



UNIVERSITI PUTRA MALAYSIA

***DEVELOPMENT OF BLAST RESISTANT RICE VARIETY DERIVED FROM
CROSSING BETWEEN MR219 AND PONGSU SERIBU 2 THROUGH
MARKER-ASSISTED BACKCROSS BREEDING***

TANWEER FATAH

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By

TANWEER FATAH

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

November 2015

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DEDICATION

I dedicate this thesis to my beloved parents, wife, son, brother, his family and sister for their kind and loving support



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 RESISTANT RICE VARIETY DERIVED FROM CROSSING BETWEEN MR219 AND PONGSU SERIBU 2 THROUGH MARKER-ASSISTED BACKCROSS BREEDING

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November 2015

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Rice blast caused by fungus *Magnaporthe oryzae* is a major rice disease due to significant yield losses worldwide as well as in Malaysia. Cultivating blast-resistant rice varieties is the most effective, economical and practical approach to prevent blast disease. Marker-assisted backcross breeding contribute an effective and vital role in incorporating blast resistant genes into blast susceptible high yielding rice varieties.

In this study two blast resistant genes (putative *Pi-b* and *Pi-kh*) were identified from the Malaysian rice variety Pongsu Seribu 2 and revealed that it contain Nucleotide Binding Site-Leucine Rich Repeat (NBS-LRR) domain. The blast resistant genes (*Pi-b* and *Pi-kh*) were introgressed from Pongsu Seribu 2 variety using marker-assisted backcross breeding strategy into a high-yielding blast susceptible rice variety MR219. Therefore crosses were made between the MR219 used as recurrent parent and Pongsu Seribu 2 as donor parent to transfer blast resistant genes into MR219 without losing their actual quality and yield sustainability. Eleven SSR molecular markers linked to rice blast resistant genes were found polymorphic between the two parental varieties and used to find the potential relation with blast resistance in the present developed backcross population. The polymorphic markers were used in the further subsequent generation for confirmation of blast resistant genes. Out of the 11 markers, only 2 markers RM208 (located on chromosome 2 linked to *Pi-b* gene) and RM206 (located on chromosome 11 linked to *Pi-kh* gene) conferring blast resistance were confirmed and used in F₁, BC₁F₁, BC₂F₁ and BC₂F₂ generations providing resistance against most virulent Malaysian rice blast fungus *M. oryzae* pathotype P7.2.

300-SSR markers were screened, out of them 72 markers were found to be polymorphic between the parental lines and used for background recovery of the recurrent parent (MR219) in each backcross population. The inheritance patterns and identification of microsatellite markers linked to the rice blast resistance were observed in BC₂F₁ and BC₂F₂ generations. The recurrent parent MR219 showed susceptibility with lesion 5 to 7 score, and donor parent PS2 showed resistivity with lesion 0 to 2 while challenging to pathotype P7.2 fungus inoculum under control conditions. In

BC₂F₁ generation, 320 plants were inoculated with pathotype P7.2 and 154 plants showed the resistance mechanism while another 166 plants showed susceptible reaction to blast. Chi-square test (χ^2 for the single-gene model was applied for testing goodness of fit of observed frequencies. The two linked markers RM208 ($\chi^2 = 1.5130$; $p = 0.2188$) and RM206 ($\chi^2 = 0.6130$; $p = 0.4338$) for blast resistance to pathotype P7.2 showed good fit with expected test cross ratio (1:1) for single-gene model analysis. The markers RM208 and RM206 found suitable for marker-assisted selection of *Pi-b* and *Pi-kh* blast resistance genes conferring resistance against the blast pathotype P7.2. Phenotypically BC₂F₂ population segregated into 3:1 ratio. The genotypic segregation of the BC₂F₂ population segregated into 1:2:1 ratio. The background selection analysis for the recovery of MR219 variety among the best improved lines ranged from 73 to 94% in BC₁F₁, 79.4 to 96.1 in the BC₂F₁ and 94 to 97.5% in BC₂F₂ generations. The average proportions of the recurrent parent genome in the selected 15 improved lines of BC₂F₂ were 96.17%, explaining that very close phenotypic resemblance to the recurrent parent MR219. The 15 homozygous lines carrying blast resistant genes with similar background to MR219 were selected for the development of improved blast resistant rice variety. The agro-morphological traits of the improved lines and recurrent parent showed no significant difference between those lines. In conclusion, from the present rice breeding program, 15 homozygous advanced blast resistant rice lines were developed with a high potential to be released as a new variety for commercial cultivation.

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

**PEMBANGUNAN VARIETI PADI RINTANG KARAH MENERUSI
KACUKAN ANTARA MR219 DAN PONGSU SERIBU 2 MELALUI
PEMBIAKBAKAN KACUKBALIK BANTUAN PENANDA**

Oleh

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Karah padi adalah disebabkan oleh fungi *Magnaporthe oryzae*, merupakan satu penyakit utama padi yang mengakibatkan penurunan hasil yang ketara di seluruh dunia serta di Malaysia. Penanaman padi rintang karah merupakan pendekatan yang paling berkesan, ekonomik dan praktikal untuk mencegah penyakit karah ini. Pembiakbakaan kacukbalik bantuan penanda menunjukkan impak yang berkesan dan penting dalam memindahkan gen kerintangan karah ke varieti padi berhasil tinggi yang rentan penyakit.

Dalam kajian ini dua gen kerintangan karah (putatif *Pi-b* dan *Pi-kh*) telah dikenalpasti dari varieti padi Malaysia, Pongsu Seribu 2 dan didapati ianya mengandungi domain *Nucleotide Binding Site-Leucine Rich Repeat* (NBS-LRR). Gen kerintangan (*Pi-b* dan *Pi-kh*) telah diintrogresikan dari varieti padi Pongsu Seribu 2, dengan menggunakan strategi pembiakbakaan kacukbalik bantuan penanda, ke dalam satu varieti padi berhasil tinggi yang rentan karah, MR219. Kacukkan telah dilakukan antara MR219 sebagai induk penerima dan Pongsu Seribu 2 sebagai induk penderma untuk memindahkan gen kerintangan karah dari Pongsu Seribu 2 ke MR219 tanpa kehilangan kualiti dan prestasi hasil induk penerima. Sebelas penanda molekul SSR yang berkait rapat dengan gen kerintangan karah padi memberikan polimorfik diantara kedua induk tersebut dan telah digunakan untuk menentukan potensinya sebagai penanda untuk gen kerintangan karah dalam populasi kacukbalik yang sedang dibangunkan ini. Dari 11 penanda polimorfik tersebut, hanya dua SSR penanda iaitu RM208 (terletak pada kromosom 2 dikaitkan dengan gen *Pi-b*) dan RM206 (terletak pada kromosom 11 dikaitkan dengan gen *Pi-kh*) menunjukkan kerintangan karah telah dikenalpasti dan telah digunakan dalam generasi F_1 , BC_1F_1 , BC_2F_1 dan BC_2F_2 terhadap patotip fungsi karah padi Malaysia *M. oryzae* yang virulen, P7.2.

Daripada 300-penanda SSR yang telah diuji, didapati 72 penanda polimorfik antara kedua-dua induk, dan penanda tersebut telah digunakan untuk pemulihan genom induk penerima (MR219) dalam setiap populasi kacukbalik. Pewarisan gen kerintangan dicerap dalam generasi BC_2F_1 dan BC_2F_2 . Induk penerima, MR219 menunjukkan

kerentanan dengan skor lesion 5 hingga 7, dan induk penderma, PS2 menunjukkan kerintangan dengan lesion 0 hingga 2 terhadap fungsi patotip P7.2 yang diinokulum di bawah keadaan terkawal. Dalam generasi BC₂F₁, 320 pokok telah diinokulasi dengan patotip P7.2, dan 154 pokok telah menunjukkan mekanisme kerintangan, manakala 166 pokok lagi menunjukkan reaksi rentan terhadap karah. Ujian Chi-NXDVDGXD\$QWXN model gen tunggal telah dijalankan ujian padanan yang baik dengan frekuensi yang GLFHUDES 'XDSHQDQGDWHUVHEXW50\$\$ GDQ50\$ (0,6130; p = 0.4338) untuk kerintangan karah bagi patotip P7.2 menunjukkan padanan yang tepat dengan nisbah dijangka (1:1) untuk analisis model gen tunggal. Kedua-dua penanda tersebut didapati sesuai untuk pemilihan bantuan penanda kerintangan gen karah, *Pi-b* dan *Pi-kh* yang memberikan rintangan terhadap patotip P7.2. Secara fenotipik populasi BC₂F₂ memberikan kadar segregasi 3:1 terhadap karah patotip P7.2. Segregasi genotip populasi BC₂F₂ adalah dengan nisbah 1:2:1. Analisis pemulihan genom bagi varieti MR219 dikalangan titisan maju terbaik adalah dari 73 hingga 94% dalam generasi BC₁F₁, 79.4 hingga 96.1% dalam BC₂F₁ dan 94 hingga 97.5% dalam BC₂F₂. Purata peratusan genom induk penerima dalam 15 titisan maju terpilih adalah 96.17%, yang menunjukkan persamaan fenotip sangat rapat dengan induk penerima, MR219. Lima belas titisan homozaigus yang mempunyai gen kerintangan serta dengan kesamaan genom MR219 yang tinggi telah dipilih untuk pembangunan varieti padi yang rintang penyakit karah. Ciri agro-morfologi diantara titisan maju tersebut dengan induk penerima MR219 menunjukkan tidak terdapat perbezaan yang bererti. Kesimpulannya, dari program pembiakbakaan padi ini, 15 titisan maju homozaigus rintang karah telah dihasilkan dan ianya sangat berpotensi untuk menghasilkan varieti padi baru bagi penanaman secara komersial.

