UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF FUSARIUM WILT RESISTANT AND HIGH YIELDING WATERMELON (*Citrullus lanatus* L.) VARIETY THROUGH MARKER-ASSISTED BACKCROSS BREEDING

OLALEKAN KAZEEM KOLAPO

IPTSM 2019 9

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By

OLALEKAN KAZEEM KOLAPO

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

February 2019

DEDICATION

This thesis is dedicated to the sweet memories of:
My parents; *Mr. Idris Olalekan Aminu* and *Mrs. Afsat Agbeke Olalekan- Aminu*
My mentor & father-like uncle, *Immam Dawood Tijani Adekilekun*, PhD
My spiritual father and guide; *Immam Asimiyu Igbayilola Ilobu*
and
My friend, brother and confidant; *Alh. (Omoba) Abdulwaheed Adewale Gbadebo.*
May Allah be pleased with their souls and count them among the dwellers of al jahnat firdaous. Aamiin.

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

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Chairman : Professor Mohd Rafii Yusop, PhD
Institute : Tropical Agriculture and Food Security

One of the major production limiting diseases of watermelon (*Citrullus lanatus* L.) is Fusarium wilt (FW) caused by *Fusarium oxysporum* f. sp. *niveum* (FON). The use of disease-free cultivars is the preferred method of controlling the disease in a sustainable way. Watermelon is a major crop in Malaysia and the country spends about RM 10 million annually for the importation of its seeds to support local production. There is therefore the need to save this huge amount by breeding for local varieties that will be high yielding and Fusarium wilt resistant. In this study, the Fusarium wilt resistant inbred line CS-19 and susceptible inbred line BL-14 were crossed to generate the F_1 population. The subsequent two backcrosses and selfing led to the transfer of the resistance gene (*fo-1*) into the susceptible inbred line BL-14 using marker-assisted backcrossing (MABC) and the subsequent development of Fusarium wilt resistant lines that still retain the desirable qualities in BL-14. Eleven microsatellite markers linked to the Fusarium wilt resistance gene were selected and two of the markers, BVWS02309 and BVWS01133 located on chromosomes 1 and 9 respectively were used for the confirmation of Fusarium wilt resistant gene in F_1,BC_1F_1, BC_2F_1 and BC_2F_2 generations. From the 380 microsatellites markers screened, 78 were found polymorphic between the parents and used for recurrent parent genome (RPG) recovery in each backcross population. From the inheritance test conducted in BC_2F_1 and BC_2F_2 generations, the recurrent parent BL-14 scored 4.5 of the 0-5 scale, and this confirmed its susceptibility to the Fusarium wilt disease. In the BC_2F_1 generation, 72 of the 150 plants showed resistance while 78 plants showed susceptibility when inoculated with the virulent *Fusarium oxysporumniveum* isolate. Chi-square test ($\chi 2$) showed that the observed frequencies in the BC_2F_1 population fitted into the single gene model. The goodness of fit ($p=0.46$) to the expected test segregation ratio (1:1) indicated that the resistance is controlled by a single dominant gene. The plants resistant to the *Fusarium oxysporum niveum* isolate from BC_2F_1 population showed good fit with the two markers BVWS02309 ($\chi 2= 0.24$; $p= 0.6892$) and BVWS01133

($\chi 2$= 0.11; p= 0.8065), with expected segregation ratio (1:1) for single gene model. These two markers were found suitable for marker-assisted selection of *fo-1* gene against Fusarium wilt disease. The BC_2F_2 population phenotypically segregated into 3:1 ratio (resistant: susceptible). The genotypic segregation of the BC_2F_2 population using the two markers was in the ratio 1:2:1. This is a confirmation of the fact that resistance to Fusarium wilt disease in CS-19 is under the control of a single dominant gene. The RPG recovery analysis for the best improved lines ranged from 74.7 to 94.4% in BC_1F_1, 86.8 to 96.8% in BC_2F_1 and 95.1 to 96.9% in BC_2F_2 generations. The 96.14% average proportion of the recurrent parent genome in selected improved lines showed the close phenotypic resemblance to the recurrent parent BL-14. Ten homozygous lines carrying Fusarium wilt resistance gene with similar genome background to BL-14 were selected as the developed improved Fusarium wilt resistant breeding lines. The agro-morphological traits showed that there was no significant difference between the recurrent parent BL-14 and Fusarium wilt resistant improved lines developed. In conclusion, this study confirmed that Fusarium wilt resistance inbred line CS-19 is under the control of a single dominant gene and it is linked with SSR markers BVWS02309 and BVWS01133. This finding is recommended for use in marker-assisted selection for further development of Fusarium wilt resistant varieties with the newly developed resistant lines serving as source of resistance.

