UNIVERSITI PUTRA MALAYSIA

# DEVELOPMENT OF BLAST, BACTERIAL LEAF BLIGHT RESISTANT AND DROUGHT TOLERANT RICE VARIETY THROUGH MARKER-ASSISTED PEDIGREE SELECTION

IBRAHIM SILAS AKOS

IPTSM 2020 5

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**By**

**IBRAHIM SILAS AKOS**

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

**March 2020**

# DEDICATION

I dedicate this thesis to the Almighty God the Father, Son and the Holy spirit for without Him I would not have achieved this. To Him alone be praise and glory forever, Amen.

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

**DEVELOPMENT OF BLAST, BACTERIAL LEAF BLIGHT RESISTANT AND DROUGHT TOLERANT RICE VARIETY THROUGH MARKER-ASSISTED PEDGREE SELECTION**

By

**IBRAHIM SILAS AKOS**

**March 2020**

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

Rice (*Oryza sativa* L.) is a staple food crop of over half of the world's population. It provides over 21% of the food needs and about 76% calorie intake of Southeast population. Blast, bacterial leaf blight (BLB) and drought are the major stresses affecting rice cultivation globally including Malaysia. The objectives of this study were to identify the polymorphic markers linked to blast, BLB and drought resistance genes, to develop rice breeding lines by introgression of BLB and drought resistance genes into high yielding blast resistant variety (Putra-1), genotyping and selection of rice breeding lines that carry the blast and BLB resistance genes and drought QTLs, and to evaluate the performance of the improved rice breeding lines. In this study high yielding and blast disease resistant variety, Putra-1 was introgressed with IRBB60, a BLB resistant variety, and MR219-PL-137 drought tolerant QTLs line through marker-assisted pedigree selection. The selected polymorphic and linked markers for blast resistance were RM6836 (*Piz, Pi2, Pi9* genes) and RM8225 (*Piz* gene), BLB resistance included, RM164, RM224 (*Xa-4* gene), RM122, RM13 (*xa-5* gene), RG136, Xa13Prom (*xa-13* gene), RM21, pTA248 (*Xa-21* gene). While tolerance comprised of QTL markers namely, RM1261, RM511; ($qDTY_{12.1}$), RM520, ($qDTY_{3.1}$) and RM236 ($qDTY_{2.2.}$). Phenotyping was carried out to determine the levels of resistance at $F_4$ generation for single cross, $F_3$ for three-way cross and $F_3$ for crossed lines. The scoring for blast, BLB disease pathogens inoculation and water deficit screening at reproductive-stage drought stress (RS) were resistant, moderately resistant and tolerant respectively. Agro-morphological and yield traits for non-drought stress (NS) showed that the progenies were similar in performance to their parental varieties but had significant differences among some of the improved lines on traits of days to 50% flowering (DTF), plant height (PH), panicle length number of tillers (TT), 100-grain weight (100-GW), fully filled grain (FFG), grain length and width ratio (GLW). At the RS, there were highly significant differences between the improved lines and susceptible variety for the traits of days to 50%

flowering, panicle length, fully filled grains and yield maturity. There was interactions between reproductive-stage drought stress (RS) treatment and genotypes on panicle length, total number of tillers, fully filled grains, 100-grain-weight and grain length and width ratio, indicating that these traits were sensitive to the drought condition. Eleven selected improved lines namely lines PD14, PD15, PB12, PB15 derived from single cross carrying blast and drought genes (line code with PD), and blast with BLB genes (line code with PB), lines PBD1, PBD3, DPB7, DPB12, DPB13, DPB20 derived from three-way cross, (line code PBD and DPB) and PDB3 from double cross carrying blast, BLB and drought genes (line code PDB). Variation pattern determined by principal component analysis (PCA) showed that PBD1, PD15, PDB3, DPB13, DPB20 improved lines were found better for high FFG under (NS) condition. Three improved lines (PD15, DPB12, PBD3) comparatively had shown better performance under reproductive-stage drought stress condition. All the selected improved lines are recommended for large scale evaluation and utilisation in the future breeding programme for rice variety development of blast and BLB resistance, and drought tolerance characteristics.

