawalan nutrien (seperti N, P, dan K), manakala hidrogel memastikan kelembapan yang konsisten untuk memastikan pokok menerima hidrasi dan nutrient yang secukupnya. Dalam menilai hasil dan atribut berkaitan hasil, kajian ini telah menjalankan analisis interaksi genotip dengan medium penanaman. Penilaian ini menunjukkan bahawa media penanaman yang ditambah dengan hidrogel iaitu

HydroStock, dan BioHydrogel sebagai persekitaran tanaman yang baik. Analisis kestabilan genotip mengenal pasti G30, G27, G26, dan G7 sebagai genotip yang sangat stabil, dengan hasil purata yang tinggi dan prestasi yang konsisten dalam pelbagai kultur tanpa tanah.

Kajian ini mengesyorkan penggunaan teknik kultur tanpa tanah, terutamanya yang menggabungkan hidrogel seperti BioHydrogel dan HydroStock, untuk meningkatkan penyerapan nutrien dan meningkatkan hasil tanaman cili. Genotip-genotip ini (G30, G27, G26, dan G7) menunjukkan kestabilan hasil yang tinggi dan boleh dicadangkan berpotensi untuk pengeluaran cili yang produktif dan mampan pada masa depan.depan.

Kata Kunci: Cili, kepelbagaian genetik, penanaman tanpa tanah, kebun bandar

SDG: MATLAMAT 1: Menghapus kemiskinan, MATLAMAT 2: Menghapus kelaparan

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

%	Percentage
µ	Micro
AMMI	Additive main effect and multiplicative interaction effect
ANOVA	Analysis of variance
bi	Regression slope
cm	Centimetre
CO_2	Carbon dioxide
et al.	et alia
g	Gram
G×E	Genotype × environmental interaction
GCV	Genotypic Coefficient
GGE	Genotype main effects plus genotype × environmental interaction model
GGL	Genotype plus genotype by location interaction
h^2_b	Broad-sense heritability
ISSR	Inter Simple Sequence Repeat
K	Selection intensity
L	Liter
LSD	Least significant difference
m	Meter
M	Molar
MARDI	Malaysian Agricultural Research and Development Institute
MET	Multiple environment trials
mg	Milligram
µg	Microgram

Min	Minute
mL	Millilitre
mol	Molarity
NaCl	Sodium Chloride
O_2	Oxygen
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction Phenotypic
PCV	Phenotypic Coefficient
P_i	Lin and Binns
S_d^2	Deviation from regression
SAS	Statistical analysis software
SVD	Singular value decomposition;
SVP	Singular value partition
W_i^2	Wricke's ecovalence
Ysi	Kang's yield stability statistics
σ^2	Variance
σ_i^2	Shukla's variance
GCA	General Combining Ability
Gy	Gray
M_1	First generation after mutagenic treatment
M_2	Second generation after mutagenic treatment
M_3	Third generation after mutagenic treatment
M_6	Sixth generation after mutagenic treatment
ng	Nano gram
ns	Non-significant
DNA	Deoxyribonucleic acid

DTF	Day to flowering
SE	Standard error
SD	Standard deviation
TE	Tris EDTA
H_e	Expected heterozygosity
H_o	Observed heterozygosity

CHAPTER 1

INTRODUCTION

1.1 Research Background

Chilli pepper (*Capsicum annuum* L.) recognized globally for its diverse range of colors, shapes, sizes, and pungency levels, is not only a culinary staple but also an important crop in the food and pharmaceutical industries due to its capsaicin content and other antioxidants. Globally, chilli peppers are among the top 10 vegetable producers, with over 30 species cultivated commercially, contributing to a production of 38.03 million metric tonnes in 2019 (Karim *et al*., 2021; Madala *et al*., 2020). Despite this global prominence, Malaysia's chilli production faces significant challenges. The Import Dependency Ratio (IDR) for farming commodities, including chilli, has increased significantly, indicating a growing reliance on imports due to issues such as inconsistent yields, reduced fruit quality, and poor nutrient uptake (Memon *et al*., 2021).

In response to these challenges, innovative solutions such as soilless farming and mutation breeding are being explored. Soilless farming, recognized for its ability to enhance nutrient and water management through inert mediums such as perlite, coconut coir, or rockwool, requires precise calibration of irrigation interval and nutrient formulations to optimize plant health and productivity, addressing some critical limitations of conventional soil cultivation (Ilahi and Ahmad, 2017). Additionally, mutation breeding shows promise in developing new chilli varieties with enhanced yields, disease resistance, and adaptability to soilless cultivation environments, offering plants that are better suited to specific challenges posed by soilless systems (Alshrouf, 2017).

Additionally, the integration of hydrogels into soilless systems is being investigated as a promising innovation to improve water retention and simplify nutrient management, thereby reducing the energy costs associated with traditional soil-based agriculture (Chiorescu, 2019; Karagoz and Yücel, 2020; Satriani *et al*., 2018; Suresh *et al.*, 2018). Together with mutation breeding, these advanced agricultural techniques are set to revolutionize chilli production in Malaysia.

