

**ISOLATION AND CHARACTERISATION OF  
ALLELIC VARIATIONS IN *FT/TFL1* GENE  
HOMOLOGS AMONG VARIOUS CULTIVARS AND  
EMS-DERIVED M2 MUTANTS OF EGGPLANT  
(*SOLANUM MELONGENA*)**



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**BIOTECHNOLOGY RESEARCH INSTITUTE  
UNIVERSITI MALAYSIA SABAH  
2023**

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EMS-DERIVED M2 MUTANTS OF EGGPLANT  
(*SOLANUM MELONGENA*)**

**RANJITA A/P SUBRAMANIAM**



**THIS IS SUBMITTED IN FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY**

**BIOTECHNOLOGY RESEARCH INSTITUTE  
UNIVERSITI MALAYSIA SABAH  
2023**

**UNIVERSITI MALAYSIA SABAH**  
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JUDUL : **ISOLATION AND CHARACTERISATION OF ALLELIC VARIATIONS IN *FT/TFL1* GENE HOMOLOGS AMONG VARIOUS CULTIVARS AND EMS-DERIVED M2 MUTANTS OF EGGPLANT (*SOLANUM MELONGENA*)**

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Tarikh : 14 September 2023

(Prof. Dr. Vijay Kumar)  
Penyelia Utama

## DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries, and references, which have been duly acknowledged.

15 June 2023



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## CERTIFICATION

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A handwritten signature in black ink, appearing to read 'Vijay Kumar', is written over a horizontal line.

## ACKNOWLEDGEMENT

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Ranjita A/P Subramaniam

15 June 2023

## ABSTRACT

*FT/TFL1* gene homologs are one of the key determinants in flowering regulation, seed dormancy and germination. Despite their utilization for crop improvements in various species, they have not been identified and characterized in eggplant (*S. melongena*). Moreover, since the allelic diversities of the gene family represent important resources for crop improvement, the identification of diverse alleles can be facilitated by different varietal cultivars and EMS-derived mutants. Therefore, the project aimed to identify *FT/TFL1* genes in eggplant using in-silico genome mining with three genome assemblies i.e. *Sme\_r2.5.1*, *S. melongena*-HQ and '67/3'. Subsequently, the deduced protein sequences were characterized. The presence of these genes was validated in four economically important cultivars Surya, EP-47 Annamalai, Pant Samrat and Arka Nidhi through Pacbio RSII amplicon sequencing, using plants grown from seeds obtained from the World Vegetable Centre. Distribution of allelic variations were identified among them. Meanwhile, initial seeds from seven cultivars were used to establish M1 mutant libraries with 0.7% EMS. In M2 generation, mutant libraries were narrowed to Surya, EP-47 Annamalai, Pant Samrat, and Arka Nidhi, with gene analysis conducted through Pacbio RSII sequencing. Morphological variations were also recorded. The results unravelled the presence of 12 *FT/TFL1* gene homologs in eggplant, with evidence of *FT*-like gene diversifications, suggesting differential responses to various environmental cues. The amplicon sequencing and variant phasing uncovered the presence of two alleles in certain genes (*SmCEN-1*, *SmCEN-2*, *SmMFT-1*, and *SmMFT-2*). An examination of *SmMFT-2* alleles across domesticated cultivars and the related wild species, *S. incanum*, indicated that the alternative allele of *S. incanum* was present in some Pant Samrat cultivar members but lacking in most other cultivars. This variation could contribute to the discrepancies in seed traits between wild and domesticated eggplants. Furthermore, EMS-mediated mutagenesis led to the discovery of a mutation in the predicted 5'UTR region which needs further verifications on their influence on the underlying traits. The mutagenesis also led to the emergence of various desirable traits. Taken together, the study provided fundamental details about the *FT/TFL1* gene family with identification of valuable allelic diversities that can lay the foundation for future functional validations and their downstream applications in alignment to the crop's improvement programs.

