UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF HIGH YIELDING, BACTERIAL LEAF BLIGHT AND BLAST DISEASE RESISTANT RICE (*Oryza sativa* L.) VARIETY THROUGH MARKER-ASSISTED BACKCROSS BREEDING

CHUKWU SAMUEL CHIBUIKE

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CHUKWU SAMUEL CHIBUIKE

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

October 2019

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DEDICATION

I strongly dedicate this work to Almighty God for His immense love, grace and mercy upon me and my family.

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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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By

CHUKWU SAMUEL CHIBUIKE

October 2019

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

Rice is an important food crop that serves as a major carbohydrate source for nearly half of the world's population. Bacteria leaf blight caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases responsible for significant yield reduction in rice. Rice blast disease is caused by the fungal pathogen *Magnaporthe oryzae*. Both are very destructive diseases of rice in Malaysia and other parts of the world's major rice growing regions causing considerable yield loss. The main objective of this study was to develop high yielding, bacterial leaf blight and blast disease resistant rice variety for commercial cultivation in Malaysia through marker-assisted backcross breeding. Parental varieties used were Putra-1 which is high yielding and blast resistant, and served as the recipient, and IRRB60 as the donor which is bacterial leaf blight resistant. Fifteen simple sequence repeats (SSR) and functional markers that were reported to be linked to *Xoo* resistance genes were screened to confirm their polymorphism between the two parents. Also, two SSR DNA-based markers that are linked to blast resistance genes were tested for their polymorphism between the two parents. A total of 472 rice SSR markers were screened out of which 79 polymorphic markers were identified between the two parents. A total of 16.74% level of polymorphism spread across the 12 rice chromosomes was recorded from the 472 markers assessed. The result showed that the number of polymorphic markers per chromosome confirmed ranged from four (chromosomes 10 and 11) to nine (chromosomes 2, 8 and 12). Out of the 72 grown F_1 plants, five F_1 hybrids were confirmed to carry all the *Xoo* (*Xa21, xa13, xa5, Xa4*) and blast (*Piz, Pi2* and *Pi9*) resistance genes. These five progenies were selected for use in the next crossing to produce BC_1F_1 population. A total of 288 BC_1F_1 progenies were obtained from a cross between the best F_1 progeny and Putra-1. The Chi-square (χ^2) result of the six foreground markers segregation analysis showed no significant difference in the BC_1F_1 progenies from a 1:1 Mendelian segregation ratio. The result indicated a goodness of fit to the single gene model. The mean recurrent parent genome recovery (RPGR) of BC_1F_1

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population was 80.11%. The best progeny in BC_1F_1 population was BC_1F_1-38, with the RPGR of 86.40% and the low heterozygous component of 8.70% and reduced donor genome of 4.90%, in addition to very negligible linkage drag. Out of 268 BC_2F_1 progenies, 14 plants were confirmed to carry all the seven *Xoo* and blast resistance genes while χ^2 result of foreground marker segregation showed a goodness of fit to the single gene model. With the result obtained on recurrent parent genome recovery from marker-assisted background selection of BC_2F_1 after genotyping, coupled with further confirmation through phenotyping, nine best BC_2F_1 progenies with recurrent parent genome recovery of more than 95.31% were selected. A total of 220 BC_2F_2 progenies were grown from the nine selected recurrent parent genome recovered BC_2F_1 lines and furthermore, the final selection was made from homozygous individuals carrying the donor (IRBB60) parent allele with highest recurrent parent genome recovery percentage. The average RPGR recorded at BC_2F_2 was approximately 96%. Sixteen progenies from the BC_2F_2 generation were selected as advanced backcross lines. In the F_1 progeny, percentage infection recorded ranged from 4.24% to 10.91%. The average percentage infection was 6.35% while the mean disease score was recorded as 1.00. This result showed that the *Xoo* resistance genes were introgressed into the F_1 progenies and as such, resistant to bacterial leaf blight infection. The *Xoo* and blast resistance genes were re-validated in BC_2F_2 populations and the selected improved lines proved to be resistant to bacterial leaf blight and blast diseases. The selection using polymorphic tightly linked functional and SSR foreground markers was successfully used to identify BC_1F_1, BC_2F_1 and BC_2F_2 progenies with the targeted alleles. The introgression of dominant (*Xa21, Xa4*) and recessive (*xa13, xa5*) *Xoo* resistance genes as well as blast R-genes (*Pi9, Pi2, Piz*) were confirmed in the selected BC_1F_1, BC_2F_1 and BC_2F_2 progenies. The high percentage of recurrent parent genome recovery in these populations was an indication of high potentials of marker-assisted backcross breeding in recovering the genomes of the recurrent parent in rice and other cereal crops. The successful pyramiding of seven *Xoo* and blast resistance genes in the newly developed rice lines will guarantee a broad spectrum and durable resistance. This breeding programme is the very first successful attempt to manipulate the genome of the Malaysian elite rice variety Putra-1 without jeopardising its high yielding characteristic and blast resistance but with additional advantage of *Xoo* resistance in the newly improved lines. The newly developed rice lines are recommended varieties suitable for commercial cultivation in Malaysia and other rice growing regions.

