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GENETIC MAPS AND MARKER ASSISTED SELECTION FOR MAJOR-GENE TRAITS IN RICE

A. W. BALOCH, A.A. RIND AND K. D. JAMALI

Nuclear Institute of Agriculture (NIA), Tando Jam, Sindh, Pakistan

ABSTRACT

Rice is an important cereal crop of Asian countries. It is a major staple food crop Southeast and eastern countries of Asian sub-continent. Knowledge on the genetic constitution of rice plant has immense importance in Plant breeding Programmes. The use of molecular markers has facilitated the selection process. Markers-assisted selection has provided a reliable source for identifying and selecting the desirable genotype in plant breeding programmes. Molecular marker can save time and labour. It is a laborious job to grow a large number of F_2 populations and practice selection for morphological markers in conventional plant breeding. Molecular markers will be more useful for selection when (1) the phenotype is difficult or expensive to measure directly, (2) genes of similar phenotype are being pyramided into a single line, or (3) markers are being used to select against the donor genome in a backcrossing programme.

INTRODUCTION:

Rice is one of the major grain crops in Pakistan. It covers an area of 2.12 m ha and producing 3.88 million tones of paddy. In Sindh province it covers an area of 0.46 m ha and producing 1.16 tones of paddy. Average yield (Kg/ha) in Pakistan is 1836 kg/ha while in Sindh province is 2514 kg/ha, which is quite low as compared to world average of 2830 kg/ha.

The existing semi-dwarf high yielding varieties like IR8, Shua-92 and Sarshar have many positive characteristics such as high yielding capacity and good plant type but they are lacking in early maturity thus affects the cultivation of paddy in rice-wheat cropping system of the country. Another problem is salinity, which is increasing with pace of time period. The marker-assisted selection would help the breeders to select the desirable genotypes such as early flowering, salinity, drought and biotic stress tolerance and could improve the rice-breeding programme in Pakistan. The aim of this review is to provide some general information to the rice breeders to adopt molecular marker techniques for rice improvement in the country. The first restriction fragment length polymorphism (RFLP) map of rice was constructed in 1980s at Cornell University (McCouch *et al* 1988) and high-density maps were subsequently developed (Causse *et al* 1994, Kurata *et al* 1994). Rice geneticists have now widely applied these map-to-map genes controlling qualitatively and quantitatively inherited traits of economic importance (Mackill 1999).

Quantitative vs qualitative traits: Plant traits can generally be classified into two categories as qualitative or quantitative. In the former, the phenotype of plants in segregating population can be classified easily into discrete classes, whereas, in the latter, the data usually show continuous variation. One or two major genes segregating in Mendelian fashion control qualitative traits. Quantitative traits are usually controlled by several genes with smaller effects that result in a continuous variation in segregating populations. These traits are typically sensitive to environmental factors, and the

genetic effects are diluted by this environmental variation and by the interaction between the genotype x environment.

In practice, there is a range of situations between the two extremes (Table 1). For example, some major genes are responsible for semi quantitative traits that segregate in a more or less discrete fashion. A common example is the semi dwarfing gene such as *sd1* and the photoperiod sensitivity gene *Se1*. In some cases, major genes can be responsible for quantitative traits showing continuous segregation, an example is the submergence tolerance gene *sub1* in the mapping of quantitative trait loci (QTL), the percent of the phenotypic variation under the control of each locus is generally used to assess the effects of the specific locus on the trait. For the purposes of this discussion, we designate QTL responsible for more than 50% of the phenotypic variation as "major genes," whereas loci controlling 25-50% of the variation would be "major QTL." For any particular QTL, the percent of the phenotype explained value would vary depending on the environment under which the trait is measured and the genetic population used.