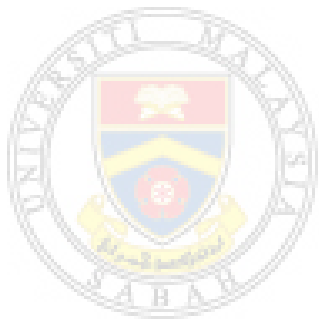
## **ABSTRAK**

### **ISOLASI DAN PENCIRIAN VARIASI ALEL KUMPULAN GEN FT/TFL1 DI KALANGAN PELBAGAI KULTIVAR DAN MUTAN EMS GENERASI M2 TERUNG (*SOLANUM MELONGENA*)**

*Kumpulan gen FT/TFL1 memainkan peranan penting dalam proses pembungaan dan percambahan benih. Walaupun kumpulan gen ini telah menambahbaikkan pelbagai spesies tumbuhan, informasi gen-gen ini tidak dikenali dalam spesies terung. Oleh kerana variasi alel dalam kumpulan gen ini bermanfaat dalam menambahbaikkan kultivar, pencarian variasi alel ini boleh dilakukan di kalangan pelbagai kultivar terung dan juga populasi mutan dirawat melalui EMS. Projek ini bertekad untuk mengekstrak kumpulan gen FT/TFL1 daripada tiga jenis data genom dan jujukan proteinnya dianalisa. Jujukan gen FT/TFL1 dan variasinya di kalangan kultivar komersial terung seperti Surya, EP-47 Annamalai, Pant Samrat dan Arka Nidhi telah dikenalpasti melalui teknologi jujukan amplicon Pacbio RSII, di mana benih-benih tamanan ini dibeli daripada 'The World Vegetable Centre'. Selain itu, benih daripada tujuh kultivar terung telah digunakan untuk rawatan kimia (0.7% EMS) untuk menghasilkan mutan generasi pertama (M1). Manakala, penghasilan tumbuhan mutan generasi M2 dihadkan kepada kultivar Surya, EP-47 Annamalai, Pant Samrat dan Arka Nidhi di mana analisa gen dilaksanakan melalui teknologi jujukan amplicon Pacbio RSII. Variasi fenotip di kalangan tumbuhan mutan telah direkodkan. Analisa menunjukkan 12 gen FT/TFL1 dalam genom terung. Gen subkelas FT menunjukkan kepelbagaian dalam domain protein, memungkinan kepelbagaian peranan terhadap pelbagai isyarat persekitaran. Selain itu, penjujukan kumpulan gen ini dan analisa variasi di antara empat jenis kultivar menunjukkan penemuan dua jenis alel untuk SmCEN-1, SmCEN-2, SmMFT-1 and SmMFT-2. Selain itu, kumpulan gen MFT telah dibandingkan dengan transkrip daripada spesies, *S. incanum* yang merupakan spesies projenitor terdekat terung. Gen MFT-2 dalam *S. incanum* terdiri daripada dua alel di mana salah satunya ditemui dalam keempat-empat kultivar yang dikaji. Manakala, yang satu lagi ditemui di kalangan populasi Pant Samrat. Kepelbagaian variasi yang ditemui dalam alel-alel gen MFT-2 berkemungkinan terlibat dalam proses dorman benih. Manakala, satu mutase telah ditemui di kawasan 5'UTR dalam gen SmMFT-2 untuk satu tumbuhan mutan yang perlu dikenalpasti impaknya terhadap ciri-cirinya. Kepelbagaian fenotip juga telah dikenalpasti. Secara menyeluruh, projek ini telah*



*menawarkan informasi asas mengenai kumpulan gen FT/TFL1 bersama dengan variasi alel di kalangannya yang boleh menjadi asas kepada pengesanan fungsi-fungsinya untuk diaplikasi dalam program penambahbaikan terung.*



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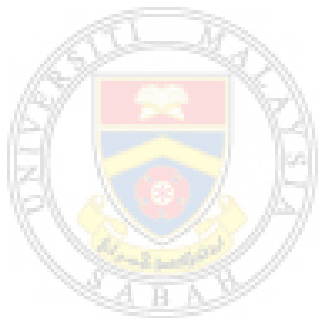
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## LIST OF ABBREVIATIONS