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

PEMBANGUNAN VARIETI PADI (*Oryza sativa* L.) BERHASIL TINGGI, RINTANG PENYAKIT HAWAR DAUN BAKTERIA DAN PENYAKIT KARAH MELALUI PEMBIAKBAKAAN KACUKBALIK BANTUAN PENANDA

Oleh

CHUKWU SAMUEL CHIBUIKE

Oktober 2019

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

Padi adalah tanaman makanan penting yang berfungsi sebagai sumber karbohidrat utama untuk hampir separuh penduduk dunia. Hawar daun bakteria yang disebabkan oleh patogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) adalah salah satu penyakit paling serius yang mengakibatkan penurunan hasil padi yang ketara. Penyakit karah padi adalah disebabkan oleh patogen fungi *Magnaporthe oryzae*. Kedua-duany penyakit ini merupakan penyakit yang sangat merosakkan padi di Malaysia dan juga di kawasan utama penanaman padi di dunia yang mengakibatkan kehilangan hasil yang tinggi. Objektif utama kajian ini adalah untuk membangunkan varieti padi berhasil tinggi serta rintang penyakit hawar daun bakteria dan karah untuk penanaman secara komersil di Malaysia melalui pembiakbakaan kacukbalik bantuan penanda. Varieti induk yang digunakan adalah Putra-1 yang berhasil tinggi dan rintang penyakit karah sebagai induk penerima, dan IRRB60 yang rintang penyakit hawar daun bakteria sebagai induk penderma. Lima belas penanda jujukan berulang mudah (SSR) dan penanda fungsian yang telah dilaporkan dikaitkan dengan gen kerintangan *Xoo* telah disaring untuk mengesahkan polimorfisme di antara kedua-dua varieti induk. Dua penanda berasaskan SSR DNA yang dikaitkan dengan gen rintangan karah juga telah diuji untuk polimorfisme di antara kedua-dua induk tersebut. Sejumlah 472 penanda SSR padi telah disaring, dan didapati 79 dikenalpasti sebagai polimorfik di antara kedua-dua induk tersebut. Daripada 472 penanda tersebut, sejumlah 16.74% tahap polimorfisme yang tersebar merentasi 12 kromosom padi. Keputusan menunjukkan bilangan penanda polimorfik per kromosom adalah di anatara empat (kromosom 10 dan 11) hingga sembilan penanda (kromosom 2, 8 dan 12). Daripada 72 pokok F_1 yang ditanam, lima hibrid F_1 telah disahkan membawa semua gen kerintangan *Xoo* (*Xa21, xa13, xa5, Xa4*) dan karah (*Piz, Pi2 dan Pi9*). Lima progeni tersebut telah dipilih untuk pengacukkan seterusnya bagi menghasilkan populasi BC_1F_1. Sejumlah 288 progeni BC_1F_1 telah dihasilkan dari kacukan antara progeni F_1 terbaik dan Putra-1. Keputusan khi kuasa dua