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

PEMBANGUNAN VARIETI TEMBIKAI (*Citrullus lanatus* L.) RINTANG LAYU FUSARIUM DAN HASIL TINGGI MELALUI PEMBIAKBAKAAN KACUKBALIK BANTUAN PENANDA

Oleh

OLALEKAN KAZEEM KOLAPO

Februari 2019

Pengerusi : Profesor Mohd Rafii Yusop, PhD
Institut : Pertanian Tropika dan Sekuriti Makanan

Satu penyakit utama tembikai (*Citrullus lanatus* L.) yang menghadkan pengeluaran tanaman ini adalah layu Fusarium (FW), yang disebabkan oleh *Fusarium oxysporum* f. sp. *niveum* (FON). Penanaman mengunakan kultivar rintang penyakit merupakan kaedah yang terbaik bagi mengawal penyakit secara lestari. Tembikai adalah merupakan satu tanaman utama di Malaysia dan negara membelanjakan kira-kira RM10 juta setiap tahun untuk mengimport bijibenih, dan oleh itu adalah perlu untuk menjimatkan jumlah yang besar ini melalui pembiakbakaan varieti yang berhasil tinggi serta kerintangan terhadap layu Fusarium. Dalam kajian ini, kacukkan antara waris inbred rintang layu Fusarium CS-19 dan waris rentan BL-14 bagi memindahkan gen rintang (*fo-1*) ke dalam waris inbred rentan BL-14 untuk menghasilkan populasi F_1. Seterusnya dua generasi kacukan balik dan swa-kacuk bagi memindahkan gen (*fo-1*) rintang ke waris inbred rentan BL-14 melalui kacuk-balik bantuan penanda molekul (MABC) untuk membangunkan waris rintang penyakit layu Fusarium yang mana ciri-ciri baik BL-14 yang dikehendaki dikekalkan. Sebelas penanda mikrosatelit yang berkaitan rapat dengan gen rintangan Fusarium telah dipilih dan dua daripada penanda polimorfik ini; penanda SSR BVWS02309 dan BVWS01133 yang terletak pada kromosom 1 dan 9 masing-masing telah digunakan untuk pengesahan gen Fusarium rintang pada generasi F_1, BC_1F_1, BC_2F_1 dan BC_2F_2. Dari 380 penanda microsatelit yang telah disaring, 78 didapati polimorfik antara kedua-dua induk yang digunakan untuk pemulihan genom induk (BL-14) penerima (RPG) dalam setiap populasi kacukbalik. Melalui ujian pewarisan yang dilakukan pada pada generasi BC_2F_1 dan BC_2F_2, didapati bahawa induk BL-14 mencatatkan 4.5 dari skala 0-5, dan ini mengesahkan kerentananya terhadap penyakit layu Fusarium. Dalam generasi BC_2F_1, 72 dari 150 pokok menunjukkan kerintangan, manakala 78 pokok menunjukkan kerentanan apabila diinokulasi dengan isolat *Fusarium oxysporum niveum* yang virulen. Ujian Khi-square ($\chi 2$) menunjukkan bahawa frekuensi yang dicerap dalam populasi BC_2F_1 menepati model gen tunggal. Ketepatan padanan (p=0.69) kepada nisbah segregasi dijangkan

(1:1) menunjukkan bahawa kerintangan ini dikawal oleh gen tunggal. Pokok yang rintang terhadap isolat *Fusarium oxysporum niveum* dari populasi BC_2F_1 menunjukkan ketepatan padanan dengan dua penanda BVWS02309 ($\chi 2$= 0.24; p= 0.6892) dan BVWS01133 ($\chi 2$= 0.11; p= 0.8065), dengan nisbah segregasi dijangkakan (1:1) untuk model gen tunggal. Kedua-dua penanda ini didapati sesuai untuk pemilihan bantuan penanda gen *fo-1* terhadap penyakit layu Fusarium. Populasi BC_2F_2 secara fenotipnya bersegregasi kepada nisbah 3:1 (rintang: rentan). Segregasi genotip populasi BC_2F_2 menggunakan penanda SSR BVWS02309 dan BVWS01133 adalah mengikut nisbah 1:2:1. Ini mengesahkan bahawa kerintangan terhadap penyakit layu Fusarium pada CS-19 adalah di bawah kawalan gen dominan tunggal. Analisis pemulihan RPG untuk waris maju terbaik adalah dengan julat dari 74.7 hingga 94.4% dalam generasi BC_1F_1, 86.8.4 hingga 96.8% dalam generasi BC_2F_1 dan 95.1 hingga 96.9% dalam generasi BC_2F_2. Purata RPG 96.14% genom induk penerima pada waris maju terpilih menunjukkan persamaan fenotip yang menyerupai induk BL-14. Sepuluh waris homozaigus yang mengandungi gen kerintangan layu Fusarium dengan genom yang sama dengan BL-14 telah dipilih sebagai waris maju yang rintang layu Fusarium. Ciri-ciri agro-morfologi menunjukkan bahawa tidak terdapat perbezaan yang ketara antara induk BL-14 dan waris maju rintang Fusarium. Kesimpulannya, kajian ini mengesahkan bahawa waris inbred CS-19 adalah rintang layu Fusarium dengan kawalan gen dominan tunggal dan ia adalah boleh disahkan dengan penanda SSR, BVWS02309 dan BVWS01133. Hasil penemuan ini serta sepuluh waris maju rintang baharu yang dibangunkan ini adalah disyorkan untuk digunakan dalam pemilihan bantuan penanda seterusnya bagi membangunkan varieti tembikai yang rintang layu Fusarium.

