# CHARACTERISTICS, GENETIC DIVERSITY AND PATHOGENIC VARIABILITY OF *Fusarium oxysporum* f. sp. *niveum* IN WATERMELON (*Citrullus lanatus* var. *lanatus* (THUNB.) MATSUM. & NAKAI)

**By**

**MUHAMMAD ZIAUR RAHMAN**

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

**August 2022**

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**DEDICATION**

***The most precious people in my life; my lovely better-half Mst. Fahomida Amin, adorable son Farraj Farihzaad Ziyaam, and daughter Feona Nusaiba Zuha, my beloved parents Late Muhammad Meher Ali, Late Mst. Aeshya Begum, for their everlasting love and prayer that encouraged me to continue it.***

***To my family members***

***And***

***To all my friends who supported me all these year***

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**CHARACTERISTICS, GENETIC DIVERSITY AND PATHOGENIC VARIABILITY OF *Fusarium oxysporum* f. sp. *niveum* IN WATERMELON (*Citrullus lanatus* var. *lanatus* (THUNB.) MATSUM. & NAKAI)**

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**MUHAMMAD ZIAUR RAHMAN**

**August 2022**

**Chairman : Associate Professor Khairulmazmi bin Ahmad, PhD**
**Faculty : Agriculture**

Watermelon (*Citrullus lanatus* L.) is one of the most economically important horticultural fruit crops. It belongs to the *Cucurbitaceae* family. Presently a major hindrance to this crop production is the soil-borne disease of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (Fon). This disease causes yield losses to growers approximately 30-80% or even more. There is limited information on the detection, genetic diversity, and pathogenic variability of the Fon pathogen in Malaysia and Bangladesh. Hence, this study was conducted with the following objectives: to survey and characterize *Fusarium oxysporum* isolates collected from different watermelon growing areas in Peninsular Malaysia and Bangladesh based on morphological and molecular methods; to determine the genetic diversity of *F. oxysporum* f. sp. *niveum* through simple sequence repeat (SSR) markers; and to determine the pathogenic variability and hosts reaction of watermelon commercial cultivars against *F. oxysporum* f. sp. *niveum*. A survey of Fusarium wilt disease of watermelon was done in major watermelon-producing areas in Peninsular Malaysia and in Bangladesh from August, 2019 to December, 2020, using a diagonal sampling method. Based on the cultural and morphological characteristics were used to identify the fungus. The Fon forma speciales-specific primers (Fn-1/Fn-2 and Fon-1/Fon-2) were used to identify the fungus based on the molecular method. The molecular characterization of *Fusarium* species was determined by PCR amplification of different gene regions (*tef1-α, IGS, and mtSSU*). The analysis of molecular variance (AMOVA) for genetic diversity was done using SSR marker. A pathogenicity test was carried out for 65 Fon isolates to determine their aggressiveness and 12 watermelon commercial cultivars were tested to identify adequate resistance to Fon. The findings of field disease surveys revealed that field disease incidence was 5-45% based on random sampling. The highest disease incidence (45%) was in Pahang, whereas, Terengganu had the lowest disease incidence (5%). The mycelia of the collected isolates were delicate, sparse to numerous, white to pinkish-white, and violet to dark violet pigmentation. Microconidia were small, oval-ellipsoid, straight to curve, non-septate and macroconidia were fusoid-subulate, scarce to abundant, mostly three-septate,

hooked apex and pedicellate base. Whereas, chlamydospores were abundant, formed terminally or intercalary, and found singly or in pairs, chains, or clusters. These typical cultural and morphological characteristics of 65 isolates were identified as *F. oxysporum*. The Fon forma speciales-specific primers (Fn-1/Fn-2 and Fon-1/Fon-2) were amplified amplicons of 320 bp and 174 bp in 100% and 91% of the isolates, respectively. The result of phylogenetic analyses of individual and combined gene sequences of *tef1-α, IGS,* and *mtSSU* revealed that 65 isolates belonging to *F. oxysporum* f. sp. *niveum*. Multigene phylogenetic analysis using *tef1-α, IGS*, *mtSSU* data set of all isolates were clustered into four main clades, indicating that Fon is polyphyletic. The analysis of molecular variance (AMOVA) showed a total variation of 67% within the population and a 33% total variation among the populations. The results of this investigation indicated that there is a maximum genetic diversity existing within the Fon populations. Based on the pathogenicity test, the 65 Fon isolates were characterized into 4 groups, viz., highly aggressive 15 isolates, moderately aggressive 7 isolates, weakly or low aggressive 29 isolates, and non-pathogenic 14 isolates. Among the 12 watermelon commercial cultivars, Black Giant and Big Family were identified as highly resistant cultivars with disease severity index (DSI) of 5.55-18.15% and 18.11-30.00%, respectively. These results indicated that these two cultivars have the potential to cultivate as resistant varieties against Fon.