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

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LIST OF ABBREVIATIONS

QTL	Quantitative trait loci
DNA	Deoxyribonucleic acid
MAS	Marker-assisted selection
MABB	Marker-assisted backcross breeding
MABC	Marker-assisted backcrossing
SSR	Simple sequence repeat
MT	Million tons
Mb	Mega base
NBS-LRR	Nucleotide binding site- Leucine rich repeat
PCR	Polymerase chain reaction
BAC	Bacterial artificial chromosome
YAC	Yeast artificial chromosome
AFLP	Amplified fragment length polymorphism
STS	Sequence tag site
RAPD	Randomly Amplified Polymorphic DNA
SNP	Single nucleotide polymorphism
RIL	Recombinant inbred line
CTAB	Cetyltrimethylammonium bromide
EDTA	Ethylenediamine Tetraacetic Acid
dNTP	Deoxynucleotide triphosphate
UV	Ultra violet
LB	Lysogeny broth
SOB	Super Optimal Broth
rpm	Revolutions per minute
Tris	tris(hydroxymethyl)aminomethane
DTT	Dithiothreitol
ATP	adenosine-triphosphate
PEG	polyethylene glycol
Taq	Thermus aquaticus
RGA	Resistance gene analogue
EST	Expressed sequence tag
RPG	Recurrent parent genome
RIL	Recombinant inbred line
RH	Relative humidity
SMA	Single marker analysis
SES	Standard evaluation score
TE	Tris/EDTA
V	volt
v/v	Volume/volume
w/v	Weight/volume
- ME	-mercaptoethanol
ddH ₂ O	Double distilled water
BLD	Blast lesion degree
BLT	Blast lesion type
%DLA	Percent diseased leaf area

CHAPTER 1

INTRODUCTION

1.1 General introduction

Rice (*Oryza sativa* L.) is the most widely consumed staple dietary food and largest cereal crops throughout the world, especially in Asia (Khush, 2005). Rice is not only used for human consumption, but it is also used for animal feeding and provides the major source of income for rural people (Datta, 2004). Rice provides nutrition for six billion people worldwide (Maclean *et al.*, 2002). Considering its nutritional value, along with carbohydrates rice also provides other essential nutrients such as protein, iron, calcium, thiamine, riboflavin, niacin and vitamin E, to the human body (Akinbile *et al.*, 2011).

In Malaysia, rice is mostly grown in eight granary areas of Peninsular Malaysia, Sabah and Sarawak, mainly under flooded conditions (Akinbile *et al.*, 2011). Malaysia is ranked 24th in terms of rice production in the world with annual production of 2.750 million tons (FAO, 2014). Total area under rice cultivation in Malaysia in 2014 was 692,340 ha, out of which 43,353 ha are situated in Sabah and 121,921 in Sarawak (DOA, 2014). Presently, Muda Agricultural Development Authority (MADA) and Kemudu Agriculture Development Authority (KADA) are two main rice growing areas in Malaysia. At present Malaysia in term of rice production is not self-sufficient. The main factor in limiting the rice production includes various biotic and abiotic stresses.

Among biotic stresses, diseases are one of the main factor that reduces rice production. The only best way to minimize the yield losses is the production of high-yielding resistant rice varieties. Blast caused by the fungal pathogen *Magnaporthe oryzae* is one of the most serious diseases because the yield loss caused by blast range from 1 to 50 % worldwide including Malaysia. On the other hand Malaysia has targeted to achieve 80% rice-sufficiency by 2020 (FAO, 2014). There is an urgent need to expand research in rice in order to assure rice sufficiency by controlling disease and increasing yield potential. Recently, several techniques have been adopted to control the blast disease, including chemical control, disease forecasting, cultivation practice, but these control measures are not very effective (Srinivasachary *et al.*, 2002). The recent advent of DNA marker technology, gene cloning, development of numerous markers, molecular breeding approaches helped plant breeders to produce rice varieties with blast resistance and higher yield potential. The cloning and characterization of blast resistant genes facilitated the exploration of mechanism of host-pathogen interaction. DNA markers also acted as a valuable resource to localize the genes/QTL controlling important traits such as plant disease resistance (Yano and Sasaki, 1997; Paran and Zamir, 2003). Currently SSR markers are widely used to screen the rice population for blast resistance. Several SSR markers tightly linked to blast resistant genes have been identified; therefore tagging of blast resistant genes until several generations are possible (Miah *et al.*, 2013a). Furthermore the application of marker-assisted selection

(MAS) and marker-assisted backcross breeding (MABB) could greatly improve the potential of modern rice varieties through introgression of novel resistant genes.

1.2 Significance of the study

Achieving stable resistance to blast is perhaps the most important goal in managing blast disease. Plant breeding has been very successful in improving blast susceptible varieties using marker-assisted selection (Zhao *et al.*, 2010). In Malaysia several upland and traditional rice varieties are available conferring strong resistance against the blast disease. Among them Pongsu Seribu 2 is one of blast resistant rice variety conferring broad spectrum resistance against the different blast pathotypes. Another Malaysia rice variety MR219 remained very popular among farmers for a long time because of high yield, long grain and good eating quality (Alias, 2002). At present MR219 is highly susceptible to blast disease. MR219 rice cultivar can be improved by the introduction of blast resistant genes from Pongsu Seribu 2 variety. Considering the above discussed advancement of marker-assisted selection the aim of this research was to identify the blast resistant genes from Pongsu Seribu 2 and tightly linked SSR marker associated with blast resistance against the most virulent pathotype P7.2 of *M. oryzae*. In the present study marker-assisted backcross breeding approach was also used to incorporate blast resistant genes into MR219 rice variety. The introgression of blast resistant genes in MR219 will retain its high yield and will be widely adopted again by local rice farmers of Malaysia.

1.3 Problem statement

In Malaysia, rice blast has become noticeable disease due to occurrence in main and off-season across the rice growing regions. Several outbreaks occur in different areas of peninsular Malaysia and about 3000 farmers lost RM5 million (DOA, 2014). The major outbreak was in the year of 2006 at KADA, Pasir Puteh, and Kelantan where 60% of 4,000 ha land cultivated with rice was affected due to blast disease and caused heavy economic loss (Ashkani *et al.*, 2011). In the recent years the frequency of blast occurrence has increased with invasion into new areas. Moreover, there is not cultivated blast resistant rice variety in Malaysia. Research for developing highly blast resistant rice varieties are urgently needed to reduce the yield losses and supporting the poor farmers by combating blast disease.

1.4 Research objectives

The main objective of the study was to develop blast resistant, high yielding rice variety suitable to local environments through marker-assisted backcrossing. Accordingly, the specific objectives were:

1. To clone and sequence the blast resistant genes from resistant rice variety Pongsu Seribu 2.

2. To identify suitable segregating SSR markers for blast resistance using inheritance and disease reaction analysis in BC₂F₁ population.
3. To estimate the recovery of recurrent parent genome (MR219) contribution in marker-assisted backcross populations for introgression of blast resistant genes.
4. To evaluate, verify and select the advanced blast resistant lines from BC₂F₂ population.



REFERENCES

- Abdullah, M.Z., Mohamad, O., Hadzim, K. and Othman, O. (1991). Vareiti padi tradisional di Malaysia. *Teknologi Padi* 7: 11-18.
- Ahn, S., Kim, Y., Han, S., Choi, H., Moon, H. and McCouch, S. (1996). Molecular mapping of a gene for resistance to a Korean isolate of rice blast. *Rice Genetics Newsletter* 13: 74-76.
- Ahn, S.N., Kim, Y.K., Hong, H.C., Choi, H.C., Moon, H.P., Han, S.S. and McCouch, S.R. (1997). Mapping of genes conferring resistance to Korean isolates of rice blast fungus using DNA markers. *Korean Journal of Breeding* 29(4): 416±423.
- Ahn, S.N., Kim, Y.K., Hong, H.C., Han, S.S., Kwon, S.J., Choi, H.C., Moon, H.P. and McCouch, S.R. (2000). Molecular mapping of a new gene for resistance to rice blast (*Pyricularia grisea* Sacc.). *Euphytica* 116(1): 17-22.
- Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T. (1996). Microsatellite DNA markers for rice chromosomes. *Theoretical and Applied Genetics* 93(7): 1071-1077.
- Akinbile, C.O. and Sangodoyin, A.Y. (2011). Evapotranspiration, soil and water quality implications on upland rice production. *Asian Journal of Crop Science* 3(4): 169-178.
- Alias, I. (2002). MR219, a new high-yielding rice variety with yields of more than 10 mt/ha. *MARDI, Malaysia. FFTC: An international information center for small scale farmers in the Asian and Pacific region.*
- Allard, R.W. (1999). Principles of Plant Breeding, 2nd edn. Wiley and Sons, New York.
- Arumuganathan, K. and Earle, E. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* 9(3): 208-218.
- Ashikawa, I., Hayashi, N., Yamane, H., Kanamori, H., Wu, J., Matsumoto, T. and Yano, M. (2008). Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics* 180(4): 2267-2276.
- Ashkani, S. (2011). Molecular dissection and QTL mapping of rice blast disease resistance using Simple Sequence repeat markers. Ph.D Thesis, Universiti Putra Malaysia.
- Ashkani, S., Rafii, M.Y., Rahim, H.R. and Latif, M.A. (2013) Mapping of the quantitative trait locus (QTL) conferring partial resistance to rice leaf blast disease. *Biotechnology Letter* 35: 799-810.
- Ashraf, M., Akram, N. and Foolad, M. (2012). Marker-assisted selection in plant breeding for salinity tolerance. *Plant Salt Tolerance*, (pp. 305-333): Springer.
- Ashraf, M. and Foolad, M.R. (2013). Crop breeding for salt tolerance in the era of molecular markers and marker-assisted selection. *Plant Breeding* 132(1): 10-20.
- Barman, S., Gowda, M., Venu, R. and Chattoo, B. (2004). Identification of a major EODVWUHVLVWDQFHJHQHLQWKHULFHFKOVLVYDQJTEBMS100-302.
- Bryan, G.T., Wu, K.S., Farrall, L., Jia, Y., Hershey, H.P., McAdams, S.A., Fauk, K.N., Donaldson, G.K., Tarchini, R. and Valent, B. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *The Plant Cell Online* 12(11): 2033-2045.