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(χ^2) dari enam analisa segregasi penanda latar hadapan yang menunjukkan tiada perbezaan bererti daripada nisbah segregasi Mendelian 1:1 bagi progeni BC_1F_1. Keputusan ini memberikan ketepatan padanan yang baik untuk model gen tunggal. Purata pemulihan genom induk berulang (RPGR) populasi BC_1F_1 adalah 80.11%. Progeni terbaik di dalam populasi BC_1F_1 ialah BC_1F_1-38, dengan RPGR sebanyak 86.40% dan mempunyai komponen heterozaigus yang rendah iaitu 8.70%, dan genom penderma berkurang kepada 4.90%, serta tambahan pula rangkaian seret yang sangat rendah. Daripada 268 progeni BC_2F_1, 14 pokok telah disahkan mempunyai kesemua tujuh gen kerintangan *Xoo* dan karah, dengan keputusan χ^2 bagi segregasi penanda latar hadapan menunjukkan ketepatan padanan dengan model gen tunggal. Dari keputusan pemulihan genom induk berulang dengan pemilihan bantuan penanda latar belakang ke atas BC_2F_1 selepas pengenotipan, serta dengan pengesahan selanjutnya melalui fenotipan, sembilan progeni BC_2F_1 yang terbaik dengan pemulihan genom induk melebihi 95.31% telah dipilih. Sejumlah 220 progeni BC_2F_2 telah ditanam dari sembilan waris terpilih tersebut dari pemulihan genom induk berulang BC_2F_1 dan seterusnya pemilihan akhir telah dibuat daripada individu homozaigus yang membawa alel dari induk penderma (IRBB60) dengan peratusan pemulihan genom induk penerima yang tertinggi. Purata RPGR tercatat pada BC_2F_2 dianggarkan adalah 96%. Enam belas progeni daripada generasi BC_2F_2 telah dipilih sebagai pembiakbakaan kacukbalik. Dalam progeni F_1, peratusan jangkitan yang direkodkan adalah berjulat antara 4.24 hingga 10.91%. Purata peratusan jangkitan adalah 6.35% dengan min skor penyakit adalah 1.00. Keputusan ini menunjukkan gen rintangan *Xoo* telah diintrogresikan ke dalam progeni F_1 dan ianya memberikan ketahanan terhadap jangkitan hawar daun bakteria. Gen kerintangan *Xoo* dan karah telah disahkan semula dalam populasi BC_2F_2 dan waris maju terpilih dan telah terbukti rintang terhadap penyakit hawar daun bakteria dan karah. Pemilihan menggunakan penanda fungsian polimorfik yang berkait rapat dan penanda latar hadapan SSR telah berjaya digunakan untuk mengenal pasti progeni BC_1F_1, BC_2F_1 dan BC_2F_2 yang membawa alel yang disasarkan. Introgresi gen kerintangan dominan (*Xa21, Xa4*) dan resesif (*xa13, xa5*) *Xoo* serta gen R karah (*Pi9, Pi2, Piz*) telah disahkan di dalam progeni terpilih BC_1F_1, BC_2F_1 dan BC_2F_2. Peratusan yang tinggi bagi pemulihan genom induk berulang dalam populasi tersebut adalah membuktikan potensi yang tinggi kaedah pembiakbakaan bantuan penanda kacukbalik bagi pemulihan genom induk berulang untuk padi dan tanaman lain. Kejayaan pempiramidan tujuh gen kerintangan *Xoo* dan karah ke dalam waris baharu yang telah dibangunkan.ini akan menjamin kerintangan secara spektrum luas dan tahan lama. Program pembiakbakaan ini merupakan satu usaha pertama yang berjaya memanipulasikaaan genom varieti padi unggul Malaysia, Putra-1 tanpa menjejaskan ciri penghasilan yang tinggi dan rintangan karah, tetapi dengan kelebihan tambahan kerintangn *Xoo* bagi waris padi baharu ini. Waris padi baharu yang telah dibangunkan ini adalah varieti yang boleh disyorkan sesuai untuk penanaman komersial di Malaysia dan di kawasan penanaman padi yang lain.