Abstraktesis yang dikemukakankepadaSenatUniversiti Putra Malaysia
sebagimemenuhikeperluanuntuk ijazah DoktorFalsafah

**CIRI-CIRI, KEPELBAGAIAN GENETIK DAN KEBOLEHUBAHAN CIRI PATOGENIK *Fusarium oxysporum* f. sp. *niveum* PADA TEMBIKAI (*Citrullus lanatus* var. *lanatus* (THUNB.) MATSUM. & NAKAI)**

Oleh

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**Ogos 2022**

**Pengerusi : Profesor Madya Khairulmazmi bin Ahmad, PhD**
**Fakulti : Pertanian**

Tembikai (*Citrullus lanatus* L.) merupakan salah satu tanaman buah hortikultur yang sangat penting dari segi ekonomi. Tembikai tergolong dalam keluarga *Cucurbitaceae*. Pada masa ini, cabaran utama pengeluaran tembikai adalah penyakit bawaan tanah iaitu layu Fusarium yang disebabkan oleh *Fusarium oxysporum* f. sp. *niveum* (Fon). Penyakit ini menyebabkan kehilangan hasil kepada penanam sekitar 30-80% atau lebih. Setakat ini, maklumat mengenai pengesanan dan kepelbagaian genetik dan kebolehubahan ciri patogenikpatogen Fon di Malaysia dan Bangladesh adalah terhad. Oleh itu, kajian ini dijalankan berdasarkan objektif berikut: untuk meninjau dan mencirikan pencilan *Fusarium oxysporum* yang dikumpul dari kawasan penanaman tembikai yang berbeza di Semenanjung Malaysia dan Bangladesh berdasarkan kaedah morfologi dan molekul; untuk menentukan kepelbagaian genetik *F. oxysporum* f. sp. *niveum* melalui penanda jujukan ringkas (SSR); dan untuk menentukan kebolehubahan kepatogenan dan tindak balas perumah kultivar komersial tembikai terhadap *F. oxysporum* f. sp. *niveum*. Tinjauan penyakit layu Fusarium pada tembikai telah dilakukan di kawasan penghasil tembikai utama di Semenanjung Malaysia dan di Bangladesh dari Ogos, 2019 hingga Disember, 2020, menggunakan kaedah pensampelan pepenjuru. Berdasarkan ciri kultura dan morfologi digunakan untuk mengenalpasti kulat. Primer khusus Fon forma (Fn-1/Fn-2 dan Fon-1/Fon-2) digunakan untuk mengenalpasti kulat berdasarkan kaedah molekul. Pencirian molekular spesies *Fusarium* ditentukan oleh amplifikasi PCR kawasan gen yang berbeza (*tef1-α, IGS,* dan *mtSSU*). Analisis varians molekul (AMOVA) untuk kepelbagaian genetik dilakukan menggunakan penanda SSR. Ujian patogenik telah dijalankan untuk 65 pencilan Fon untuk menentukan keagresifannya dan 12 kultivar komersial tembikai telah diuji untuk mengenal pasti rintangan yang mencukupi terhadap Fon. Dapatan tinjauan penyakit di lapangan mendedahkan bahawa kejadian penyakit di lapangan adalah 5-45% berdasarkan persampelan rawak. Kejadian penyakit tertinggi (45%) adalah di Pahang, manakala, Terengganu mempunyai kejadian penyakit terendah (5%). Miselia bagi pencilan yang dikumpul adalah halus, jarang kepada banyak, putih hingga putih merah jambu, dan pigmentasi ungu hingga ungu gelap. Mikrokonidia adalah kecil, bujur-