- Basavaraj, S., Singh, V. K., Singh, A., Singh, A., Singh, A., Anand, D., Yadav, S., Ellur, R.K., Singh D. and Krishnan, S.G. (2010). Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Molecular breeding* 26(2): 293-305.
- Bennetzen, J. (2002). The rice genome-Opening the door to comparative plant biology. *Science* 296, 5.
- Berruyer, R., Adreit, H., Milazzo, J., Gaillard, S., Berger, A., Dioh, W., Lebrun M.H. and Tharreau, D. (2003). Identification and fine mapping of *Pi33*, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene ACE1. *Theoretical and Applied Genetics* 107(6):1139-1147.
- Biswas, J.K., Hossain, M.A., Sarkar, B.C., Hassan, M. and Haque, M.Z. (1998). Yield performance of several rice varieties seeded directly as a late crop. *Bangladesh Journal of Life Science* 10: 47-52.
- Bonman, J., Vergel de Dios, T. and Khin, M. (1986). Physiologic specialization of *Pyricularia oryzae* in the Philippines. *Plant Disease* 70(8): 767-769.
- Bonman, J.M. and Mackill, D. (1988). Durable resistance to rice blast disease. *Oryza*, 25(2): 103-110.
- Bonman, J. (1992). Durable resistance to rice blast disease-environmental influences. *Euphytica* 63: 115-123.
- Bradbury, L.M., Fitzgerald, T.L., Henry, R.J., Jin, Q. and Waters, D.L. (2005). The gene for fragrance in rice. *Plant Biotechnology Journal* 3(3): 363-370.
- Bres-Patry, C., Lorieux, M., Clement, G., Bangratz, M. and Ghesquière, A. (2001). Heredity and genetic mapping of domestication-related traits in a temperate *japonica* weedy rice. *Theoretical and Applied Genetics*, 102(1): 118-126.
- Bryan, G.T., Wu, K., Farrall, L., Jia, Y., Hershey, H.P., McAdams, S.A., Tarchini, R., Donaldson, G., Faulk, K., and Valent, B. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* 12: 2033±2045.
- Causse, M.A., Fulton, T.M., Cho, Y.G., Ahn, S.N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P.C. and Harrington, S.E. (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 1274: 138: 1251.
- Chauhan, R., Farman, M., Zhang, H.B. and Leong, S. (2002). Genetic and physical mapping of a rice blast resistance locus, *Pi-CO39(t)* that corresponds to the avirulence gene AVR1-CO39 of *Magnaporthe grisea*. *Molecular Genetics and Genomics* 267(5): 603-612.
- Chen, D.H., Dela Vina, M., Inukai, T., Mackill, D., Ronald, P. and Nelson, R. (1999). Molecular mapping of the blast resistance gene, *Pi44 (t)*, in a line derived from a durably resistant rice cultivar. *Theoretical and Applied Genetics* 98(6-7): 1046-1053.
- Chen, S., Lin, X.H., Xu, C.G. and Zhang, Q.F. (2000). Improvement of bacterial blight resistance of Minghui 63 an elite restorer line of hybrid rice, by molecular marker-assisted selection. *Crop Science* 40: 239±44.
- Chen, H. (2001). Population structure of *Pyricularia grisea* from central and southern China and comparative mapping of QTL for blast-and bacterial blight-resistance in rice and barley. Wuhan, China, Huazhong Agriculture University, PhD thesis.
- Chen, X.W., Li, S.G., Xu, J.C., Zhai, W.X., Ling, Z.Z., Ma, B.T., Wang, Y.P., Wang, W.M., Cao, G., Ma, Y.q., Shang, J.J., Zhao, X.F., Zhou, K.D. and Zhu, L.H. .GHQWLFDWLRQ RI WZR EODVW UHVLVWDQFH JHQHV LQ D ULFH YDULHW\|JX *Phytopathology* 152: 77±85.

- Chen, S., Wang, L., Que, Z., Pan, R. and Pan, Q. (2005). Genetic and physical mapping of *Pi37(t)*, a new gene conferring resistance to rice blast in the famous cultivar St. No. 1. *Theoretical and Applied Genetics* 111(8): 1563-1570.
- Chen, X., Shang, J., Chen, D., Lei, C., Zou, Y., Zhai, W., Liu, G., Xu, J., Ling, Z., Cao, G., Ma, B., Wang, Y., Zhao, X., Li, S. and Zhu, L. (2006). AB-lectin receptor kinase gene conferring rice blast resistance. *The Plant Journal* 46(5): 794-804.
- Chen, H.Q., Chen, Z.X., Ni, S., Zuo, S.M., Pan, X.B. and ZHU, X.D. (2008). Pyramiding Three Genes with Resistance to Blast by marker-assisted selection to Improve Rice Blast Resistance of Jin 23B, application. *Chinese Journal of Rice Science* 22:(1), 23-27.
- Cho, Y.G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., McCouch, S.R., Park, W.D., Ayres, N. and Cartinhour, S. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 100(5): 713-722.
- Collard, B.C.Y. and Mackill, D.J. (2007). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 17: 1-16.
- Collard, B.C., Vera Cruz, C.M., McNally, K.L., Virk, P.S. and Mackill, D.J. (2008a). Rice molecular breeding laboratories in the genomics era: current status and future considerations. *International Journal of Plant Genomics* 2008: 1.
- Collard, B.C. and Mackill, D.J. (2008b). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences* 363(1491): 557-572.
- &ROOLQV)6 3RVLWLRQDO FORQLQJ OHWY QRW FDOO LW UHYHWHW DQPRUH
Genetics 1: 3±6.
- Conaway-Bormans, C., Marchetti, M., Johnson, C., McClung, A. and Park, W. (2003). Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker-assisted selection. *Theoretical and Applied Genetics* 107(6): 1014-1020.
- Correa-Victoria, F.J. and Zeigler, R.S. (1995). Stability of partial and complete resistance in rice to *Pyricularia grisea* under rainfed upland conditions in eastern Colombia. *Phytopathology* 85(9): 977-982.
- Correll, J.C., Harp, T.L., Guerber, J.C., Zeigler, R.S., Liu, B., Cartwright, R.D. and Lee, F.N. (2000). Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. *Phytopathology* 90: 1396±1404.
- Couch, B.C. and Kohn, L.M. (2002). A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia* 94:683-693.
- Courtois, B., Nelson, R. and Roumen, E. (2002). Creation of a gene pool to improve *Pyricularia* on Resistance partial secanano rice through recurrent selection. In: Guimara es EP (ed) Recurrent selection in rice. *International Centre for Tropical Agriculture, Cali* 189-202.
- Cuc, L.M., Huyen, L.T., Hien, P.T., Hang, V.T., Dam, N.Q., Mui, P.T., Quang, V.D., Ismail, A.M. and Ham, L.H. (2012). Application of marker-assisted backcrossing to introgress the submergence tolerance QTL *SUB1* into the Vietnam elite rice variety-AS996. *American Journal of Plant Sciences* 3(4):528-536.

- Dally, A. and Second, G. (1990). Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza*, section *Oryza*). Cladistic-mutation and genetic-distance analysis. *Theoretical and Applied Genetics* 80(2): 209-222.
- Datta, S.K. (2004). Rice Biotechnology- a need for developing countries. *AgBio Froum* 7: 31.
- Daviewwala, A.P., Reddy, A.P.K., Lagu, M.D., Ranjekar, P.K. and Gupta, V.S.(2001). Marker-assisted selection of bacterial blight resistance genes in rice. *Biochemical Genetics* 39: 261-278.
- Dawe, D., Pandey, S. and Nelson, A. (2010). Emerging trends and spatial patterns of rice production. Rice in the Global Economy: Strategic Research and Policy Issues for Food Security. Los Baños, Philippines: International Rice Research Institute (IRRI).
- Dean, R. A., Talbot, N.J., Ebbole, D.J., Farman, M.L., Mitchell, T.K., Orbach, M.J., Thon, M., Kulkarni, R., Xu, J.R., Pan, H., Read, N.D., Lee, Y.H., Carbone, I., Brown, D., Oh, Y.Y., Donofrio, N., Jeong, J.S., Soanes, D.M., Djonovic, S., Kolomiets, E., Rehmeyer, C., Li, W., Harding, M., Kim, S., Lebrun, M.H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L.J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J.E. and Birren, B.W. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434: 980-986.
- Deng, Y., Zhu, X., Shen, Y. and He, Z. (2006). Genetic characterization and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety. *Theoretical and Applied Genetics* 113(4): 705-713.
- Department of Agriculture (DOA), (2014). Annual rice production of rice in Malaysia. Kuala Lumpur, Malaysia.
- Divya, B., Robin, S., Rabindran, R., Senthil, S., Raveendran, M. and Joel, A.J. (2014). Marker assisted backcross breeding approach to improve blast resistance in Indian rice (*Oryza sativa*) variety ADT43. *Euphytica* 200(1): 61-77.
- Doi, K., Iwata, N. and Yoshimura, A. (1997). The construction of chromosome introgression lines of African (*Oryza glaberrima* Steud.) in the background of *Japonica* (*O. sativa* L.). *Rice Genetics Newsletter* 1: 39-41.
- Dokku, P., Das, K.M. and Rao, G.J.N. (2013). Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. *Euphytica* 192: 87±96.
- Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.
- Dufresne, M. and Osbourn, A.E. (2001). Definition of tissue-specific and general requirements for plant infection in a phytopathogenic fungus. *Molecular Plant Microbe Interaction* 14: 300-307.
- Ebbole, D.J. (2007). *Magnaporthe* as a model for understanding host-pathogen interactions *Annual Review of Phytopathology* 45: 437-456.
- Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* 5(6): 435-445.
- Ellingboe, A.H. and Chao, C.C.T. (1994). Genetic Interactions in *Magnaporthe grisea* that Affect Cultivar 463 Specific Avirulence/Virulence in Rice. In: Rice Blast Disease (Zeigler RS, Leong SA and 464 TengP S, eds.). CAB International, Wallingford, 51-64.
- FAOSTAT. (2015). (online) <http://faostat.fao.org/faostat> [11 May 2015].
- FAO (2014). World rice production. Food and Agriculture organization of United Nations. <http://FAOSTAT.fao.org>. Accessed on 11 December 2014.
- Farooq, S. and Azam, F. (2002). Molecular markers in plant breeding-I: Concepts and characterization. *Pakistan Journal of Biological Sciences* 5(10): 1135-1140.