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Finally, to everyone that has contributed one way or the other in making me who I am today, I appreciate and pray God reward you in folds.

I certify that a Thesis Examination Committee has met on 8 February 2019 to conduct the final examination of Olalekan Kazeem Kolapo on his thesis entitled "Development of Fusarium Wilt Resistant and High Yielding Watermelon (*Citrullus lanatus* L.) Variety Through Marker-Assisted Backcross Breeding" 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 Doctor of Philosophy.

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

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This is to confirm that:

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- Supervision responsibilities as slated in Rule 41 in Rules 2003(Revision 2012-2013) were adhered to.

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QTL	Quantitative trait loci
DNA	Deoxyribonucleic acid
MAS	Marker-assisted selection
MABC	Marker-assisted backcross
SSR	Simple sequence repeat
PCR	Polymerase chain reaction
AFLP	Amplified fragment length polymorphism
RAPD	Randomly Amplified Polymorphic DNA
SNP	Single nucleotide polymorphism
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediamine Tetraacetic Acid
LB	Lysogeny broth
rpm	Revolutions per minute
Tris	tris(hydroxymethyl)aminomethane
HCl	Hydrochloric acid
$MgCl_2$	Magnesium chloride
Taq	Thermus aquaticus
NaCl	Sodium chloride
EST	Expressed sequence tag
RPG	Recurrent parent genome
RIL	Recombinant inbred line
RPG	Recurrent parent genome
TE	Tris/EDTA
%	Percentage
^0C	Degree Celsius
NaOCl	Sodium hypochlorite
V	Voltage
DI	Disease incidence
DSI	Disease severity index
FW	Fusarium wilt
FON	Fusarium oxysporum niveum

CHAPTER 1

INTRODUCTION

1.1 Background

Watermelon (*Citrullus lanatus* L) is a vegetable fruit and the largest among the fruits eaten in hot weather (Zhao *et al.*, 2013). It is an economically important vegetable crop, providing source of income for small–scale farmers worldwide, particularly in China that is rated as the highest producing country (FAOSTAT, 2018; Zhang *et al.*, 2016; Nimmakayala *et al.*, 2014). It is cherished for its sweet-flesh, good source of vitamin A and C, minerals including potassium, iron and calcium, and its possession of high amounts of citrulline and lycopene (its lycopene content is next only to that of tomato) (Reetu and Tomar, 2017; Ren *et al.*, 2012). The fact that high diversity of watermelon are found growing wild in Southern Africa makes many people attributed its origin to the place (Pitrat *et al.*, 1999). World production amounted to 117 million tonnes in the year 2016 and Malaysia, with production of 192,910 tonnes, occupied the 41st position among the producing countries (FAOSTAT, 2018).

Watermelon was reported to have been brought to Peninsular Malaysia in the 14th century through merchandise of the early Indian and Chinese (Salleh, 1986). Though first planted in Kelantan in the 1940s, it gained popularity in Malaysia around 1970s when much sweeter hybdrids were introduced from Taiwan and Japan. Breeding for hybrid watermelon has not been very successful in Malaysia and this has been attributed to lack of genetic resources. There were few choices of parental inbred lines to work with and thus the breeders depend on commercial hybrids released by foreign seed companies (Zainab and Hasnah, 2000). Other challenges militating against successful breeding in watermelon include high humidity and rainfall as well as the outbreak of diseases (Razmunah and Nik, 2016; Salleh, 1986). A number of open-pollinated watermelon varieties that are disease resistant have been developed in Malaysia, these include Super Dragon, Jade Dew and Glamour (Muhammad and Masdek, 2016). Besides being of less vigour, most of these varieties are poor in fruit quality; their flesh is neither dark red in colour nor do they have high sugar content. The emphasis of breeding work on watermelon has therefore shifted to the production of F_1 hybrid varieties and more recently, development of hybrid triploid varieties (seedless). In an effort to produce F_1 hybrid in Malaysia, Zainab and Hasnah (2000), generated four inbred lines (CS-19, BL-14, 6372-4, and CH-8) through pedigree breeding. These inbred lines possessed varying desirable qualities and showed different combining abilities (Bahari *et al.*, 2012).