elipsoid, lurus ke lengkung, tanpa septat dan makrokonidia adalah fusoid-subulate, jarang kepada banyak, kebanyakannya tiga septa, puncak bercangkuk dan tapak pediselata. Manakala, klamidospora banyak, terbentuk secara terminal atau interkalari, dan ditemui secara tunggal atau berpasangan, rantai, atau kelompok. Ciri-ciri tipikal kultura dan morfologi bagi 65 isolat ini dikenalpasti sebagai *F. oxysporum*. Primer khusus Fon forma (Fn-1/Fn-2 dan Fon-1/Fon-2) masing-masing menghasilkan amplikon yang bersaiz 320 bp dan 174 bp dengan 100% dan 91% kesamaan bagi isolat masing-masing. Hasil analisis filogenetik bagi jujukan gen individu dan gabungan *tef1-α, IGS,* dan *mtSSU* mendedahkan bahawa 65 isolat kepunyaan *F. oxysporum* f. sp. *niveum*. Analisis filogenetik pelbagai gen menggunakan set data *tef1-α, IGS, mtSSU* bagi semua pencilan dikelompokkan kepada empat klad utama, menunjukkan bahawa Fon adalah polifiletik. Analisis varians molekul (AMOVA) menunjukkan jumlah variasi 67% dalam populasi dan 33% jumlah variasi di kalangan populasi. Keputusan penyiasatan ini menunjukkan bahawa terdapat kepelbagaian genetik maksimum yang wujud dalam populasi Fon. Berdasarkan ujian patogenik, 65 Fon pencilan telah dicirikan kepada 4 kumpulan, iaitu, sangat agresif 15 pencilan, sederhana agresif 7 pencilan, lemah atau rendah agresif 29 pencilan, dan bukan patogenik 14 pencilan. Antara 12 kultivar komersil tembikai, “Black Giant” dan “Big Family” dikenal pasti sebagai kultivar sangat tahan dengan indeks keterukan penyakit (DSI) masing-masing 5.55-18.15% dan 18.11-30.00%. Keputusan ini menunjukkan bahawa kedua-dua kultivar tersebut berpotensi untuk ditanam sebagai varieti tembikai tahan terhadap jangkitan Fon.

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I certify that a thesis examination committee has met on 12 August 2022 to conduct the final examination of name Muhammad Ziaur Rahman on his Doctor of Philosophy thesis entitled "Characterization, Genetic Diversity and Pathogenic Variability of *Fusarium oxysporum* f. sp. *niveum* in Watermelon (*Citrullus lanatus* var. *Lanatus* (Thunb.) Matsum. & Naki)" 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 candidate be awarded the Doctor of Philosophy.

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| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| ANOVA | Analysis of Variance |
| AMOVA | Analysis of Molecular Variance |
| a.i. | Active Ingredient |
| AUDPC | The area under the disease progression curve |
| BLASTn | Nucleotide Basic Local Alignment Search Tool |
| Bp | Base pair |
| β-tubulin | Beta tubulin |
| CABI | Centre for Agriculture and Bioscience International |
| CRD | Completely Randomized Design |
| CTAB | Cetyltrimethylammonium Bromide |
| Cm | Centimetre |
| DAI | Days after inoculation |
| Df | Degree of freedom |
| DI | Disease Incidence |
| DNA | Deoxyribonucleic acid |
| dH2O | Distilled water |
| ddH2O | Double distilled water |
| DSI | Disease Severity Index |
| EDTA | Ethylenediaminetetraacetic acid |
| Fon | *Fusarium oxysporum* f. sp. *niveum* |
| GDP | Gross Domestic Product |
| G | Gram |
| GS | Genetic similarity |
| H | Nei's Gene Diversity |

| | |
|---|---|
| Ho | Expected Homozygosity |
| He | Expected Heterozygosity |
| Ha | Hectares |
| I | Shannon's Information Index |
| ITS | Internal Transcribed Spacer |
| IGS | Intergenic spacer |
| kb | Kilobase pair |
| LSD | Least significant difference |
| Mg | Milligram |
| Min | Minute |
| Ml | Millilitre |
| Mm | Millimetre |
| MS | Means of Sum Squares |
| *mtSSU* | Mitochondrial small subunit |
| Na | Observed Number of Alleles |
| NaOCl | Sodium hypochlorite |
| NCBI | National Centre for Biotechnology Institute |
| Ne | The effective number of alleles |
| NTSYS | Numerical Taxonomy and Multivariate Analysis System |
| PCA | Principal component analysis |
| PCoA | Principal Coordinate Analysis |
| PCR | Polymerase Chain Reaction |
| PDA | Potato dextrose agar |
| PIC | Polymorphic Information Content |
| POPGENE | Population Genetic Analysis |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |

| | |
|---|---|
| R | Correlation Coefficient |
| rDNA | Ribosomal DNA |
| Rpm | Revolutions per minute |
| SAS | Statistical Analysis Software |
| SDS | Sodium Dodecyl Sulfate |
| SDW | Sterile distilled water |
| SIX | Secreted in Xylem Gene |
| SS | Sum of Squares |
| SSR | Simple Sequence Repeats |
| Secs | Second |
| TBE | Tris-borate EDTA |
| Tukey's HSD | Tukey's Honestly Significant Difference |
| tef-1 α | Translation Elongation Factor -1 Alpha |
| uHe | Unbiased Expected Heterozygosity |
| Mg | Microgram |
| µl | Microliter |
| Mm | Micrometre |
| UV | Ultraviolet |
| uHe | Unbiased Expected Heterozygosity |
| V | Voltage |
| VCG | Vegetative Compatibility Group |
| UPM | Universiti Putra Malaysia |
| UPMGA | Unweighted Pair Group Methods with Arithmetic Mean |
| % | Percentage |
| % P | Percentage of polymorphic loci |
| ℃ | Degree Celsius |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the study