- Fasahat, P., Kharidah, M., Aminah, A. and Ratnam, W. (2012). Proximate nutritional composition and 16 antioxidant properties of *Oryza rufipogon*, a wild rice collected from Malaysia compared 17 to cultivated rice, MR219. *Australian Journal of Crop Science* 6: 1502-18.
- Filippi, M.C. and Prabhu, A.S. (2001). Phenotypic virulence analysis of *Pyricularia grisea* isolates from Brazilian upland rice cultivars. *Pesquisa Agropecuária Brasileira* 36(1): 27-35.
- Fjellstrom, R., Conaway-Bormans, C.A., McClung, A.M., Marchetti, M.A., Shank, A. R. and Park, W.D. (2004). Development of DNA markers suitable for marker-assisted selection of three genes conferring Resistance to multiple pathotypes. *Crop Science* 44(5): 1790-1798.
- Fjellstrom, R., McClung, A.M. and Shank, A.R. (2006). SSR markers closely linked to the *Pi-z* locus are useful for selection of blast resistance in a broad array of rice germplasm. *Molecular Breeding* 17(2): 149-157.
- Frisch, M., Bohn, M. and Melchinger, A.E. (1999). Comparison of selection strategies for marker-assisted backcrossing of a gene. *Crop science* 39(5): 1295-1301.
- Frisch, M. and Melchinger, A.E. (2005). Selection theory for marker-assisted backcrossing. *Genetics* 170(2): 909-917.
- Fu, C., Wu, T., Liu, W., Wang, F., Li, J., Zhu, X., Zhu, X., Huang, H., Liu, Z.R., Liao, Y., Zhu, M., Chen, J. and Huang, Y. (2012). Genetic improvement of resistance to blast and bacterial blight of the elite maintainer line Rongfeng B in hybrid rice (*Oryza sativa* L.) by using marker-assisted selection. *African Journal of Biotechnology* 11(67): 13104-13114.
- Fujii, K., Hayano-Saito, Y., Shumiya, A. and Inoue, M. (1995). Genetical mapping based on the RFLP analysis for the panicle blast resistance derived from a rice parental line St. No. 1. *Breeding Science* 45(1): 209.
- Fujita, D., Ebron, L.A., Kobayashi, N. and Fukuta, Y. (2006). Comparison of DNA marker analysis of the blast resistance genes *Pi-b* and *Pi-ta* in IRRI-bred rice varieties with gene estimation by conventional genetic analysis. Development and characterization of blast resistance using differential varieties in rice. Japan International Research Centre for Agricultural Science (JIRCAS), Japan, working report No. 63.
- Fukuoka, S. and Okuno, K. (2001). QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice. *Theoretical and Applied Genetics* 103(2-3): 185-190.
- Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. and McCouch, S. (2005). Genetic structure and diversity in *Oryza sativa* L. *Genetics* 169(3): 1631-1638.
- Ghislain, M., Spooner, D.M., Rodriguez, F., Villamón, F., Nunez, J., Vásquez, C., Waugh, R. and Bonierbale, M. (2004). Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theoretical and Applied Genetics* 108(5): 881-890.
- Glaszmann, J. (1987). Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics* 74(1): 21-30.
- Gnanamanickam, S.S. (2009). "Major Diseases of Rice." Biological Control of Rice Diseases: 13-42.
- Gopalakrishnan, S., Sharma, R.K., Rajkumar, K.A., Joseph, M., Singh, V.P., Singh, A.K., Bhat, K.V., Singh, N.K. and Mohapatra, T. (2008). Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breeding* 127: 131-139.

- Goto, K., Kozaka, T., Yanagita, K., Takahshi, Y., Suzuki, H., Yamada, M., Matsumoto, S., Shindo, K., Atkins, J.G. and Robert, A.L. (1967). US-Japan cooperative research on the international pathogenic races of the rice blast fungus, *Pyricularia oryzae* Cav. and their international differentials. *Annals of the Phytopathological Society of Japan* 33: 1-87.
- Goto, I. (1970). Genetic studies on the resistance of Rice plant to the blast fungus. I. Inheritance of resistance in crosses SenshoXH-79 and Imochi-shirazu XH-79. *Annals of the Phytopathological Society of Japan* 36(5): 304-312.
- Goto, I. (1976). Genetic studies on resistance of rice plant to blast fungus. ii. difference in resistance to the blast disease between Fukunishiki and its parental cultivar, Zenith. *Annals of the Phytopathological Society of Japan*.
- Goto, I. (1988). Genetic studies on resistance of rice plant to blast fungus, 7: Blast resistance genes of Kuroka. *Annals of the Phytopathological Society of Japan (Japan)* 42(3): 253-260.
- Gowda, M., Roy-Barman, S. and Chattoo, B.B. (2006). Molecular mapping of a novel blast resistance gene *Pi38* in rice using SSLP and AFLP markers. *Plant Breeding* 125: 596±599.
- Greer, C.A. and Webster, R.K. (2001). Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California. *Plant Disease* 85: 1096±1102
- Hari, Y., Srinivasarao, K., Viraktamath, B.C., Hariprasad, A.S., Laha, G.S., Ahmed, M. I., Natarajkumar, P., Ramesha, M.S., Neeraja, C.N. and Balachandran, S. M. (2011). Marker-assisted improvement of a stable restorer line, KMR-3R and its derived hybrid KRH2 for bacterial blight resistance and grain quality. *Plant Breeding* 130(6):608-616.
- Hari, Y., Srinivasarao, K., Viraktamath, B.C., Prasad, H., Arremsetty, S., Laha, G. S., Ahmed, M.I., Natarajkumar, P., Sujatha, K., and Prasad, M.S. (2013). Marker-assisted introgression of bacterial blight and blast resistance into IR 58025B, an elite maintainer line of rice. *Plant Breeding* 132(6): 586-594.
- Harrington, S.E. (2000). A survey of genetic diversity of eight AA genome species of *Oryza* using microsatellite markers: Cornell University, Jan.
- Hayasaka, H., Miyao, A., Yano, M., Matsunaga, K. and Sasaki, T. (1996). RFLP mapping of a rice blast resistance gene *Pi-k*. *Breeding Science* 46(Suppl 2): 68.
- Hayashi, K., Hashimoto, N., Daigen, M. and Ashikawa. I. (2004). Development of PCR-based SNP markers for rice blast resistance genes at the *Pi-z* locus. *Theoretical and Applied Genetics* 108: 1212±1220.
- Hayashi, K., Yoshida, H. and Ashikawa, I. (2006). Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes. *Theoretical and Applied Genetics* 113(2): 251-260.
- Hayashi, N., Inoue, H., Kato, T., Funao, T., Shiota, M., Shimizu, T., Kanamori, H., Yamane, H., Hayano-Saito, T. and Matsumoto, T. (2010). Durable panicle blast-resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *The Plant Journal* 64(3): 498-510.
- Herzog, E. and Frisch, M. (2011). Selection strategies for marker-assisted backcrossing with high-throughput marker systems. *Theoretical and Applied Genetics* 123(2):251-260.

- Hirabayashi, H., Sato, H., Nonoue, Y., Kuno-Takemoto, Y., Takeuchi, Y., Kato, H., Nemoto, H., Ogawa, T., Yano, M. and Imbe, T. (2010). Development of introgression lines derived from *Oryza rufipogon* and *O. glumaepatula* in the genetic background of japonica cultivated rice (*O. sativa* L.) and evaluation of resistance to rice blast. *Breeding Science* 60(5): 604-612.
- Hittalmani, S., Parco, A., Mew, T., Zeigler, R. and Huang, N. (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. *Theoretical and Applied Genetics* 100(7): 1121-1128.
- Hospital, F., Chevalet, C. and Mulsant, P. (1992). Using markers in gene introgression breeding programs. *Genetics* 132:1199-1210.
- Hospital F. and Charcosset, A. (1997). Marker-assisted introgression of quantitative trait loci. *Genetics* 147(3): 1469-1485.
- Hospital, F. (2003) Marker-assisted breeding, In H.J. Newbury (ed.) Plant molecular breeding. Blackwell Publishing and CRC Press, Oxford and Boca Raton, pp.30-59.
- Hospital, F. (2005). Selection in backcross programs. *Philosophical Transactions of the Royal Society B: Biological Science* 360: 1503±1511.
- Hossain, M.B., Islam, M.O. and Hasanuzzaman, M. (2008). Influence of different nitrogen levels on the performance of four aromatic rice varieties. *International Journal of Agriculture and Biology* 10: 693-696.
- Huang, N., Angeles, E.R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J., and Khush, G.S., (1997). Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. *Theoretical and Applied Genetics* 95: 313-320.
- Huyen, L.T.N., Cuc, L.M., Ismail, A.M. and Ham, L.H. (2012). Introgression the salinity tolerance QTLs *Saltol* into AS996, the Elite rice variety of Vietnam. *American Journal of Plant Sciences* 3: 981-987.
- Iftekharaudaula, K.M. (2008). Comparison of new selection strategies or marker assisted backcrossing for a submergence tolerant gene in rice. Doctoral dissertation. Bangladesh Agriculture University, Mymensingh, Bangladesh, 1-205.
- International Rice Genome Sequencing Project (IRGSP) (2005). The map-based sequence of the rice genome. *Nature* 436(7052): 793-800.
- Inukai, T., Nelson, R., Zeigler, R., Sarkarung, S., Mackill, D., Bonman, J. and Kinoshita, T. (1996). Genetic analysis of blast resistance in tropical rice cultivars using near isogenic lines. in *Rice Genetics Iii* pp. 447-50.
- IRRI, (1996). Standard Evaluation System for Rice. 4th edn. The International Network for Genetic Evaluation of Rice, Genetic Resources Center. International Rice Research Institute, Manila, 52.
- Ise, K. (1991). Linkage analysis of some blast resistance gene in rice, *Oryza sativa* L. *Japanese Journal of Breeding* 42(Suppl 2): 388-389.
- Iwata, N. (1996). Report of the committee on gene symbolization, nomenclature and linkage groups. *Rice Genetic Newsletter* 13: 12-18.
- ,JDZDOO7DQG,ZDVDNLJO0,GHQWLFWDWLRQRID5/3 marker tightly linked to the panicle blast resistance gene, *Pb1*, in Rice. *Breeding Science* 50: 183-188.
- Jena, K. and Mackill, D. (2008). Molecular markers and their use in marker-assisted selection in rice. *Crop Science* 48(4): 1266-1276.
- Jeon, J.S., Chen, D., Yi, G.H., Wang, G. and Ronald, P. (2003). Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. *Molecular Genetics and Genomics* 269(2): 280-289.