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

**PEMBANGUNAN VARIETI PADI RINTANG KARAH, HAWAR DAUN BAKTERIA, DAN TOLERAN KEMARAU MELALUI PEMILIHAN PEDIGRI BANTUAN PENANDA**

Oleh

**IBRAHIM SILAS AKOS**

**Mac 2020**

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

Padi (*Oryza sativa* L.) adalah tanaman makanan ruji bagi lebih separuh penduduk dunia. Ia menyediakan lebih daripada 21% daripada keperluan makanan penduduk dunia dan lebih kurang 76% pengambilan kalori bagi penduduk Asia Tenggara. Penyakit karah dan hawar daun bakteria (BLB), dan kemarau merupakan tekanan utama yang mempengaruhi penanaman padi di seluruh dunia termasuk Malaysia. Objektif kajian ini adalah untuk mengenal pasti penanda polimorfik yang berpautan dengan gen-gen karah, BLB dan rintangan kemarau, untuk membangunkan titisan biakbaka padi dengan mengintrogresi gen-gen BLB dan kerintangan kemarau kedalam varieti berhasil tinggi serta rintang karah (Putra-1), untuk pengenotipan dan pemilihan titisan biakbaka padi yang membawa gen kerintangan karah dan BLB, dan QTLs toleran kemarau dan untuk menilai prestasi titisan maju biakbaka padi tersebut. Dalam kajian ini varieti berhasil tinggi dan rintang penyakit karah, Putra-1 telah diintrogresi ke dalam varieti IRBB60, varieti rintang BLB dan MR219-PL-137 titisan QTL toleran kemarau dengan melalui pemilihan pedigri bantuan penanda. Penanda polimorfik dan penanda pautan yang terpilih untuk kerintangan karah adalah RM6836 (gen *Piz, Pi2, Pi9*) dan RM8225 (gen *Piz*), rintangan BLB termasuk, RM164, RM224 (gen *Xa-4*), RM122, RM13 (gen *xa-5*) RG136, Xa13Prom (gen *xa-13*), RM21, pTA248 (gen *Xa-21*). Untuk toleransi kemarau pula terdiri daripada penanda QTL iaitu RM1261 ($qDTY_{12.1}$) RM511; ($qDTY_{12.1}$), RM520, ($qDTY_{3.1}$) dan RM236 ($qDTY_{2.2}$.). Pengenotipan telah dijalankan untuk menentukan tahap kerintangan pada generasi $F_4$ untuk kacukan tunggal, $F_3$ untuk kacukan tiga hala dan $F_3$ untuk kacukan ganda dua. Skor dari saringan secara inokulasi induksi penyakit karah dan BLB serta tekanan defisit air pada tahap kemarau di peringkat pembiakan (RS) menunjukkan masing-masing adalah rintang, serderhana rintang, dan toleran. Ciri-ciri agro-morfologi dan hasil untuk tanpa tekanan kemarau menunjukkan progeni tersebut mempunyai prestasi yang sama dengan varieti induknya tetapi mempunyai perbezaan yang ketara dikalangan beberapa titisan maju bagi ciri-ciri bilangan hari 50% berbunga, panjang tangkai, bijian berisi penuh, tempoh kematangan hasil, ketinggian pokok, bilangan anak pokok, berat 100 bijian dan nisbah panjang dan lebar bijian. Tekanan kemarau di peringkat reproduktif, terdapat perbezaan yang sangat ketara di antara titisan-titisan maju dengan varieti rentan bagi ciri tempoh hari 50% berbunga, panjang tangkai, bijian berisi penuh dan tempoh kematangan hasil. Terdapat interaksi antara rawatan tekanan kemarau dan genotip ke atas ciri panjang tangkai, jumlah bilangan anak pokok seperdu, bijian berisi penuh dan nisbah panjang dan lebar bijian, yang menunjukkan bahawa ciri-ciri ini sensitif terhadap keadaan kemarau. Sebelas titisan maju yang dipilih ialah titisan PD14, PD15, PB12, PB15 yang dihasilkan daripada kacukan tunggal yang membawa gen-gen karah dan kemarau (kod titisan PD), dan gen karah dengan gen BLB (kod titisan PB), titisan PBD1, PBD3, DPB7, DPB12, DPB13, DPB20 yang dihasikan dari kacukan tiga hala (titisan kod PBD dan DPB), dan PDB3 dari kacukan ganda dua, BLB dan gen kemarau (kod titisan PDB). Corak variasi yang telah didapati melalui analisa komponen utama (PCA) menunjukkan titisan maju PBD1, PD15, PDB3, DPB13, DPB20 didapati lebih baik untuk ciri bijian berisi penuh di bawah keadaan bukan tekanan kemarau. Tiga titisan maju (PD15, DPB12, PBD3) secara komperatifnya telah menunjukkan prestasi yang lebih baik di bawah keadaan tekanan kemarau di peringkat reproduktif. Semua titisan maju terpilih tersebut adalah disyorkan untuk penilaian berskala besar dan digunakan dalam program pembiakbakaan dimasa hadapan bagi pembangunan varieti padi yang bercirikan keringantan karah dan BLB serta toleran kemarau.