Watermelon (*Citrullus lanatus* L.) is one of the most economically important horticultural fruit crops belonging to the *Cucurbitaceae* family. It is widely cultivated for its sugary, fleshy edible fruit (Lv et al., 2018; Wehner, 2008; Xie et al., 2019). The advantages of watermelon cultivation over other fruit crops are its short duration, ease of handling, quick financial returns, and low production costs (Costa et al., 2018). Various factors limit watermelon production, but diseases are critical. However, the wilt disease caused by *F. oxysporum* f. sp. *niveum* (Fon) is the most serious disease that affects watermelon productivity (Egel and Martyn, 2013; Zitter et al., 1996). Fon causes infection only in watermelon. The pathogen (Fon) can rapidly disperse in watermelon cultivating areas and is liable for about 30-80% yield losses (Egel and Martyn, 2013; Lü et al., 2011).

Rapid and accurate identification of pathogenic micro-organisms is essential for successful disease management. Traditionally, Fusarium species are identified based on their morphological characteristics, which are usually influenced by environmental and geographical factors (Das et al., 2012). However, these methods are mostly documented to be unreliable and unstable (Bosland & Williams, 1987; Watanabe et al., 2011). On the other hand, molecular methods are progressively used to detect and identify pathogens due to the assurance of better results (accuracy), and phylogenetic analyses (McCartney et al., 2003; Saikia & Kadoo, 2010).

PCR amplification based on the nucleotide sequences which is important in identifying species and distinguishing among the formae speciales of *F. oxysporum*. The important genes are translation elongation factor 1-α (*tef1-α*), calmodulin (*cmdA*), actin, intergenic spacer (*IGS*), the mitochondrial small subunit (*mtSSU*), RNA polymerase II subunits 1 and 2 (RPB1 and RPB2) and *β-tubulin* (TUB) region (Lombard et al., 2019; O'Donnell et al., 2010). These genes provide phylogenetic data that are somewhat similar, and only one of them is usually recommended for giving adequate genetic resolution (O'Donnell et al., 2015; Ramdial et al., 2017).

Knowledge of the genetic diversity within the *F. oxysporum* formae speciales is crucial and is investigated as a primary vestige for the sketch of disease management strategies (McDonald et al., 2002). To evaluate genetic variation within a population of *F. oxysporum* forma speciales, various techniques have already been developed such as vegetative compatibility grouping (VCGs), random amplified polymorphism DNA (RAPD) analysis, amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphism (RFLP), single-nucleotide polymorphism (SNP), inter-

simple sequence repeats (ISSR), and simple sequence repeat (SSR) (Aguilar-Hawod et al., 2019; Merzoug & Belabid, 2018; Petkar et al., 2019; Zang et al., 2018).
Of these methods, SSRs marker can help understand significant function and evolution based on their abundance and density. As a result, SSR techniques have been extensively used for estimating genetic mapping and diversity analysis (Mahfooz et al., 2015).

## 1.2 Problem Statements

Fon is a predominant, highly diversified fungus comprising pathogenic and non-pathogenic individuals that affect watermelon yield and quality (Das et al., 2012; LeBlanc et al., 2017; Xiong & Zhan, 2018). In Malaysia, almost 13.0% of the total area of fruit production which is equivalent to 8308 ha and produced 144213 Mt watermelon. The disease incidence of Fusarium wilt in major production areas namely Johor, Kedah, Kelantan, Pahang and Terengganu were 10-45% and export decrease around 2.1%. In Bangladesh watermelon is grown on 16,542 ha and about 345955 Mt are produced annually. Fusarium wilt disease incidence in commercial fields of main watermelon growing regions in Barishal, Chattagram, and Rajshahi were 30-70% and yield loss as a result of disease is around 23%. Thus, concerning effective pathogen management, current status of the disease and identification up to the species level is essential. In addition, there are many reports of characterization, genetic diversity and pathogenic variability in other species of *Fusarium*. Still, less information is available on the relative importance of Fon, their distribution, diversity and pathogenic variability in Peninsular Malaysia and Bangladesh. For this reason, the present study was undertaken; to evaluate the characterization, genetic diversity, and pathogenic variability of Fon isolates in watermelon growing areas in Peninsular Malaysia and Bangladesh for effective management strategies.

## 1.3 The objectives of the studies were as follows:

1) To survey and characterize *Fusarium oxysporum* isolates collected from different watermelon growing areas in Peninsular Malaysia and Bangladesh based on morphological and molecular methods.

2) To determine the genetic diversity of *Fusarium oxysporum* f. sp. *niveum* through Simple Sequence Repeat (SSR) markers.

3) To determine the pathogenic variability and host reaction of watermelon commercial cultivars against *Fusarium oxysporum* f. sp. *niveum*.

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