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LIST OF ABBREVIATIONS

BAC	Bacterial artificial chromosome libraries
BC	Backcross
BLB	Bacterial leaf blight
BLDs	Blast/blight lesion degrees
BLT	Blast/blight lesion
CTAB	Cetyltrimethylammonium bromide
DLA	Disease leaf area percentage
DNA	Deoxyribonucleic acid
DSN	Donor segment number
EDTA	Ethylenediaminetetraacetate
ETI	Effector-triggered immunity
F_1	First filial generation
GAS	Gene assisted selection
GGT 2.0	Graphical Genotyper version 2.0
HCl	Hydrochloric acid
HR	Hypersensitive response
LD	Linkage disequilibrium
LE	Linkage equilibrium
MABB	Marker-assisted backcross breeding
MABC	Marker-assisted backcrossing
MAS	Marker-assisted selection
NA	Nutrient agar
NaCl	Sodium chloride

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NBSLRR	Nucleotide binding site-leucine-rich region
ND	NanoDrop
NILs	Near-isogenic lines
OMAP `	*Oryzae* map alignment project
P7.2	*Magnaporthe oryzae* pathotype 7.2
P7.7	*Xanthomonas oryzae* pv *oryzae* pathotype 7.7
PAMP	Pathogen associated molecular patterns
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PSA	Potato sucrose agar
PRR	Pattern recognition receptors
PTI	Pathogen triggered immunity
PVP	Polyvinylepyrrolidone
Q-Q plot	Quantile-quantile plot
R-genes	Resistance genes
RPGR	Recurrent parent genome recovery
SSLP	Simple sequence length polymorphism
SSR	Simple sequence repeat
Xoo	*Xanthomonas oryzae* pv *oryzae*
YAC	Yeast artificial chromosome

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Rice (*Oryza* spp.) is an important food crop that serves as a major carbohydrate source for nearly half of the world's population. It is a monocotyledonous plant that belongs to the family Poaceae. Rice, being a part of balanced diet and the most consumable staple food throughout the world, it is necessary to focus on the production of improved variety of rice. Statistics suggest that rice is one of the most demanding cereals in the world (Asia-RiCE, 2018). Bacteria leaf blight (BLB) which is caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases responsible for significant yield reduction in rice. The disease constraints the photosynthetic area of the crop thereby causing partial grain filling, and this leads to poor yield (Pradhan *et al*., 2015). Use of resistant varieties of rice is the best approach to guard against the disease as chemical control is not effective. About 42 BLB resistance genes have been identified in rice and some of them have been incorporated into new varieties (Kumar *et al*., 2014).

The blast disease is caused by a fungal pathogen called *Magnaporthe oryzae*. It is among the most destructive diseases affecting rice in Malaysia and other parts of the world's major rice growing regions. The disease causes considerably large grain yield losses in almost all major ecosystems where it is grown (Ashkani *et al*., 2011). Molecular markers that co-segregate with the gene are a powerful method to develop a resistant cultivar.

Farmers in Japan, specifically, Fukuoka prefecture, Kyushu Island, were the first to observe the BLB in their rice fields as early as 1884–1885 (Mannam and Hameed, 2013). The disease is nearly affecting all the major rice growing areas in Malaysia and causes mild-to-severe infection and may lead to total crop failure. In December 2016, about 4440 ha of rice fields located in Sabak Bernam were infected with *Xoo* resulting to a direct loss in rice yield of about 30–50% (AsiaRice, 2018). Bacterial leaf blight is a vascular disease and infection may be noticed at seedling, vegetative growth and reproductive stages. The symptoms are characterized by drying and yellowing of leaves which start from the tips of leaves and proceeds downward. In general, the disease is favoured by temperatures at 25 to 34 °C, with relative humidity more than 70%. The blast and BLB pathogen distribution and expansion could be modified by climate change, thereby adjusting the metabolism, growth and resistance of the host plant. Both diseases occur together most times but can as well occur singly. Where both diseases occur together, BLB becomes a serious challenge as no chemical is available to manage bacteria unlike fungal infection. Also, low temperature in humid tropical and subtropical regions accelerates the chances of blast epidemics (Bevitori and Ghini, 2014). The BLB disease is visualized with small droplets of bacterial ooze (pale amber in colour) found from the affected portions. The most destructive phase of the disease in the tropics is 'kresek' or wilt phase resulting from early systemic infection in the nursery or from seed

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infection. Host plant resistance offers the most viable, low cost effective and sustainable strategy to manage the disease than chemical control and biological method. Use of multiple R-genes is the most attractive approach to combat BLB (Arunakumari *et al*., 2016). Limitations for natural screening of BLB are labour intensive and time consuming and due to variation in degree of natural infection. Artificial inoculation of BLB could be the most effective method of screening and it could be performed by a number of strategies such as prick inoculation of the leaves, spraying the plants with bacterial suspension, dipping the seedlings in bacterial suspension before transplanting and cutting the leaves and then spraying with bacterial suspension (Laha *et al*., 2009).