Major-gene traits mapped in rice

Since the first RFLP map in rice was constructed (McCouch *et al* 1988), hundreds of genes and QTL have been mapped. The QTLs have been discussed in a number of previous reviews (McCouch and Doerge 1995, Yano and Sasaki 1997). The following major genes have been tagged and listed in below (Table 2). Approximately 50% of the tagged genes are resistant to biotic stresses. The tagged genes will be helpful for marker-assisted selections for economic important characters (Young and Tanksley 1989). Table 2. Tagging and mapping of some major genes in rice using molecular markers Application of markers in breeding and genetics

There are three major applications of molecular markers in rice breeding and genetics, which are (1) identifying allelism of genes that confer identical phenotypes (for example, blast resistance genes identified in different countries with different isolates), (2) use in marker-assisted selection (MAS) or marker-assisted backcrossing (MAB) programmes, and (3) use in positional cloning of genes. This paper focuses on their use in selection programmes.

Advantages of MAS or MAB

Markers assisted selections will be helpful under the conditions such as (1) the trait is difficult or expensive to score, (2) several genes are being selected that confer similar phenotypes, or (3) background markers are being used to select against genes from the donor parent.

The benefit of MAS is that selections could be made without phenotypic expression, but on the basis of DNA markers for desired character. In MAS selections could be done in a juvenile stage without environmental effects (Liu et al 2002). Several traits, although being controlled by a single gene, are quite laborious to measure. Most traits that fall under this category are considered QTL that affect agronomic traits. Some types of disease resistance are quantitative or partial in nature and require replicated testing to measure accurately. Examples include resistance to blast (Wang *et al* 1994) and stem rot (Ni *et al* 2001).

Traits that can only be measured after the reproductive stage would be good candidates for marker-assisted selection. For example, amylose content is currently measured after harvest using chemical methods or sophisticated equipment. A microsatellite repeat that is part of the *Wx* gene (Ayres *et al* 1997) can be effective in selecting for this trait. PCR-based markers proved 85% accurate for identifying the thermosensitive male

sterility gene *tms3* in the juvenile stage (Lang *et al* 1999). The ultimate example of this concept is genes that control traits that can only be observed in the progeny of testcrosses of individual plants. Two examples are the wide compatibility allele $S5_n$ (Liu *et al* 1997) and restorer genes for cytoplasmic male sterility (Akagi *et al* 1996, Ichikawa *et al* 1997, Yao *et al* 1997). The ability to identify such genes at the seedling stage during a backcrossing program would offer a remarkable savings in time and effort to transfer these genes into a specific genetic background.

Pyramiding multiple genes. Gene pyramiding is considered a viable approach to attaining durable resistance to rice diseases or insect pests. Different resistance genes often confer resistance to different isolates, races, or biotypes. Combining these resistances, the number of races or biotypes that a variety can resist, and there is evidence that multiple resistance genes make it more difficult for virulent races to evolve. Furthermore, combining major-gene and minor-gene resistance may lead to increased durability (Wang *et al* 1994). When partial resistance is present in breeding lines, scoring for major-gene resistance can also be more difficult (Kelly and Miklas 1998).

Hittalmani *et al* (2000) used marker-assisted selection to combine three blast resistance genes, *Pil* on chromosome 11, *Piz-5* on chromosome 6, and *Pita* on chromosome 12, in a single genotype. For *Piz-5*, a single marker was used, whereas flanking markers were used for the other two. The authors confirmed that the markers were efficient in developing gene pyramids and that the line containing all three resistance genes had a broader resistance spectrum than lines with individual genes.

Markers have been used to pyramid several bacterial blight resistance genes. Huang *et al* (1997) pyramided four resistance genes, *Xa4, xa5, xa13,* and *Xa21,* using PCR- based markers. Sanchez *et al* (2000) transferred three bacterial blight resistance genes into three susceptible rice lines possessing desirable agronomic charac-teristics. Two RFLP markers, linked to the recessive gene *xa5* were converted to sequence tagged site (STS) markers based on their DNA sequences. The RFLP marker RG136 was converted to an STS marker for selection for the resistance gene *xa13.* The sequence of the genomic clone RAPAD248 was used to develop an STS marker for *Xa21.* This work showed the effectiveness of using markers linked to recessive genes in a backcrossing program, particularly in the presence of a dominant resistance gene. In an F_2 population, selection efficiency was as high as 95% for *xa5* and 96% for *xa13.*

Selecting against the donor genotype during backcrossing. To expand the number of genes available for rice breeding, exotic cultivars or even wild species with advantageous traits are being employed as parents more frequently in breeding programs. Unfortunately, these new genes are often associated with alleles from the donor. High-density molecular maps and the graphical genotype (Young and Tanksley 1989) can be used to determine the genotype of different progenies throughout the entire genome and make it possible to select the individuals with the most genetic constitution from the progenies of backcrosses.