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**Mohd Rafii Yusop, PhD**
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Chairman)

**Mohd Razi Ismail, PhD**
Professor
Institute of Tropical Agriculture and Food Security
Universiti Putra Malaysia
(Member)

**Shairul Izan Ramlee, PhD**
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

**Noraziyah Abd Aziz Shamsudin, PhD**
Senior Lecturer
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Member)

**Asfaliza Binti Ramli, PhD**
Director
Rice Research Centre
Malaysia Agricultural Research and Development Institute (MARDI)
(Member)

**ZALILAH MOHD SHARIFF**, **PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Name of Chairman of Supervisory Committee: Professor Dr Mohd Rafii Yusop

Signature: ______________________________
Name of Member of Supervisory Committee: Professor Dr Mohd Razi Ismail

Signature: ______________________________
Name of Member of Supervisory Committee: Dr Shairul Izan Ramlee

Signature: ______________________________
Name of Member of Supervisory Committee: Dr Noraziyah Abd Aziz Shamsudin

Signature: ______________________________
Name of Member of Supervisory Committee: Dr Asfaliza Binti Ramli

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| | |
|---|---|
| AFLPs | Amplified Fragment Length Polymorphism |
| ANOVA | Analysis of Variance |
| BC | Backcross |
| BCF | Backcross, Filial generation |
| BLB | Bacterial Leaf Blight |
| CV | Coefficient of Variation |
| DB | Double Cross |
| DF | Degree of Freedom |
| DTF | Days to 50% Flowering |
| DNA | Deoxyribonucleic Acid |
| DPB | MR219-PL-137 drought tolerant, Putra--1 and IRBB60 |
| DS | Drought stress |
| EDTA | Ethylenediaminetetracetic Acid |
| ET | Effective Tillers |
| FAO | Food and Agricultural Organization |
| FFG | Fully Filled Grain |
| FM | Flanking Markers |
| $F_1$ | First filial generation |
| GLW | Grain Length and Width ratio |
| PH | Plant Height |
| IBL | Interbreeding Line |
| IRRI | International Rice Research Institute |
| LSD | Least Significant Difference |
| LL | Lesion Length |
| MABC | Marker Assisted Backcross |
| MARDI | Malaysia Agricultural Research and Development Institute |
| MR | Moderately Resistant |

| | |
|---|---|
| MS | Moderately Susceptible |
| NS | Non-Drought stress |
| NTSYS | Numerical Taxonomy Multivariate Analysis System |
| PB | Putra-1 and IRBB60 varieties |
| PBD | Putra-1, IRBB60 and MR219-PL-137 drought tolerance line |
| PCA | Principal Component Analysis |
| PCR | Polymerised Chain Reaction |
| PD | Putra-1 crossed with MR219-PL-137 drought tolerant line |
| PL | Panicle length |
| POP. | Population |
| QTLs | Quantitative Trait Loci |
| RAP | Random Amplified Polymorphic |
| RAPD | Random Amplified Polymorphic DNA |
| RFLP | Restriction Fragment Length Polymorphism |
| RP | Recovery Parent |
| RRS | Reciprocal Recurrent Selection |
| S | Susceptible |
| SAHN | Agglomerative, Hierarchic and Non-overlapping |
| SNPs | Single Nucleotide Polymorphism |
| SSR | Simple Sequence Repeat |
| TT | Total number of Tillers per hill |
| TRIS-HCl | Aminomethane hydrochloride |
| UV | UltraViolet |
| UPGMA | Unweighted Pair Group Method using Arithmetic Average |
| VAR. | Variety |
| *Xoo* | *Xanthomonas oryzae* |
| YM | Yield Maturity |
| 100GW | 100-Grain weight |

# CHAPTER 1

# INTRODUCTION

## 1.1 Introduction

Rice (*Oryza sativa* L) is an important, staple food crop and one of the oldest cultivated crop for more than half of the world's human population. One hundred and sixty-seven million and twenty five thousand hectares of cultivated area yielded a global production of approximately 770 million tons (FAO, 2019) with Asia producing 90%, Africa and Americas producing 4.8% and 4.6% respectively. The human population is expected to rise to 10 billion by 2050 and 50% increase in food production is expected in order to feed this expected population. Asia alone produces up to 90% of the world consumable rice. Seventy-nine million hectares of arable land representing 75% of world production was basically for irrigated low land (Maclean et al., 2002; Bourman et al., 2007), while Southeast Asia had recorded 31% global production in 48 million hectares of land in 2010 (Redfern et al., 2012).