- Jeung, J., Kim, B., Cho, Y., Han, S., Moon, H., Lee, Y. and Jena, K. (2007). A novel gene, *Pi40(t)*, linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. *Theoretical and Applied Genetics* 115(8): 1163-1177.
- Jia, Y., Wang, Z. and Singh, P. (2002). Development of Dominant Rice Blast Resistance Gene Markers. *Crop Science* 42(6): 2145-2149.
- Jia, Y., Bryan, G.T., Farrall, L. and Valent, B. (2003). Natural variation at the *Pi-ta* rice blast resistance locus. *Phytopathology* 93(11): 1452-1459.
- Jiang, N., Li, Z., Wu, J., Wang, Y., Wu, L., Wang, S. and Sun, P. (2012). Molecular mapping of the *Pi2/9* allelic gene *Pi2-2* conferring broad-spectrum resistance to *Magnaporthe oryzae* in the rice cultivar Jefferson. *Rice*, 5(1): 1-7.
- Jin, Q., Waters, D., Cordeiro, G. M., Henry, R.J. and Reinke, R.F. (2003). A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.). *Plant Science* 165(2): 359-364.
- Johnston, T.H. (1958). Registration of rice varieties. *Agron* 50: 694-700.
- Joseph, M., Gopalakrishnan, S., Sharma, R., Singh, V., Singh, A., Singh, N. and Mohapatra, T. (2004). Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Molecular breeding* 13(4): 377-387.
- Khanh, T.D., Linh, L.H., Linh, T.H., Ham, L.H. and Xuan, T.D. (2013). Rapid and high-precision marker assisted backcrossing to introgress the SUB1 QTL into the Vietnamese elite rice variety. *Journal of Plant Breeding and Crop Science*, 5(2): 26-33.
- Khush, G.S. (2005). "What it will take to feed 5.0 billion rice consumers in 2030." *Plant Molecular Biology* 59(1): 1-6.
- Khush, G.S. and Jena, K. (2009). Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.) Advances in genetics, genomics and control of rice blast disease (pp. 1-10): Springer.
- Kiyosawa, S. (1982a). Gene analysis for blast resistance. *Oryza* 18: 196-203.
- Kiyosawa, S. (1982b). Genetics and epidemiological modeling of breakdown of plant disease resistance. *Annual Review of phytopathology* 20(1): 93-117.
- Kinoshita, T. (1993). Naming and symbolization of blast resistance genes. *Rice Genetics Newsletter* 10(11).
- Kinoshita, T. and Kiyosawa, S. (1997). Some considerations on linkage relationships between *Pii* and *Piz* in the blast resistance of rice. *Rice Genetics Newsletter* 14: 57-59.
- Kiyosawa, S. (1967). Genetic studies on host-pathogen relationship in the rice blast disease. Proc. Symp. Rice Diseases and their Control by Growing Resistant Varieties and Other Measures. Tokyo, p. 137-153.
- Koide, Y., Kobayashi, N., Xu, D. and Fukuta, Y. (2009). Resistance genes and selection DNA markers for blast disease in rice. *Oryza sativa* 255-280.
- Koizumi, S. (2007). Durability of resistance to rice blast disease. A differential system for blast resistance for stable rice production environment. *JIRCAS working report* (53): 1-10.
- Korinsak, S., Sirithunya, P., Meakwatanakarn, P., Sarkerung, S., Vanavichit, A. and Toojinda, T. (2011). Changing allele frequencies associated with specific resistance genes to leaf blast in backcross introgression lines of Khao Dawk Mali 105 developed from a conventional selection program. *Field Crops Research* 122(1): 32-39.

- Kumar, M., Mishra, G.P., Singh, R., Kumar, J., Naik, P.K. and Singh, S.B. (2009). Correspondence of ISSR and RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas. *Physiology and Molecular Biology of Plants* 15(3): 225-236.
- Kwon, S.W., Cho, Y.C., Kim, Y.G., Suh, J.P., Jeung, J.U., Roh, J.H., Lee, S.K., Jeon, J.S., Yang, S.J. and Lee, Y.T. (2008). Development of near-isogenic *Japonica* rice lines with enhanced resistance to *Magnaporthe grisea*. *Molecules and cells* 25(3): 407-416.
- Latif, M., Yusop, M.R., Rahman, M.M. and Talukdar, M.B. (2011). Microsatellite and minisatellite markers based DNA fingerprinting and genetic diversity of blast and ufra resistant genotypes. *Comptes Rendus Biologies* 334(4): 282-289.
- Lei, C., Wu, j., Ling, Z., Zhaung, J., Wang, J., Zheng, K. and Wan, J. (2006). Research progress on rice blast disease and resistance breeding in China. A differential system for blast resistance for stable rice production environment. Japan International Research Centre for Agriculture Science (JIRCAS), Japan, working report No. 53.
- Lei, C.L., Huang, D.Y., Li, W., Wang, J.L., Liu, Z.L., Wang, X.T., Shi, K., Cheng, Z.J., Zhang, X. and Ling, Z.Z. (2005). Molecular mapping of a blast resistance gene in an *indica* rice cultivar Yanxian No. 1. *Rice Genetics Newsletter* 22: 76-77.
- Leung, H., Borromeo, E.S., Bernardo, M.A. and Nottoghem, J.L. (1988). Genetic analysis of virulence in the rice blast fungus *Magnaporthe grisea*. *Phytopathology* 78(9): 1227-1233.
- Leung, H., Wu, J., Liu, B., Bustaman, M., Sridhar, R., Singh, K., Redona, E., Quang, V.D., Zheng, K., Bernardo, M., Wang, G., Leach, J., Choi, I.R. and Vera Cruz, C. (2004). Sustainable disease resistance in rice: current and future strategies. In: New directions for a diverse planet". Proceedings of the 4th International Crop Science Congress, 26 Sep1±Oct 2004, Brisbane, Australia. Published on CDROM. Web site www.cropsience.org.au.
- Li, L.Y., Wang, L., Jing, J.X., Li, Z.Q., Lin, F., Huang, L.F. and Pan, Q.H. (2007). The *Pik-m* gene, conferring stable resistance to isolates of *Magnaporthe oryzae*, was finely mapped in a crossover-cold region on rice chromosome 11. *Molecular Breeding* 20(2): 179-188.
- Lin, F., Chen, S., Que, Z., Wang, L., Liu, X. and Pan, Q. (2007). The blast resistance gene *Pi37* encodes a nucleotide binding site±leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* 177(3): 1871-1880.
- Linh, L.H., Linh, T.H., Xuan, T.D., Ham, L.H., Ismail, A.M. and Khanh, T.D. (2012). Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *International Journal of Plant Genomics* 2012: 9.
- Liu, G., Lu, G., Zeng, L. and Wang, G.L. (2002). Two broad-spectrum blast resistance genes, *Pi9(t)* and *Pi2(t)*, are physically linked on rice chromosome 6. *Molecular Genetics and Genomics* 267(4): 472-480.
- Liu, D.W., Oard, S.V. and Oard, J.H. (2003). High transgene expression levels in sugarcane (*Saccharum officinarurn* L) driven by rice ubiquitin promoter RUBQ2. *Plant Science* 165: 743-750.
- Liu, X., Wang, L., Chen, S., Lin, F. and Pan, Q. (2005). Genetic and physical mapping of *Pi36(t)*, a novel rice blast resistance gene located on rice chromosome 8. *Molecular Genetics and Genomics* 274(4): 394-401.

- Liu, J., Liu, X. and Dai, L. (2007a) Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. *Journal of Genetic and Genomics* 34: 765-776.
- Liu, X., Yang, Q., Lin, F., Hua, L., Wang, C., Wang, L. and Pan, Q. (2007b). Identification and fine mapping of *Pi39(t)*, a major gene conferring the broad-spectrum resistance to *Magnaporthe oryzae*. *Molecular Genetics and Genomics* 278(4): 403-410.
- Liu, W.G., Jin, S.J., Zhu, X.Y., Wang, F., Li, J.H., Liu, Z.R. and Liu, Y.B. (2008). Improving blast resistance of a thermo-sensitive genic male sterile rice line GD-8S by molecular marker-assisted selection. *Rice Science* 15(3): 179-185.
- Lopez-Gerena, L. (2006). Mapping QTL Controlling Durable Resistance to Rice Blast in Cultivar Oryza llanos 5. Doctoral dissertation, Kansas State University, Manhattan, KS, USA.
- Mackill, D., Bonman, J., Suh, H. and Srilingam, R. (1985). Genes for resistance to Philippine isolates of the rice blast pathogen. *Rice Genetics Newsletter* 2: 80-81.
- Mackill, D. and Bonman, J. (1992). Inheritance of blast resistance in near-isogenic lines of rice. *Phytopathology* 82(7): 746-749.
- Mackill, D.J. (2007). Molecular markers and marker-assisted selection in rice. *Genomics-Assisted Crop Improvement* (pp. 147-168): Springer.
- Maclean, J.L., Dawe, D.C. and Hettel, G.P. (2002). Rice almanac: Source book for the most important economic activity on earth: International Rice Research Institute.
- McCouch, S., Kochert, G., Yu, Z., Wang, Z., Khush, G., Coffman, W. and Tanksley, S. (1988). Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* 76(6): 815-829.
- McCouch S.R., Nelson R.J., Thome, J. and Zeigler, R.S. (1994). Mapping of blast-resistance genes in rice. 167±187. doi: In: Zeigler RS, Leong SA, Teng PS (eds) Rice blast disease. CAB International, Wallingford, pp.
- McCouch, S.R., Teytelman, L., Xu, Y., Lobos, K.B., Clare, K., Walton, M. and Xing, Y. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA Research* 9(6): 199-207.
- McHale, L., Tan, X., Koehl, P., and Michelmore, R.W. (2006). Plant NBS-LRR proteins: adaptable guards. *Genome Biology* 7(4): 212.
- Melchinger, A. (1990). Use of molecular markers in breeding for oligogenic disease resistance. *Plant breeding* 104(1): 1-19.
- Miah, G., Rafii, M., Ismail, M., Puteh, A., Rahim, H., Asfaliza, R. and Latif, M. (2013a). Blast resistance in rice: a review of conventional breeding to molecular approaches. *Molecular Biology Reports* 40(3): 2369-2388.
- Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., Islam, K.N. and Latif, M.A. (2013b). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International Journal of Molecular Sciences* 14(11): 22499-22528.
- Miyamoto, M., Ando, I., Rybka, K., Kodama, O. and Kawasaki S, (1996). High resolution mapping of the *indica*-derived rice blast resistance genes. I. *Pi-b*. *Molecular Plant-Microbe Interactions* 9: 6±13.
- Mohan, M., Nair, S., Bhagwat, A., Krishna, T., Yano, M., Bhatia, C. and Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. *Molecular Breeding* 3(2): 87-103.