Resistance genes for BLB and blast are usually obtained from rice varieties, land races, primitive cultivars and wild species. To date, 42 BLB R-genes have been discovered and deployed in rice varieties while about 100 blast resistance genes have been identified, and many of them have been cloned and characterized (Devanna *et al*., 2014). Variability in pathogens leading to overcoming of resistance effect of major gene after 4–5 years, has forced the breeder for continuous search of novel R-genes. Widespread use of resistant cultivars leads to development of selection pressure in the pathogen that resulted into breaking of resistance barrier. Majority of R-genes are dominant in nature, but recessive gene has also been reported. Some dominant R-genes are *Xa-1, Xa-3, Xa-4, Xa-7, Xa-10, Xa-14, Xa-21* and *Xa-22(t)* and some major recessive genes are *xa-5, xa-13*. Pyramiding of genes is an effective strategy for enhancing the durability of R-genes over time and space. Combination of dominant and recessive genes could perform more durable resistance but pyramiding of these genes is hampered by conventional plant breeding due to masking of dominant gene to recessive one. Marker assisted selection is one of the most sustainable and attractive idea and it facilitates incorporation of two or more major genes in different genetic background of rice.

Backcrossing is a conventional means of inserting a specific gene controlling a particular trait into an elite variety. It involves the use of two parents known as donor and recipient. The recipient parent is referred to as recurrent parent when it is used repeatedly in the crossing scheme (Hasan *et al*., 2015). A disease resistance gene could be transferred from one cultivar (usually not improved) to another cultivar being an elite variety (Matthew, 2012). Marker-assisted backcross breeding offers an efficient and precise method for breeding that preserves the vital characteristics of the recurrent parent such as high yielding trait. The underlying principle of marker-assisted backcrossing is to incorporate the specific gene of interest obtained from the donor into the defined locus of the recurrent parent. Marker-assisted backcrossing reduces linkage drag and helps in recovering the recurrent parent genome while simultaneously reducing the donor parent genome. Useful molecular markers could be functional markers and/or simple sequence repeats (SSR or microsatellite) markers, etc. (Miah *et al*., 2013).

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1.2 Problem Statement

Crop losses caused by major biotic stressors such as bacterial leaf blight and blast are quite high (Hasan *et al*., 2015). Bacterial leaf blight and blast are both destructive diseases of rice in Malaysia and other rice growing regions in the world. Both diseases are responsible for significant yield reduction in rice production. Previous research has focused on breeding new varieties that are resistant to blast disease and high yielding e.g., Putra1 rice variety. Currently in Malaysia, there is no single variety known to have multiple disease resistance as well as high yielding (BLB + blast + high yielding). The occurrence of new stresses necessitates development of highly improved and novel approaches to enhance the capability of various rice varieties that can survive attacks caused by several pathogens at once while also surviving in unfavourable environments with high level of grain quality. Molecular marker techniques are currently the most advanced method available for the transfer of desired gene in desired rice variety with required combination. Marker-assisted backcross breeding (MABB) is gaining considerable importance as it can improve the efficiency of plant breeding through precise transfer of genomic regions of interest and acceleration of the recovery of the recurrent parent genome (Wijerathna, 2015).

1.3 Research Questions

i. Are there available markers that could be useful in both foreground and background selection?
ii. Is MABB a useful tool for introgression of the target genes?
iii. Does MABB method has the potential of reducing linkage drag in the process of introgressing target genes?
iv. What are the potentials of MABB in recovering the recurrent parent genome?

1.4 Research Hypothesis

i. MABB does not have the potentials of fixing the target genes in the newly developed lines and recovering the high yielding trait of the recurrent parent (H_O: $X_A = X_B$).
ii. MABB has the potentials of fixing the target genes in the newly developed lines and recovering the high yielding trait of the recurrent parent (H_A: $X_A \neq X_B$).

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1.5 Research Objectives

1.5.1 Main objective

The main objective of this study was to develop high yielding, bacterial leaf blight and blast resistant rice variety for commercial cultivation in Malaysia.