Rice cultivation has challenges that affects its yield performance annually, these can be broadly classified as; (1) artificial and (2) natural factors. Artificial factors could be viewed as human deliberate action or inaction which consist of poor knowledge of rice farming practices. Land tenure system which gives land title to individuals who might not be interested in farming and leasing out for farming, industrialization, urbanization, scarce labour for non-mechanized agricultural economy, lack of improved seeds peculiar to certain environment, cropping system, lack of agricultural financing policy by financial institution for fear of untimely repayment, government priority which might not be in favour of rice farming, poor irrigation facilities. Natural factors could be categorized in to abiotic and biotic. Abiotic factors include drought stress which is one of the major global constraints to rice production caused by effect of climate change. biotic factors for consideration in our study are mainly fungi and bacteria. These natural factors often have limits to which human could be responsible for it.

Blast is a fungal disease caused by *Magnaporthe grisea* which affect plant parts resulting in poor yield, and bacterial leaf blight caused by *Xanthomonas oryzae pv oryzae* often affects the leaves causing it to dry, this also affect yield because photosynthesis is impeded due to absence of chlorophyll. Drought which is one of the three traits considered in this study affects rice, causing severe yield losses depending on growth phase and stage when stress occurs and the duration (Pandey and Shukla, 2015). Yield losses ranging from 1-100% has been recorded for all of these stresses, depending on the incidence of diseases and severity of water deficit condition (Scardaci et al., 2003; Zhai and Zhu, 1999; Bray et al., 2000).

Breeding of rice could be conventional which takes longer time and resources or by modern approach of molecular marker assisted selection, which is precise and less time is required to developed improved and new lines. Pyramiding approach is a systematic process of assembling gene/QTLs into desired single elite variety, which confers multi-functional characteristics on the new variety. This is particularly employed in conferring resistance and or tolerance abilities in a single high yielding variety in order to keep in check the devastating effect of stresses that affect the yield potential of crop plants and especially rice. The new multiple gene/QTLs variety becomes a host resistant and, or tolerant variety with wider broad spectrum of resistance and or tolerance with the introgressed traits and at the same time high yielding potential (Pradhan et al., 2015). There are basically three approaches in pyramiding for the development of host stresses resistance and or tolerant variety of rice; backcross method, recurrent and pedigree selections (Ruengphayak et al., 2015).

Basically, backcross method is used in recovery of recurrent parent (RP) genome, it is simply considered as a breeding technique use by breeders to recover a desired trait from a donor parent, say high yielding but susceptible to say blast disease into a blast resistance variety referred to as recipient parent but low yielding, through repeated backcross of the donor in to the recipient. The conventional approach takes time and requires good knowledge of morphological markers.

Recurrent selection is another method use in pyramiding by self-pollinating crops. It is basically selecting for certain traits of interest generation after generation by interbreeding the reselected plants, this allows access to selection of favourable recombinant at the same stabilize traits in the gene pool. At each interbreeding line (IBL) ideotype is selected, although caution must be exercised to avoid over reliance on the phenotype because it is not an absolute indicator of the genotypic expression usually, yield and quality traits along with test crosses are carried out to select the outstanding ten lines. At the end of recurrent selection new individuals should be selected to be used as new parents of IBLs. These should be recurrently selected up to four or five generations. An $F_1$ single cross (A×A) of two lines is produced after recurrent selection is carried in two separate programs.

Pedigree breeding begins by crossing two genotypes and each of them have one or more traits of interest that is lacking in the other. In a situation where the two parents are not able to provide all the traits or characters of interest in the $F_{1,}$ a third parent with a desired trait can be crossed with one of the hybrid progeny ($F_1$). In this method, only superior types are often selected in successive generations, a good record of parent-progeny relationship is maintained. Individuals having undesirable major genes are eliminated. Successive generations of self-pollination produce hybrid conditions that will enable the emergence of pure breeding because of natural self-pollination. Record keeping helps in decision making for elimination. The selected families at this stage are usually harvested in mass for the purpose of gathering large quantity of seeds required for quantitative character evaluation, thereafter by usual selection the number of families would be reduced to a manageable proportion by the $F_7$ or $F_8$ generation of conventional breeding, and less generations for marker assisted selection, which leads to evaluation for performance and quality. The final evaluation that leads to selection of best strains involves genotype by environment evaluation.