There are two ways to use markers to assist in backcrossing a gene into a recurrent parent: (1) use markers to select for the recurrent parent markers on non-carrier chromosomes and (2) use markers to select against markers linked to the locus of interest to avoid linkage drag. When linkage drag is not a problem, selection against non-linked markers can be performed easily. This procedure has been shown in simulation studies to

reduce the number of backcrosses from six to three to transfer a gene of interest (Frisch *et al* 1999a). In most cases, even after six backcrosses, the size of the introgressed chromosomal segment can be quite large (Stam and Zeven 1981). Selecting for recombination on either side of the target gene requires a marker that cosegregates with the gene and two markers that flank the gene on either side. After the first backcross, BC_1F_1 plant recombinant for the gene and one of the flanking markers are selected. In the BC_2F_2 plants, recombinants for the other flanking marker are selected. The genetic distance between the flanking markers and the gene will determine the population size necessary to obtain the desirable recombinant. As the genetic distance decreases, the number of BC F_1 plants needed becomes prohibitively large (Frisch *et al* 1999b). For example, if the flanking markers are 5 cM from the target gene, 100 Bc_nF_1 individuals would need to be assayed to obtain a recombinant between the target gene and one of the markers at the 0.99 level of probability. If one of the flanking markers is homozygous for the recurrent parent allele, the number rises to 192 for obtaining a recombinant with the other flanking marker (Frisch *et al* 1999b).

Practical considerations in marker-assisted selection: Molecular marker technology has immense importance in plant breeding programs nowadays, however, several factors should be considered while conducting the molecular marker studies.

Choice of molecular markers: Application of markers in selection schemes depends on identifying a closely linked marker or flanking markers near the gene or genes of interest. The suitability of any particular marker would depend on several factors:

1. **DNA quality and quantity required:** Markers such as RFLP, RAPD, and AFLP require high-quality DNA, and are more laborious intensive. On comparison with PCR-based methods, RFLPs need a large quantity of DNA.
2. **Difficulty of assay:** RFLP markers are the most laborious, requiring preparation of filters and Southern blots and development of film. AFLP markers do not require blotting, but the assay is relatively laborious, requires higher skills, and must be detected on polyacrylamide gels. Simple PCR-based markers such as RAPD, microsatellite, and CAPS (cleaved amplified polymorphic sequence) or STS are the simplest to detect, although microsatellite markers often require polyacrylamide gels.
3. **Degree of polymorphism:** Ideally, a marker should be useful within the germplasm pool to be used by breeders for crossing purpose. Thus, although RFLP and AFLP markers are generally suitable for indica-japonica crosses, they are not highly polymorphic within a subspecies. Microsatellite markers are by far the most polymorphic and can be used within subspecies.
4. **Reliability:** RAPD markers have the reputation of being the most unreliable, although they are still being used because of their simplicity. The other types of markers tend to be more reliable; however, none of them could be called to be error-free.

Many new types of markers are becoming available. As the rice genome sequence is completed, markers based on specific sequence differences, such as CAPS and SNPs (single nucleotide polymorphisms) are likely to become the markers of choice. Now, microsatellite markers are certainly the best choice for most purposes. These markers are highly polymorphic, reliable, and abundantly available (see McCouch *et al* 1988). Those SSRs that can be scored using agrose gels would be particularly useful in

marker-assisted breeding (Gupta *et al* 1999).

False-positives in MAS: If the distance between the linked marker and the target gene is not small enough, a crossover will result in false-positives during MAS. Another reason for the false-positive screening is incorrect results of gene mapping. Fine mapping or high-resolution mapping of the gene and the discovery of the more closely linked markers using larger populations will reduce the occurrence of false-positives. Furthermore, phenotypic evaluation should be performed with more reliable methods, with multiple replications, and under different environments in order to locate genes more precisely. New efficient gene / QTL mapping strategies and quantitative genetic analysis methods should also be proposed and adopted.