<b>EMS</b>	-	Ethyl methanesulphonate
<b>LAA</b>	-	Long Amplicon Analysis
<b>MNU</b>	-	Methylnitrosourea
<b>Az</b>	-	Azide
<b>FT</b>	-	<i>FLOWERING LOCUS T</i>
<b>TFL1</b>	-	<i>TERMINAL FLOWER 1</i>
<b>CEN</b>	-	<i>Centroradialis</i>
<b>ATC</b>	-	<i>ARABIDOPSIS THALIANA CENTRORADIALIS</i>
<b>MFT</b>	-	<i>MOTHER OF FT AND TFL1</i>
<b>BFT</b>	-	<i>BROTHER OF FT AND TFL1</i>
<b>TSF</b>	-	<i>TWIN SISTER OF FT</i>
<b>TILLING</b>	-	Targeting Induced Local Lesion in Genomes
<b>RAPD</b>	-	Randaom Amplified Polymorphic DNA
<b>ISSR</b>	-	Inter Simple Sequence Repeat
<b>SRAP</b>	-	Sequence Related Amplified Polymorphism
<b>AFLP</b>	-	Amplified Fragment Length Polymorphism
<b>SSR</b>	-	Simple Sequence Repeat
<b>ORF</b>	-	Open Reading Frame
<b>CDS</b>	-	Coding Sequence
<b>TSS</b>	-	Transcription Start Site
<b>Aa</b>	-	Amino Acid
<b>Bp</b>	-	Base pair



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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

The eggplant (*Solanum melongena* L;  $2n= 24$ ) which is also known by other regional names such as brinjal, aubergine, melanzana, garden egg and patlican, is a crop species of tropical and subtropical regions. Countries like China, India, Egypt, Turkey and Iran are known to be the largest producers in the global scale and it is among the most widely cultivated vegetables worldwide. The global production of eggplant reached around 58.6 million tons in 2021 (FAOSTAT, 2023).

Nutritionally, eggplant contains myriad health benefits due to its abundant nutrient contents such as phenolic compounds like anthocyanin and chlorogenic acid (CGS), proteins, fibers, minerals and vitamins. In addition, the decholesterolating activity of eggplant due to the polyunsaturated fatty acids has been a remedial in treating of asthma and liver issues. The high fibre and low soluble-carbohydrate content of eggplant has made it suitable for the management of type 2 diabetes and hypertension and has been recommended by the National Diabetes Education Program of NIH, Mayo Clinic, and American Diabetes Association. It has also been ranked as the top ten vegetables with oxygen radical absorbance capacity owing to the fruit phenolic component.

Flowering time regulation is one among the most indispensable agronomic trait that has yet to be uncovered its underlying genetics, in eggplant. This is a morphologically complex phase in angiosperms where transition from vegetative growth to reproductive development occurs in the plants. *FLOWERING LOCUS T (FT)/TERMINAL FLOWER 1 (TFL1)* gene homologs act as key players in the regulation

of flowering time and the members of this gene family are considered as elite targets in crop breeding. Genetic manipulations of these genes have been tailored to advances in crop adaptations as in fine-tuning flowering time and flowering responses and optimizations of crop yield, and the impact created varies based on individual species. Gene duplications of these homologs in different plant species produce differing number of paralogs where functional diversifications are not a rare phenomenon. The diversifications represent strategies of the plants to refine their floral responses upon environmental and endogenous cues, intrinsic to every species. Thus, identification and characterization of *FT/TFL1* gene family of individual plant species is of paramount importance as it caters the basic understanding of this gene family underlying flowering mechanisms.

In this context, next generation sequencing technologies can come in handy. Plant breeding has undergone a revolution particularly in terms of molecular aspects have revolutionized as there have been a surge in the genomic resources shared among the scientific community parallel to the advent and growth of these advanced technological platforms. As per eggplant, multiple genome assemblies of various qualities have been released especially within the last decade. The first draft genome of eggplant was constructed using cultivar 'Nakate-Shinkuro' in the year 2014 and covered 833.1 Mb. In subsequent years, more genome assemblies from different cultivars were released. The genome assemblies conducted on inbred line '67/3' and 'HQ-1315' had genome sizes of 1.16 Gb and 1.17 Gb, respectively. These resources are gateways to accelerate the understanding of genetic traits of interest. The publicly available transcriptomes of eggplant cultivars and its wild relative, *S. incanum* also provide additional benefits in identifying and characterizing members of eggplant *FT/TFL1* gene sequences.

Apart from that, analysis of the genes of interest, *FT/TFL1* homologs across various commercial cultivars would allow the identification of variations within the pre-existing gene pool by screening for polymorphisms. Polymorphisms or introduction of artificially introduced mutations in the *FT/TFL1* family members represent potent resources of genetic variants, providing further avenues to investigate the dynamics of roles played by these genes in eggplant. Introduction of artificial mutations in eggplant in a manner of applying chemical mutagenesis would