- Moncada, P., Martinez, C.P., Borrero, J., Châtel, M., Gauch, H., Guimaraes, E.P., Tohme, J. and McCouch, S.R. (2001). Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC₂F₂ population evaluated in an upland environment. *Theoretical and Applied Genetics* 102(1): 41-52.
- Mondal, U., Khanom, M.S.R., Hassan, L. and Begum, S.N. (2013). Foreground selection through SSRs markers for the development of salt tolerant rice variety. *Journal of Bangladesh Agriculture University* 11: 67-72.
- Monna, L., Miyao, A., Zhong, H., Yano, M., Iwamoto, M., Umehara, Y., Kurata, N., Hayasaka H. and Sasaki, T. (1997). Saturation mapping with subclones of YACs: DNA marker production targeting the rice blast disease resistance gene, *Pi-b*. *Theoretical and Applied Genetics* 94(2): 170-176.
- Morgante, M., Hanafey, M. and Powell, W. (2002). Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. *Nature Genetics* 30(2): 194-200.
- Morishima, H., Sano, Y. and Oka, H. (1984). Differentiation of perennial and annual types due to habitat conditions in the wild rice *Oryza perennis*. *Plant Systematics and Evolution* 144(2): 119-135.
- Nakamura, S., Asakawa, S., Ohmido, N., Fukui, K., Shimizu, N. and Kawasaki, S. (1997). Construction of an 800-kb contig in the near-centromeric region of the rice blast resistance gene *Pi-ta 2* using a highly representative rice BAC library. *Molecular and General Genetics* 254(6): 611-620.
- Naqvi, N.I., Bonman, J.M., Mackill, D.J., Nelson, R.J. and Chattoo, B.B. (1995). Identification of RAPD markers linked to a major blast resistance gene in rice. *Molecular Breeding* 1(4): 341-348.
- Naqvi, N.I. and Chattoo, B.B. (1996). Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice. *Genome* 39(1): 26-30.
- Narayanan, N.N., Baisakh, N., Vera Cruz, N., Gnananmanickam, S.S., Datta, K. and Datta, S.K. 2002. Molecular breeding for the development of blast and bacterial blight resistance in rice cv. IR50. *Crop Science* 42(6): 2072± 2079.
- Neeraja, C.N., Maghirang-Rodriguez, R., Pamplona, A., Heuer, S., Collard, B.C., Septiningsih, E.M., Vergara, G., Sanchez, D., Xu, K., Ismail, A.M. and Mackill, D.J. (2007). A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theoretical and Applied Genetics* 115(6): 767-776.
- Newton, A.C., Torrance, L., Holden, N., Toth, I.K., Cooke, D.E., Blok, V. and Gilroy, E.M. (2012). Climate change and defense against pathogens in plants. *Advances in Applied Microbiology* 81: 89.
- Nguyen, T., Koizumi, S., La, T.N., Zenbayashi, K.S., Ashizawa, T., Yasuda, N., Imazaki, I. and Miyasaka, A. (2006). *Pi35(t)*, a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188. *Theoretical and Applied Genetics* 113(4): 697-704.
- Okuyama, Y., Kanzaki, H., Abe, A., Yoshida, K., Tamiru, M., Saitoh, H. and Galam, D.C. (2011). A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *The Plant Journal* 66(3): 467-479.
- Oliveira, E.J., Pádua, J.G., Zucchi, M.I., Vencovsky, R. and Vieira, M.L.C. (2006). Origin, evolution and genome distribution of microsatellites. *Genetics and Molecular Biology* 29(2): 294-307.

- Ou, S. and Jennings, P. (1969). Progress in the development of disease-resistant rice. *Annual Review of Phytopathology* 7(1): 383-410.
- Ou, S., Nuque, F. and Bandong, J. (1975). Relation between qualitative and quantitative resistance to rice blast [*Pyricularia oryzae*, fungus diseases]. *Phytopathology* (USA).
- Ou, S. (1980). Pathogen variability and host resistance in rice blast disease. *Annual Review of phytopathology* 18(1): 167-187.
- Ou, S.H. (1985). Rice Diseases, 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England.
- Pan, Q., Wang, L., Ikehashi, H. and Tanisaka, T. (1996). Identification of a new blast resistance gene in the *indica* rice cultivar Kasalath using Japanese differential cultivars and isozyme markers. *Phytopathology* 86(10): 1071-1075.
- Pan, Q., Tanisaka, T. and Ikehashi, H. (1997). Studies on the genetics and breeding of blast resistance in rice VII. Gene analysis for the blast resistance of Indian native cultivar, Aus 373. *Breeding Science* 47(Suppl 1): 35.
- Pandey, M.K., Shobha Rani, N., Sundaram, R.M., Laha, G.S., Madhav, M.S., Srinivasa Rao, K., Injey Sudharshan., Yadla Hari., Varaprasad, G.S., Subba Rao, L.V., Kota Suneetha., Sivaranjani, A.K.P. and Viraktamath, B.C.(2013). Improvement of two traditional Basmati rice varieties for bacterial blight resistance and plant stature through morphological and marker-assisted selection. *Molecular Breeding* 31: 239±246.
- Paran, I. and Zamir, D. (2003). "Quantitative traits in plants: beyond the QTL." *Trends in Genetics* 19(6): 303-306.
- Parlevliet, J. and Zadoks, J. (1979). The integrated concept of disease resistance: a new view including horizontal and vertical resistance in plants. *Euphytica* 26(1): 5-21.
- Pemberton, J., Slate, J., Bancroft, D. and Barrett, J. (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Molecular Ecology* 4(2): 249-252.
- Perez, D.R., Lopez, G.E., Rivera, J., Ferreira, A. and Fontan, G.(2008). Naturally RFXUULQJ %UXWRQW WURSLQJHLN have no dominant negative effect in an Xlinked agammaglobulinaemia cellular model. *Clinical and Experimental Immunology* 152: 33-38.
- Persaud, M., Kumar, A., Sengar, R.B.S., Abhinav, S., Dantre, R.K., and Shrivastava, M.N. (2007). Genetic analysis of blast (*Pyricularia grisea* Sacc.) resistance in rice (*Oryza sativa* L.). *Journal of Biological Science* 7(1): 215-217.
- Powell, W., Machray, G.C. and Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 1(7): 215-222.
- Prabhu, A.S., Castro, E.D.M.D., Araújo, L.G.D. and Berni, R.F. (2003a). Resistance spectra of six elite breeding lines of upland rice to *Pyricularia grisea*. *Pesquisa Agropecuária Brasileira* 38(2): 203-210.
- Prabhu, M., Veeraragavathatham, D. and Srinivasa, K. (2003b). Effect of nitrogen and phosphorous on growth and yield of brinjal. *South-Indian-Horticulture*, 51: 152-156.
- Prigge, V., Maurer, H.P., Mackill, D.J., Melchinger, A.E. and Frisch, M. (2008). Comparison of the observed with the simulated distributions of the parental genome contribution in two marker-assisted backcross programs in rice. *Theoretical and Applied Genetics* 116(5): 739-744.

- Qu, S., Liu, G., Zhou, B., Bellizzi, M., Zeng, L., Dai, L., Han, B. and Wang, G.L. (2006). The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site±leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* 172(3): 1901-1914.
- Rafii, M.Y., Zakiah, M.Z., Asfaliza, R., Haifaa, I., Latif, M.I. and Malek, M.A. (2014). Grain quality performance and heritability estimation in selected F₁ rice genotypes. *Sains Malaysians* 43: 1-7.
- Ragimekula, N., Varadarajula, N.N., Mallapuram, S.P., Gangimani, G., Reddy, R.K. and Kondreddy, H.R. (2013). Marker-assisted selection in disease resistance breeding. *Journal of Plant Breeding and Genetics* 1(2): 90-109.
- Rahim, H.A. (2010). Genetic studies on blast disease (*Magnaporthe grisea*) resistance in Malaysian rice. Doctoral dissertation Universiti Kebangsaan Malaysia.
- Rahim, H.A., Zarifith, S.K., Bhuiyan., M.A.R, Narimah, M.K., Wickneswari, R., Abdullah, M.Z., Anna, L.P.K., Sobri, H., Rusli, and Khairuddin, A.R. (2012). Evaluation and characterization of advanced rice mutant line of rice (*Oryza sativa*), MR219-4 and MR219-9 under drought condition. Research and Development seminar 26-28 september 2012, 44; 1.15.
- Rahim, H.A., Bhuiyan, M.A.R., Saad, A., Azhar, M. and Wickneswari, R. (2013). Identification of virulent pathotypes causing rice blast diseases (*Magnaporthe oryzae*) and study on single nuclear gene inheritance of blast resistance in F₂ population derived from Pongsu Seribu 2 Mahshuri. *Australian Journal of Crop Science* 7(11): 1597-1605.
- Rajpurohit, D., Kumar, R., Kumar, M., Paul, P., Awasthi, A., Basha, P.O., Puri, A., Jhang, T., Singh, K. Dhaliwal, H.S. (2011). Pyramiding of two bacterial blight resistance and a semidwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* 178(1): 111-126.
- Ramkumar, G., Madhav, M.S., Rama Devi, S.J., Manimaran, P., Mohan, K.M., Prasad, M.S., Balachandran, S.M., Neeraja, C.N., Sundaram, R.M. and Viraktamath, B.C. (2014). Nucleotide diversity of *Pita*, a major blast resistance gene and identification of its minimal promoter. *Gene* 546(2): 250-256.
- Reddy, J.N., Baroidan, M.R., Bernardo, M.A., George, M.L.C. and Sridhar, R., (1997). Application of marker-assisted selection in rice for bacterial blight resistance gene, *Xa-21*. *Current Science* 73: 873±5.
- Ribaut, J.M., Jiang, C. and Hoisington, D. (2002). Simulation experiments on efficiencies of gene introgression by backcrossing. *Crop science* 42(2): 557-565.
- Ronald, P.C., Albano, B., Tabien, R., Abenes, M.L.P., Wu, K.S., McCouch, S.R. and Tanksley, S.D. (1992). Genetic and physical analysis of the rice bacterial blight disease resistance locus *Xa-21*. *Molecular General Genetic* 236: 113-120.
- RoyChowdhury, M., Jia, Y., Jia, M., Fjellstrom, R. and Cartwright, R. (2012). Identification of the rice blast resistance gene *Pib* in the national small grains collection. *Phytopathology* 102(7): 700-706.
- Sabu, K.K., Abdullah, M.Z., Lim, L.S. and Wickneswari, R. (2006). Development and evaluation of advanced backcross families of rice for agronomically important traits. *Communication in Biometry and Crop Science* 1: 111-123.
- Sakai, H., Lee, S.S., Tanaka, T., Numa, H., Kim, J., Kawahara, Y., Wakimoto, H., Yang, C., Iwamoto, M. and Abe, T. (2013). Rice annotation project database (RAP-DB): an integrative and interactive database for rice genomics. *Plant and Cell Physiology*, 54(2): e6.