## 1.2 Justification of the study

The devastating effects of blast pathogen resulted in 50-70% yield loss recorded in about 4033 ha (less than 5% of paddy planted area) of rice field in Malaysia (Zakaria and Misman, 2011). Bacterial leaf blight recorded the most severe outbreak of the last 30 years with 50-70% yield loss in 2016 at Sekinchan, Selangor, Malaysia when paddy rice field had more than 90% of 1,296ha of padi fields affected (Toh et al., 2019). Drought stress has the potential to cause up to 100% yield loss depending on stage of absence of water, duration/length. Abiotic stress was recorded to have caused loss of over 50% of rice globally (Bray et al., 2000; Iqbal et al., 2013; Li et al., 2014). In a season of normal supply of water and no stress, yield of over 8000 kg $ha^{-1}$ was recorded (Shamsudin et al., 2016). The attendant effect of drought on crop yield losses, accentuate the need to develop resistance/tolerant rice variety to combat the threat to food security of the world's population and economy of farmers, as well as the proximate production demand of 40% before 2030 and 50% before 2050 to meet expected increase population (Maclean et al., 2002; Khush, 2005; Bourman et al., 2007).

## 1.3 Research objectives

The main objective of this research was to develop a high yielding, blast and bacterial leaf blight resistance, and drought tolerant rice variety for Malaysia rice ecosystems. The specific objectives were:

1. To identify the polymorphic markers, linked to blast, BLB and drought resistance genes/QTLs
2. To develop rice breeding lines by introgression of BLB and drought resistance genes/QTLs into high yielding blast resistant variety (Putra-1)
3. To genotype and select the rice breeding lines that carry blast, BLB and drought resistance genes/QTLs
4. To evaluate the performance of the rice breeding lines that carry blast, BLB and drought resistance genes/QTLs

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# BIODATA OF STUDENT

I, Ibrahim Silas Akos was born on the 3rd of April, 1978 to the family of Mr Silas Wuyah Akos and Late Damaris Silas Akos (Mrs) (who passed away on the 27th February, 2012). I attended L.E.A Nasarawa North Primary School, Ungwan Gwari, Nasarawa L.G.A., Nasarawa State (then Plateau State), Nigeria from 1986-1991. I then sat for Common Entrance Examination and secured admission to the famous Government Science Secondary School, Kuru, Jos South L.G.A., Plateau State, Nigeria, from 1991-1998. I sat for Senior School Certificate Examination (SSCE) and passed with the requisite passes which qualified me to sit for University Matriculation Examination (UME) which gained me offer to study Botany at the Nasarawa State University, Keffi, from 2001-2005 for Bachelor of Science (Honours) Botany. I was deployed to Delta State, Nigeria for my one year compulsory National Youth Service Corps (NYSC), 2006/2007. I secured admission in 2008 for a Master of Science degree (M.Sc) Cytogenetics and Plant Breeding, University of Jos, Jos, Plateau State, Nigeria and graduated in 2012. I also obtained a Postgraduate Diploma in Education (PGDE) from Usman Danfodiyo University, Sokoto, Sokoto State, Nigeria through the National Teachers' Institute, Kaduna, Keffi Study Centre and Advanced Diploma in Public Administration (ADPA) from the Nasarawa State University, Keffi, both between 2011/2012.

I worked with the Nasarawa State Government in the Ministry of Environment and Natural Resources (formerly Ministry of Agriculture and Natural Resources) as Forestry Officer and then to Zonal Forestry Officer between 2007-2013. In December 2013, I was offered employment into Kaduna State University as an Assistant Lecturer in the Department of Crop Science, Faculty of Agriculture. While on the job, I gained this admission offer for Ph.D. Plant Breeding and Genetics, Universiti Putra Malaysia. I am married to Grace Ibrahim Akos (Mrs) and we are blessed with two children, a boy and girl namely Master Elyon Ibrahim Akos and Miss Sharon Ibrahim Akos respectively.

## LIST OF PUBLICATIONS

Akos, I.S., Rafii, M.Y., Razi, M.I., Ramlee, S.I., Shamsudin, N.A.A., Ramli, A.B., Momodu, J., Halidu, J. and Swaray. S. 2019. Genetic inheritance of multiple traits of blast, bacteria leaf blight resistant and drought tolerant rice lines. *International Journal of Applied Biology,* 3(2):1-35

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**Conference Attendance and Paper Presented**

Akos, I. S., Rafii, M.Y., Razi, M.I., Ramlee, S.I., Shamsudin, N.AA., Ramli, A.B., Chukwu, S.C. 2019. Genotyping and Phenotyping for Rice Selection Resistant to Blast, Bacteria Leaf Blight and Tolerant to Drought. Book of proceedings of Malaysia International Genetic Congress (MiGC13): Exploring Innovation In Genetics, Selangor, Malaysia. $19^{th}$-21st November, 2020.

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