Expense of MAS: The relatively high expense is another factor limiting the development and application of MAS. The expense include not only the materials and supplies but also less definable costs such as quality of technical support, lab. space, and radioisotope permits
(Mohan *et al* 1997a). However, advances in technologies would result in a decrease in the costs of MAS. PCR-based markers such as microsatellites and AFLP are amenable to automation. In addition, DNA extraction methods have been improved. Not only have rapid DNA extraction methods for rice been developed (Williams and Ronald 1994, Zheng *et al* 1995, Lange *et al* 1998), but it is also possible to isolate DNA directly from the seeds before sowing (Chunwongse *et al* 1993). With these developments, DNA marker technology without electrophoresis should come into use in the future.

Restriction of number of genes in the screening programme: The number of genes (loci) involved in the MAS program is another factor that should be considered. For example, with only four or five loci being selected, the population size and number of F_1 seeds needed for a MAS programme will be considerable and any further addition will lead to an exponential increase (Mackill *et al* 1999). This indicates that only the most important traits or loci should be identified and selected in a MAS programme. More importantly, marker-assisted selection should be considered as a complement to conventional breeding rather than a replacement for it.

Conclusions:

Molecular marker technology has immense importance in rice breeding programme. The technology has lead cloning of very important genes. The challenging task is to integrate both the molecular markers and conventional breeding methods would be an important aspect in rice breeding program.

The molecular marker breeding programs will map the major genes and also help in sequencing the DNA. Map based cloning of genes is difficult task but with the availability of complete genome sequencing of rice it will be much easier. The molecular marker technology cannot replace the plant breeding certainly but it will help the plant breeders by providing new tools to ease the many problems faced by plant breeders.

Table–1: Classification of major and minor gene traits.

Trait	Segregation	Percent of Phenotypic variation explained	Examples	Classification
Qualitative	Discrete	100	Purple leaf, blast resistance	Major gene
Semi-quantitative	Discrete	100	Semi-dwarfism, *sd1*	Major gene
Quantitative	Continuous	>50	Submergence tolerance gene, *Sub1*	Major gene
Quantitative	Continuous	25-50	Stem rot resistance	Major QTL
Quantitative	Continuous	<25	QTL for most agronomic and Physiological traits	QTL

Table-2:Tagging and mapping of some major genes in rice using molecular markers

Trait	Gene	Chromosome	Linked markers	Reference
1. Grain quality				
Grain aroma				
Fgr	8	RG28	Ahn et al (1992)	
Cooked-kernel elongation				
KNE		8	RZ323	Ahn et al (1993)
Amylose				
Wx		6	WX	Wang et al (1992)
2. Abiotic Stresses				
Submergence tolerance				
Sub1		9	RZ698	Xu and Mackill (1996)
Salt tolerance				
Salt		7	RG4	Zhang et al (1995)
Osa3		12	RG457/Y12817R	Zhang et al (1999)
3. Heterosis and wide compatibility				
tgms1.2		8	RZ562	Wang et al (1995)
Tms2		7	R643A/R1440	Yamaguchi et al (1997)
Tms3		6	RZ144	Subudhi et al (1997)
Tgms		9	RM257	Reddy et al (2000)
Tgms-vn1 (tms4)		2	RM2	Dong et al (2000)
Pms1		7	RG477	Zhang et al (1994)
Pms2		3	RG191	Zhang et al