- Salina, E., Dobrovolskaya, O., Efremova, T., Leonova, I. and Röüder, M. (2003). Microsatellite monitoring of recombination around the *Vrn-B1* locus of wheat during early backcross breeding. *Plant breeding* 122(2): 116-119.
- Sallaud, C., Lorieux, M., Roumen, E., Tharreau, D., Berruyer, R., Svestasrani, P., Garsmeur, O., Ghesquière, A. and Notteghem J.L. (2003). Identification of five new blast-resistance genes in highly resistant blast resistant cultivar IR-64 using a QTL mapping strategy. *Theoretical and Applied Genetics* 106: 794±803.
- Sambrook, J., Fritschi, E.F. and Maniatis, T. (1989). *Molecular Cloning: Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York.
- Sanchez, A.C., Brar, D.S., Huang, N., Li, Z. and Khush, G.S. (2000). Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Science* 40: 792±797.
- Sasaki, R. (1922). Existence of strains in rice blast fungus. *Japanese Journal of Plant Protection* 9: 631±644.
- Sasaki, T.A. and Sedoroff, R. (2003). Genome studies and molecular genetics the rice genome and comparative genomics of higher plants. *Current Opinion in Plant Biology* 6: 97-100.
- Septiningsih, E.M., Pamplona, A.M., Sanchez, D.L., Neeraja, C.N., Vergara, G.V., Heuer, S. Ismail, A.M. and Mackill, D.J. (2009). Development of submergence-tolerant rice cultivars: the *Sub1* locus and beyond. *Annals of Botany* 103(2): 151-160.
- Shang, J., Tao, Y., Chen, X., Zou, Y., Lei, C., Wang, J., Li, X., Zhao, X., Zhang, M. and Lu, Z. (2009). Identification of a new rice blast resistance gene, *Pid3*, by genomewide comparison of paired nucleotide-binding site±leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* 182(4): 1303-1311.
- Shanti, M., Shenoy, V., Devi, G.L., Kumar, V.M., Premalatha, P., Kumar, G.N., Shashidar, H.E., Zehr, U.B. and Freeman, W.H. (2010). Marker-assisted breeding for resistance to bacterial leaf blight in popular cultivar and parental lines of hybrid rice. *Journal of Plant Pathology* 92: 495-501.
- Shapiro, S.S. and Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52: 591-611.
- Sharma, T.R., Chauhan, R.S., Singh, B.M., Paul, R., Sagar, V. and Rathore, R. (2002). RAPD and pathotype analysis of *Magnaporthe grisea* population from North-western Himalayan region of India. *Phytopathology* 150: 649±656.
- Sharma, T., Madhav, M., Singh, B., Shanker, P., Jana, T., Dalal, V. and Upreti, H. (2005). High-resolution mapping, cloning and molecular characterization of the *Pi-kh* gene of rice, which confers resistance to *Magnaporthe grisea*. *Molecular Genetics and Genomics* 274(6): 569-578.
- Sharma, R., Shrestha, S. and Pandey, M. (2007). Inheritance of blast resistance and DVVRFLDWHG PLFURVDWPOOLWH PDUNHUV LQ ULEH EXOWLYDU μ/D[PL. *phytopathology* 155(11-12): 749-753.
- Sharma, O. and Bambawale, O. (2008). Integrated management of key diseases of cotton and rice. In: *Integrated Management of Diseases Caused by Fungi, Phytoplasm ad Bacteria*, pp: 271±302. Ciancio, A. and K.G. Mukerji (eds.). Springer, Dordrecht
- Sharma, T., Rai, A., Gupta, S., Vijayan, J., Devanna, B. and Ray, S. (2012). Rice blast management through host-plant resistance: retrospect and prospects. *Agricultural Research* 1(1): 37-52.

- Shi, C., Zhu, J., Wu, J. and Fan, L. (2000). Genetic and genotype×environment interaction effects from embryo, endosperm, cytoplasm and maternal plant for rice grain shape traits of indica rice. *Field Crops Research* 68(3): 191-198.
- Shinoda, H., Toriyama, K., Yunoki, T., Ezuka, A. and Sakurai, Y. (1971). Studies on the varietal resistance of rice to blast. 6. Linkage relationship of blast resistance genes. *Jap Chugoku Nogyo Shikengo Fukuyama Bull Ser A*.
- Shu, Q.Y. (2009). Induced plant mutations in the genomics era. Proceedings of an International Joint FAO/IAEA Symposium, Vienna, Austria, 2008. Paper presented at the Induced plant mutations in the genomics era. Proceedings of an International Joint FAO/IAEA Symposium, Vienna, Austria, 2008.
- Silué, D., Notteghem, J.L. and Tharreau, D. (1992). Evidence of a gene-for-gene relationship in the *Oryza sativa Magnaporthe grisea* pathosystem. *Phytopathology* 82(5): 577-580.
- Singh, S., Sidhu, J. S., Huang, N., Vikal, Y., Li, Z., Brar, D.S., Dhaliwal, H.S. and Khush, G.S. (2001). Pyramiding three bacterial blight resistance genes (*xa5*, *xa13* and *Xa21*) using marker-assisted selection into *indica* rice cultivar PR106. *Theoretical and Applied Genetics* 102(6-7): 1011-1015.
- Singh, A., Singh, V.K., Singh, S., Pandian, R., Ellur, R.K., Singh, D., Bhowmick, P.K., Krishnan, S.G., Nagarajan, M. and Vinod, K. (2012a). Molecular breeding for the development of multiple disease resistance in Basmati rice. *AoB plant*, 2012: pls029.
- Singh, V. K., Singh, A., Singh, S., Ellur, R. K., Choudhary, V., Sarkel, S., Singh, D., Krishnan, S.G., Nagarajan, M. and Vinod, K. (2012b). Incorporation of blast UHVLVWDQFHLQWR 355 DQ HOLWH %DVPDWL ULFH UHVWRUHU OLQH WKURXJ assisted backcross breeding. *Field Crops Research* 128: 8-16.
- Singh, V.K., Singh, A., Singh, S.P., Ellur, R.K., Singh, D., Krishnan, S.G., Bhowmick, P.K., Nagarajan, M., Vinod, K.K. and Singh, U.D. (2013). Marker-assisted simultaneous but stepwise backcross breeding for pyramiding blast resistance genes *Piz5* and *Pi54* LQWR DQ HOLWH %DVPDWL ULFH UHVWRUHU OLQH μ355¶ *Breeding* 132(5), 486-495.
- Singh, K.G. (1971). Transmission studies on orange leaf virus disease of rice in Malaysia. *Malaysian Agriculture Journal* 48: 122-132.
- Sirithunya, P., Tragoonrun, S., Vanavichit, A., Pain, N., Vongsaprom, C. and Toojinda, T. (2002). Quantitative trait loci associated with leaf and neck blast resistance in recombinant inbred line population of rice (*Oryza sativa*). *DNA research* 9(3): 79-88.
- Sreewongchai, T., Toojinda, T., Thanintorn, N., Kosawang, C., Vanavichit, A., Tharreau, D. and Sirithunya, P. (2010). Development of elite *indica* rice lines with wide spectrum of resistance to Thai blast isolates by pyramiding multiple resistance QTLs. *Plant Breeding* 129(2): 176-180.
- Srinivasachary, H.S., Shivayogi, S., Vaishali, M.G., Shashidar, H.E. and Kumar, K.G. (2002). Genetic analysis of rice blast fungus of souther Karnataka using DNA marker and reaction of popular rice genotypes. *Current Science* 82(6): 732-735.
- Steele, K.A., Price, A.H., Shashidhar, H.E. and Witcombe, J.R. (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theoretical and Applied Genetics* 112(2): 208-221.
- Sundaram, R.M., Vishnupriya, M.R., Biradar, S.K., Laha, G.S., Reddy, G.A., Rani, N. S., Sarma, N.P. and Sonti, R.V. (2008). Marker assisted introgression of bacterial blight resistance in Samba Mahsuri, an elite *indica* rice variety. *Euphytica* 160(3): 411-422.