The significance of this study lies in its focus on leveraging genetic diversity and advanced cultivation technologies to address the challenges of chilli cultivation. By exploring the genetic diversity and stability of chilli genotypes using both morpho-physiological traits and molecular markers like Inter-Simple Sequence Repeats (ISSR), this research aims to identify genotypes that perform well under advanced soilless cultivation conditions. Such insights are crucial for developing effective breeding programs and creating robust chilli varieties that are well-suited to local conditions, thereby supporting sustainable agricultural advancements and ensuring food security (Hatami *et al.*, 2019; Mafakheri *et al.*, 2020).

Understanding these genetic foundations enables effective evaluation of genotype by environment interactions (G×E), essential for assessing how different genotypes perform under various soilless cultivation conditions. The insights gained from G×E analysis prove invaluable in multilocation trials, aiding breeders in identifying chilli genotypes that consistently yield high across different environmental conditions or planting systems. This analysis is instrumental in developing robust chilli varieties that are adaptable to specific agricultural practices and climate variabilities (Chowdhury *et al.*, 2020; Hidayatullah *et al.*, 2021; Sran *et al.*, 2021). By integrating these findings with innovative cultivation techniques like soilless farming and hydrogel integration, this research supports the establishment of sustainable agricultural systems that enhance productivity and ensure food security. This comprehensive approach not only enriches our understanding of genetic diversity and its impact on crop performance but also enhances breeding programs, supporting sustainable agricultural advancements.

1.2 Problem Statement

The rising interest in soilless agriculture has been driven by its multiple advantages, including enhanced fertiliser utilization (Al Rohily *et al.*, 2021), improved plant performance (Calcagnile *et al.*, 2019; Monfared *et al.*, 2018), economical nutrient cycling, and reduced environmental impact (Coello *et al.*, 2018). Superabsorbent hydrogels acting as soil conditioners have emerged as a promising alternative plant medium, capable of increasing the water-holding capacity (WHC) by 154% and doubling the nitrogen content by enhancing nutrient retention and release, thus preventing nitrogen leaching and improving its availability to plants (Iftime *et al.*, 2019). This excellent innovation has spurred researchers to optimize water usage and yield in chilli cultivation.

Despite these advancements, significant research gaps in *Capsicum annuum* L. cultivation remian, particularly concerning the use of soilless systems with hydrogel amendments. Currently, acrylate-based superabsorbent hydrogels dominate the commercial market. Their potential toxicity for agriculture and human consumption raises alarming concerns, highlighting the need to develop safer and biodegradable alternative planting mediums that can efficiently conserve water and nutrients, thus promoting enhanced chilli productivity in soilless systems.

Moreover, the lack of robust information among small-scale farmers about the benefits of diverse and innovative soilless cultivation systems often results in the underutilization of such technologies, which could otherwise help in overcoming the limitations of traditional farming. This scenario complicates the development of efficient breeding programs and the selection of planting systems that are resilient and productive across diverse environmental conditions.

1.3 Significance of Study

The proposed study holds significant potential in advancing sustainable agriculture and improving the sufficient supply of *Capsicum annuum* L. through the soilless cultivation approach. By determining optimal irrigation interval and comparing various soilless cultivation systems, this research aims to improve agricultural efficiency and enable farmers and industries to select the most effective and economically viable techniques. These efforts directly address the need for alternative cultivation methods that better manage resources and reduce environmental impacts, as outlined in the problem statement.

Moreover, investigating the genetic diversity and stability of chilli genotypes using morpho-physiological and yield attributes will contribute to the development of high-yield, stable, and disease-resistant chilli varieties. This aspect is crucial for overcoming the current limitations in breeding programs caused by a lack of robust genotypic information and the variability of performance in different environments.

Finally, employing ISSR markers to assess genetic polymorphism within a soilless system provides vital insights into the genetic adaptability of superior chilli genotypes. This method demonstrates effective management and exploitation of genetic diversity, which aids in selecting genotypes that perform well across diverse conditions, thereby directly supporting global sustainability efforts and food security.

1.4 Objective of Study

This study aims to investigate the genetic diversity and selection of high-yield chilli (*Capsicum annuum* L.) genotypes under hydrogel amendment soilless culture. To achieve this aim, the study sets the following specific objectives:

i. To determine the optimal irrigation interval on hydrogel amended planting medium on chilli growth performance.

ii. To compare the growth performance of chilli across various soilless culture, including conventional fertigation with cocopeat, fertigation with BioHydrogel, fertigation with HydroStock, and hydroponics.

iii. To determine the genetic diversity and genotype stability of chilli genotypes based on the morpho-physiological and yield attributes.

iv. To evaluate the genetic polymorphism of chilli in a soilless planting system using the ISSR marker.

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