1.5.2 Specific objectives

i. To identify foreground and background SSR markers suitable for use in developing the bacterial leaf blight and blast resistant rice variety.
ii. To introgress the bacterial leaf blight resistance genes from a donor parent IRBB60 into the genetic background of Putra-1 with incorporated blast resistance genes.
iii. To quantify the percentage recurrent parent genome recovery in the selected bacterial leaf blight and blast disease resistant lines.
iv. To validate the bacterial leaf blight and blast disease resistance genes (*Xa4, xa5, xa13, Xa21* and *Pi2, Piz, Pi9*) in the selected lines developed from the cross between Putra-1 × IRBB60 varieties.
v. To select high yielding, bacterial leaf blight and blast resistant lines for commercial cultivation in Malaysia.

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BIODATA OF STUDENT

Born at Amuda autonomous community in Umunneochi LGA of Abia State, Nigeria, Chukwu Samuel Chibuikem attended Isuochi Central School, Amuda, for his primary education (1992-1998), Umuaku Secondary School for his junior secondary education (1998-2001), Mater Christi Academy and later Methodist College Nneochi between 2001 and 2004 where he obtained his O'level (WASSCE) with distinctions. Between 2004 and 2005, he had a brief auxiliary teaching experience at Peace Comprehensive Secondary School, Amuda.

Later in 2005, he proceeded to Ebonyi State University where he obtained B.Agric (Crop Production and Landscape Management) with First Class Honours in 2010, the first of its kind in the Faculty of Agriculture and Natural Resources Management since the inception of the present Ebonyi State University. During the 3rd convocation ceremony of the Ebonyi State University held in 2012, he emerged best graduate of the University and was awarded automatic employment in the same University, in furtherance to the policy of the State government, under the watch of His Excellency, the former Executive Governor of Ebonyi State, Chief Martin N. Elechi, in rewarding excellence. As a Corps member at the Obafemi Awolowo University, Ile-Ife, Osun State, he was assigned to Dr. H. Mohammed who delegated him to handle tutorial classes in Statistics for Engineers (Factorial Experiments).

He also obtained MSc in Genetics and Plant Breeding from the Ebonyi State University in 2015. Soon after completing his Master's Degree, he was again nominated by Ebonyi State University for TETFund scholarship sponsored by the Nigerian government to study genetics and breeding at the Universiti Putra Malaysia at doctoral level. He was a participant in the Global Citizens Consultation on Biodiversity, Denmark 2012. He has over 20 publications to his credit including books and book chapters. Chukwu is happily married with children.

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LIST OF PUBLICATIONS

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Hasan, M. M., Oladosu, Y. A., ... & Olalekan, K. K. (2019). Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Molecular biology reports*, 46(1): 1519-1532.

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SC Chukwu, MY Rafii, SI, Ramlee, SI, Ismail, Y, Oladosu (2019). Addition of bacterial leaf blight resistance genes into the genetic background of Putra-1 rice variety with recurrent parent genome recovery. Under Review.

SC Chukwu, MY Rafii, SI, Ramlee, SI, Ismail, Y, Oladosu, Muhammad Isma'ila (2019).Marker-assisted introgression of multiple resistance genes confers broad spectrum resistance against bacterial leaf blight and blast diseases in Putra-1 rice variety. Under Review.

SC Chukwu, MY Rafii, SI, Ramlee, SI, Ismail, Y, Oladosu (2019). Genetic analysis of microsatellites associated with resistance against bacterial leaf blight and blast diseases of rice. Under Review.

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CONFERENCE ATTENDED

"Towards Excellence in Genetics" In conjunction with PGM's 24th Annual General Meeting. A conference organised by the Genetic Society of Malaysia (PGM), held at the Auditorium Rashdan Baba, TNCPI Building Universiti Putra Malaysia (UPM) on 20th March 2019. **Participant**

"Food Security Sustainability in South East Asia". Universiti Putra Malaysia-Kasetsart University Post Graduate Colloquium and Research Forum held at Mini Auditorium 2, 3rd Floor, Building of the DVC (Research and Innovation), UPM, from 14 – 15 August 2019. **Oral Presenter**

"Reshaping Agriculture for Sustainable Development". International Congress & General Meeting of the International Society for Southeast Asian Agricultural Sciences (ISSAAS) held at Universiti Putra Malaysia, Selangor Malaysia from 18 – 20 October 2019. **Oral Presenter.**

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