			(1994)
Pms3	12	C751/RZ261	Mei et al (1999)
Ms-h(t)	9	RG451/RZ404	Koh et al (1999)
Rf-1	10	OSRRF	Akagi et al (1996)
Rf?	10	C1361	Tan et al (1998)
Rf-2	2	CD0686/RZ58	Yang et al (1997)
Rf3	1	RG532	Zhang et al (1997)
Rf5	1	RG374/RG394	Shen et al (1998)
Rfu	10	C4003	Yao et al (1997)
Rf?	10	RM258	Huang et al (1999)
Hybrid breakdown			
Hwd1	10	C701/R2309	Fukuoka et al (1998)
Hwd2	7	C796B/R1382/C492/C145	Fukuoka et al (1998)
Wide compatibility			
S5	6	RG213	Yanagihara et al (1995)
4. Insect resistance			
Gall Midge			
Gm2	4	RG329	Mohan et al (1994)
Gm4(t)	8	R1813	Mohan et al (1997b)
Brown Planthopper			
Bph1	12	XNpb248	Hirabayashi and Ogawa (1995)
Bph10	12	RG457	Ishii et al (1994)
Bph(t)	9	RZ404/UCH170	Mei et al (1996)
Green leafhopper			
GLH	4	RZ262	Sebastian et al (1996)
Grip3	3	XNpb144	Fukuta et al (1998)
Grip11	11	G1465	Fukuta et al (1998)
Grh1	5	R566	Tamura et al (1999)
Whitebacked planthopper			

WBPH	6	R1954/L668	Yamasaki et al (1999)
WBPH	11	RG103/RG167	Kadirvel et al (1999)
5.Disease Resistance			
Blast			
Pi1	11	RZ536	Yu et al (1996)
Pi2	6	RG64	Yu et al (1991)
Pi4	12	RG869	Yu et al (1991)
Pi5	4	RZ788	Wang et al (1994)
Pi7	11	RG16	Wang et al (1994)
Pi10	5	RG13	Naqvi et al (1995)
Pi11	8	RG181IB/BP127	Zhu et al (1993)
Pi12	12	RG869/RG81/RZ397	Zheng et al (1996)
Pi18	11	RZ536	Ahn et al (1996)
Pi20	12	XNpb88	Imbe et al (1997)
Pi44	11	CD0520	Chen et al (1999)
Pib	2	RZ123	Miyamoto et al (1996)
Pita-2, Pita	12	XNpb088	Rybka et al (1997)
PiK^m	11	L190/R1506	Kaji & Ogawa (1996)
Pb1	11	S723/CD0226/C189	Fujii et al (1999)
Bacterial blight			
Xa1	4	XNpb235	Yoshimura et al (1992)
Xa3	11	XNpb181	Yoshimura et al (1995
Xa4	11	XNpb181	Yoshimura et al (1995)
Xa5	5	RZ390/RG550/RG207	Yoshimura et al (1995)
Xa10	11	RG303	Yoshimura et al (1995)
Xa13	8	RG136	Zhang et al (1996)
Xa21	11	RG103	Ronald et al (1992)

Xa22(t)	11	R543/RZ536	Lin et al (1996)
Yellow mottle virus			
RYMV	12	RG341/RG869	Ghesquiere et al (1997)
Tungro (RTSV)			
RTSV	4	RZ262	Sebastian et al (1996)
Rice stripe			
Stv-b(I)	11	XNpb220	Hayano Saito et al (1998)
6. Other traits			
Photoperiod sensitivity			
Sd1	6	RG64	Mackill et al (1993)
Semidwarf gene			
Sd1	1	XNpb363	Ogi et al (1993)
Sdg	5	RZ182	Liang et al (1994)
Shattering-resistance gene			
Sh2	1	XNpb174	Ogi et al (1993)
Sh4	3	R250	Fukuta and Yagi (1998)
Sht	4	R1427/C107	Sobrizal et al (1999)

References

Ahn, S.N., C.N.Bollich, A.M..McClung and S.D. Sley, RFLP analysis of genomic regions associated with cooked-Kernel elongation in rice. Theor. Appl. Genet. 87:27-32 (1993)

Ahn, S.N., C.N.Bollich and S.D.Tanksley, RFLP tagging of a gene for aroma in rice. Theor. Appl. Genet. 84:825-828 (1992)

Ahn, S.N., Y.K.Kim, S.S.Han, H.C.Choi, H.P. Moon and S.R.McCouch, Molecular mapping of a gene for resistance to a Korean isolate of rice blast. Rice Genet. Newsl. 13: 74-76 (1996)

Akagi, H., Y.Yokozeki, A.Inagaki, A.Nakamura and T.Fujimura, A condominant DNA marker closely linked to the rice nuclear restorer gene, *Rf-1*, identified with inter-SSR fingerprinting. Genome 39:1205-1209 (1996)