- Sundaram, R.M., Priya, M.R.V., Laha, G.S., Shobha Rani, N., Srinivasa Rao, P., Balachandran, S.M., Ashok Reddy, G., Sarma, N.P. and Sonti, R.V. (2009). Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety by molecular marker assisted breeding. *Biotechnology Journal* 4: 400-407.
- Tabien, R., Li, Z., Paterson, A., Marchetti, M., Stansel, J. and Pinson, S. (2002). Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theoretical and Applied Genetics* 105(2-3): 313-324.
- Takeuchi, Y., Ebitani, T., Yamamoto, T., Sato, H., Ohta, H., Hirabayashi, H., Kato, H., Ando, I., Nemoto, H. and Imbe, T. (2006). Development of isogenic lines of rice cultivar Koshihikari with early and late heading by marker-assisted selection. *Breeding Science* 56(4): 405-413.
- Takken, F.L.W., Albrecht, M. and Tameling, W.I.L. (2006). Resistance proteins: molecular switches of plant defense. *Current Opinion in Plant Biology* 9: 383±390.
- Talbot, N.J. (1995). Having a blast: exploring the pathogenicity of *Magnaporthe grisea*. *Trends in Microbiology* 3(1): 9-16.
- Talbot, N.J. (2003). On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology* 57: 177-202.
- Tanksley, S.D. (1983). Molecular markers in plant breeding. *Plant Molecular Biology Reporter* 1(1): 3-8.
- Tanksley, S., Young, N., Paterson, A. and Bonierbale, M. (1989). RFLP mapping in plant breeding: new tools for an old science. *Nature Biotechnology* 7(3): 257-264.
- Tanweer, F.A., Rafii, M.Y., Sijam, K., Rahim, H.A., Ahmed, F. and Latif, M.A. (2015). Current advance methods for the identification of blast resistance genes in rice. *Comptes rendus biologiques* 338(5): 321-334.
- Tanweer, F.A., Rafii, M.Y., Sijam, K., Rahim, H.A., Ahmed, F., Ashkani, S., and Latif, M.A. (2015). Identification of suitable segregating SSR markers for blast resistance in rice using inheritance and disease reaction analysis in backcross families. *Australasian Plant Pathology*, 44(6): 619-627.
- Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic acids research* 17(16): 6463-6471.
- Temnykh, S., Park, W.D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y.G., Ishii, T. and McCouch, S.R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 100(5): 697-712.
- Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S. and McCouch, S. (2001). Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. *Genome Research* 11(8): 1441-1452.
- Teng, P.S. (1994). The epidemiological basis for blast management. In: Zeigler, R. S. et al. (eds.), *Rice Blast Disease*. CAB International.
- Tian, F., Li, D.J., Fu, Q., Zhu, Z.F., Fu, Y.C., Wang, X.K. and Sun, C.Q. (2006). Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. *Theoretical and Applied Genetics* 112(3): 570-580.

- ~~Wang, C.~~ (2003). Advances in plant biotechnology and its adoption in developing countries. *Current Opinion in Plant Biology* 6: 191-198.
- Totad, A.S., Fakrudin, B. and Kuruvinashetti, M.S. (2005). Isolation and characterization of resistance gene analogs (RGAs) from sorghum (*Sorghum bicolor* L. Moench). *Euphytica* 143: 179-188.
- USDA, N. (2007). The PLANTS Database (<http://plants.usda.gov>). National Plant Data Center, Baton Rouge: La.
- Valent, B., Farrall, L. and Chumley, F.G. (1991). *Magnaporthe grisea* genes for pathogenicity and virulence identified through a series of backcrosses. *Genetics* 127(1): 87-101.
- Valent, B. (1997). The rice blast fungus, *Magnaporthe grisea*. In Carroll/Tudzynski (Eds.). *The Mycota V Part B. Plant Relationships*, Berlin Heidelberg: Springer-Verlag. pp. 37-54.
- Van Berloo, R. (2008). GGT 2.0: Verstile software for visualization and analysis of genetic data. *Journal of Heredity* 99: 223-236.
- Vaughan, D.A., Lu, B.R. and Tomooka, N. (2008). The evolving story of rice evolution. *Plant Science* 174(4): 394-408.
- Visscher, P. (1996a). Proportion of the variation in genetic composition in backcrossing programs explained by genetic markers. *Journal of Heredity* 87(2): 136-138.
- Visscher, P.M., Haley, C.S. and Thompson, R. (1996b). Marker-assisted introgression in backcross breeding programs. *Genetics*, 144(4): 1923-1932.
- Wang, G.L., Mackill, D.J., Bonman, J.M., McCouch, S.R., Champoux, M.C. and Nelson, R.J. (1994). RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* 136(4): 1421-1434.
- Wang, Z.X., Yano, M., Yamanouchi, U., Iwamoto, M., Monna, L., Hayasaka, H., Katayosi, Y. and Sasaki, T. (1999). The *Pi-b* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *The Plant Journal* 19(1): 55-64.
- Wang, X., Jia, Y., Shu, Q., and Wu, D. (2008) Haplotype diversity at the *Pi-ta* locus in cultivated rice and its wild relatives. *Phytopathology* 98: 1305-1311.
- Wang, L., Xu, X., Lin, F. and Pan, Q. (2009). Characterization of rice blast resistance genes in the *Pik* cluster and fine mapping of the *Pik-p* locus. *Phytopathology* 99(8):900-905.
- Ware, D., Jaiswal, P., Ni, J., Pan, X., Chang, K., Clark, K. and Cartinhour, S. (2002). Gramene: a resource for comparative grass genomics. *Nucleic Acids Research* 30(1): 103-105.
- Wen, S. and Gao, B. (2012). Introgressing Blast Resistant Gene *Pi-9(t)* into Elite Rice Restorer Luhui17 by Marker-Assisted Selection, *Rice Genomics and Genetics* 2(4): 31-36.
- Wilson, R.A. and Talbot, N.J. (2009). Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nature Reviews Microbiology* 7(3): 185-195.
- Wu, K.S. and Tanksley, S.D. (1993). Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular and General Genetics* 241(1-2): 225-235.
- Wu, K., Martinez, C., Lentini, Z., Tohme, J., Chumley, F., Scolnik, P. and Valent, B. (1996). Cloning a blast resistance gene by chromosome walking. *Rice Genetics*, 3: 669-674.

- Wu, J.L., Fan, Y.Y., Li, D.B., Zheng, K.L., Leung, H. and Zhuang, J.Y. (2005). Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates. *Theoretical and Applied Genetics* 111(1): 50-56.
- Xu, Y. and Crouch, J.H. (2008). Marker-assisted selection in plant breeding: from publications to practice. *Crop science* 48(2): 391-407.
- Yano M, and Sasaki T. (1997). Genetic and molecular dissection of quantitative traits in rice. *Plant Molecular Biology* 35:145-153.
- Yang, J.H., Liu, H. X., Zhu, G.M., Pan, Y.L., Xu, L.P. and Guo, J.H. (2008). Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. *Journal of Applied Microbiology* 104(1): 91-104.
- Yang, Q., Lin, F., Feng, S., Wang, L. and Pan, Q. (2009). Recent progress on molecular mapping and cloning of Blast resistance genes in rice (*Oryza sativa* L.). *Scientia Agricultura Sinica* 42: 1601±1615.
- Yokoo, M. and Fujimaki, H. (1971). Tight linkage of blast-resistance with late maturity observed in different *indica* varieties of rice. *Japanese Journal of Breeding* 21(1): 35-39.
- Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S., Abenes, M.L., Baraoidan, M.R., Mew, T.W. and Nelson, R.J. (1995a). Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Molecular Breeding* 1(4):375-387.
- Yoshimura, S., Yoshimura, A., Nelson, R.J., Mew, T.W. and Iwata, N., (1995b). Tagging *Xa-1*, the bacterial blight resistance gene in rice, by using RAPD markers. *Japanese Journal of Breeding* 45: 81-85.
- Young, N. and Tanksley, S. (1989). RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. *Theoretical and Applied Genetics* 77(3): 353-359.
- Yu, Z., Mackill, D. and Bonman, J. (1987). Inheritance of resistance to blast in some traditional and improved rice cultivars. *Phytopathology* (USA).
- Yu, Z., Mackill, D., Bonman, J. and Tanksley, S. (1991). Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theoretical and Applied Genetics* 81(4): 471-476.
- Yunoki, T., Ezuka, A., Morinaka, T. and Sakurai, Y. (1970). Studies on the varietal resistance to Rice blast. 4. Variation of field resistance due to fungus strains. Bulletin of the Chugoku Agricultural Experiment Station A(6): 21-41.
- Zenbayashi, K., Ashizawa, T., Tani, T. and Koizumi, S. (2002). Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. *Theoretical and Applied Genetics* 104(4): 547-552.
- Zenbayashi-Sawata, K., Ashizawa, T. and Koizumi, S. (2005). *Pi34-AVRPi34*: a new gene-for-gene interaction for partial resistance in rice to blast caused by *Magnaporthe grisea*. *Journal of General Plant Pathology* 71(6): 395-401.
- Zhan X.D., Zhou H.P., Chai R.Y., Zhuang J.Y., Cheng S.H. and Cao L.Y. (2012). Breeding of R8012, a rice restorer line resistant to blast and bacterial blight through marker-assisted selection. *Rice Science* 19(1): 29-35.
- Zhang, J., Li, X., Jiang, G., Xu, Y. and He, Y. (2006). Pyramiding of *Xa7* and *Xa21* for the improvement of disease resistance to bacterial blight in hybrid rice. *Plant Breeding* 125(6): 600±605.
- Zhang, Z., Deng, Y., Tan, J., Hu, S., Yu, J. and Xue, Q. (2007). A genome-wide microsatellite polymorphism database for the *indica* and *japonica* rice. *DNA Research* 14(1): 37-45.

- Zhao, F., Cai, Z., Hu, T., Yao, H., Wang, L., Dong, N., Wang, B., Ru, Z. and Zhai, W. (2010). Genetic analysis and molecular mapping of a novel gene conferring resistance to rice stripe virus. *Plant Molecular Biology Reporter* 28(3): 512-518.
- Zhou, P., Tan, Y., He, Y., Xu, C. and Zhang, Q. (2003). Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. *Theoretical and Applied Genetics* 106(2): 326-331.
- Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Leung, H. and Leung, H. (2000). Genetic diversity and disease control in rice. *Nature* 406(6797): 718-722.
- Zhu, M., Wang, L. and Pan Q. (2004a). Identification and characterization of a new blast resistance gene located on rice chromosome 1 through linkage and differential analyses. *Phytopathology* 94: 515±519.
- Zhu, X., Yang, Q., Yang, J., Lei, C., Wang, J. and Ling, Z. (2004b). Differentiation ability of monogenic lines to *Magnaporthe grisea* in *indica* rice. *Acta Phytopathologica Sinica* 34(4): 361-368.
- Zhuang, J.Y., Ma, W.B., Wu, J.L., Chai, R.Y., Lu, J., Fan, Y.Y., Jin, M.Z., Lueng, H. and Zheng, K.L. (2002). Mapping of leaf and neck blast resistance genes with resistance gene analog, RAPD and RFLP in rice. *Euphytica* 128(3): 363-370.
- Zou, J., Pan, X., Chen, Z., Xu, J., Lu, J., Zhai, W. and Zhu, L. (2000). Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*Oryza sativa* L.). *Theoretical and Applied Genetics* 101(4): 569-573.
- Zuraida, A., Zulkifli, A., Habibuddin, H. and Naziah, B. (2012). Regeneration of Malaysian rice variety MR 219 via somatic embryogenesis. *Journal of Tropical Agriculture and Food Science* 39(2): 167-177.