1.2 Significance of the study

Watermelon is a very popular short-term, non-seasonal fruit in Malaysia. However, most varieties in the country are of low fruit quality. Therefore, there is a need to breed for improved varieties with high yield and good fruit quality. One of the major factors responsible for low yield of watermelon in Malaysia is the outbreak of diseases, mainly, Fusarium wilt. Use of resistant varieties has been found to be the most effective method in controlling the disease in a sustainable manner (Mcgregor, 2013; Park and Cho, 2012). However, there are little varieties that are resistant to Fusarium wilt disease in Malaysia and other ASEAN countries. It is believed that the use of marker-assisted backcross breeding technique would lead to the (timely) development of varieties that are disease resistant and high yielding. This will lead to an increase in production of watermelon for local consumption as well as increase source of more foreign reserves through export. Also, the identification of the polymorphic simple sequence repeat (SSR) markers linked with the disease resistance will add to the pool of knowledge about the genetic base of watermelon in Malaysia.

1.3 Problem Statement

Watermelon contributes about twenty percent of the total fruit exports of Malaysia and it is therefore classified under major fruits by the Ministry of Agriculture and Agro-Based Industry (MOA), Malaysia. In spite of its contribution to the export earning of the country, its production still depends on hybrid seeds imported from other countries. The country spends about RM 10 million annually to import about 1.5 tonnes of seeds needed to meet up its production (Bahari *et al.,* 2012; Mahmood, 2006). However, it has been observed that the imported seeds do not produce high yield in the local environments and are susceptible to Fusarium wilt disease. This soil-borne disease caused by the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON) is recorded to be one of the important diseases of watermelon. It is widespread worldwide except in Antarctica (Everts and Himmelstein, 2015; Egel and Martyn, 2013; Zhou *et al.,* 2010). The pathogen can survive for a long time in the soil and new races continously evolve; these make the control of the disease challenging (Lin *et al.,* 2009). So far, there is little information on the availability of Fusarium wilt resistant variety in Malaysia and this has led to a reduction in production and yield loss of the crop. It is believed that the use of improved varieties will lead to increase yield and subsequently, more income for the local farmers and availability of high quality variety of watermelon for domestic consumption.

1.4 Research objectives

The main objective of this study was to develop a variety of watermelon that is resistant to Fusarium wilt disease and high yielding through marker-assisted backcross breeding using SSR markers.

The specific objectives were to:

1. Identify the polymorphic SSR markers between inbred lines CS-19 and BL-14 for foreground and background selections.
2. Introgress Fusarium wilt resistance gene from CS-19 resistant line into the inbred line BL-14 through marker-assisted backcrossing method.
3. Quantify the genome recovery of the recurrent parent (BL-14) in marker-assisted backcross population

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Kazeem Kolapo was born on the 12th October 1974 in Ede, Osun State, Nigeria. On completing his primary and secondary education, he was admitted to University of Ibadan, Ibadan, Nigeria where he had his B.Sc Agriculture (Agronomy) and M.Sc Forest Resources Management (Forest Biology and Silviculture) in the years 2001 and 2005 respectively.

He worked briefly as an agricultural extension officer with the Osun State Government bewtween 2006 and 2009 before taking up a lecturing job in the Department of Agronomy, Osun State University, Osogbo, Nigeria from year 2009 till date.

Kazeem was awarded Nigeria's government sponsored Tertiary Education Trust Fund (TETFUND) to pursue his PhD. He started the programme in 2015 at the Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia under the supervision of Prof Dr. Mohd Rafii Yusop.

He is happily married to Mrs Idayat Idowu and the marriage is blessed with promising children.

LIST OF PUBLICATIONS

Kazeem K. Olalekan, Mohd Y. Rafii, Azrul M. Salleh, Mahmud TM. Mohamed, Khairulmazmi Ahmad, Azizah Misran,Tanweer F. Abro, Yusuff Oladosu, Ibrahim W. Arolu, Chukwu Samuel, Magaji Usman. (2019). Analysis of Recurrent Parent Genome Recovery in Marker-Assisted Backcross Breeding Program in Watermelon. *International Journal of Scientific & Technology Research*. 8(08): 945-955.

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