Anonymous, Agricultural statistics of Pakistan Pp. 13-17 (2001-2002)

Ayres, N.M., A.M.McClung, P.D.Larkin, H.F.J. Bligh, C.A.Jones and W.D.Park, Micro-satellites and single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of US rice germplasm. Theor. Appl. Genet. 94:773-781 (1997)

Botstein, D., R.L.White, M.Skolinck and R.W. Davis, Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32:314-331 (1980)

Causse, M.A., T.M.Fulton, Y.G.Cho, S.N.Ahn, J.Chunwongse, K.S.Wu, J.H.Xiao, Z.H.Yu, P.C.Ronald, S.E.Harrington, G.Second, S.R. McCouch and S. D. Tanksley, Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251-1274 (1994)

Chen,D.H., M. Dela Vina, T.Inukai, D.J.Mackill, P. C. Ronald and R. J. Nelson, Molecular mapping of the blast resistance gene. *Pi44(t),* in a line derived from a durably resistant rice cultivar. Theor. Appl. Genet. 98:1046-1053 (1999)

Chunwongse,J., G.B.Martin and S.D. Tanksley, Pre-germination genotypic screening using PCR amplification of half-seeds. Theor. Appl. Genet, 86:694-698 (1993)

Dong,N.V., P.K.Subudhi, P.N.Luong, V. D.Quang, T.D.Quy, H.G.Zheng, B. Wang and H.T.Nguyen, Molecular mapping or rice gene conditioning thermo-sensitive genic male sterility using AFLP,RFLP & SSR techniques. Theor. Appl.Genet. 100:727-734 (2000)

Frisch, M., M.Bohn and A.E.Melchinger, Comparison of selection strategies for marker-assisted backcrossing of a gene. Crop Sci. 39:1295-1301 (1999a).

Frisch, M., M.Bohn and A.E.Melchinger, Minimum sample size and optimal positioning of flanking markers in marker-assisted backcrossing for transfer of a target gene. Crop Sci. 39:967-975 (1999b)

Fujii, K., Y.Hayano-Saito, N.Sugiura, N. Hayashi, N.Saka, T.Tooyama, T.Izawa, and A. Shumiya, Gene analysis of panicle blast resistance in rice cultivars with rice stripe resistance. Breed. Res. 1: 203-210 (1999)

Fukuoka,S., H.Namai and K.Okuno, FRLP mapping of the genes controlling hybrid break-down in rice *(Oryza sativa* L.*).* Theor. Appl. Genet. 97:446-449 (1998)

Fukuta, Y., K.Tamura, M.Hirae, S.Oya, Genetic analysis of resistance to green rice leaf-hopper *(Nephotettix cincticeps* UHL WE) in rice parental line, Norin-PL6, using FRLP markers. Breed. Sci. 48:243-249 (1998)

Fukuta, Y. and T.Yagi, Mapping of a shattering resistance gene in a mutant line SR-5 induced from an indica rice variety, Nan-jing11. Breed.Sci. 48:345-348 (1998)

Ghesquiere, A., L.Albar, M.Lorieux, N. Ahmadi, D.Fargette, N.Huang, S.R. McCouch and J.L.Notteghem, A major quantitative trait locus for rice yellow mottle virus resistance maps to a cluster of blast resistance genes on chromosome 12. Phytopathology 87: 1243 -1249 (1997)

Gupta,P.K., R.K.Varshney, P>C>Shara, and B.Ramesh, Molecular markers and their applications in wheat breeding. Plant Breed. 118:369-390 (1999)

Harrington,S.E., H.F.J.Bligh, W.D.Park, C.A.Jones and S.R.McCouch, Linkage mapping of starch branching enzyme III in rice *(Oryza sativa* L.) and prediction of location of orthologous genes in other grasses. Theor. Appl. Genet. 94:564-568 (1997)

Hayano,S.Y., T.Tsuji, K.Fujii, K.Saito, M. Iwasaki and A.Saito, Localization of the rice stripe disease resistance gene, *Stv-b(i),* by graphical genotyping and linkage analyses with molecular markers. Theor. App. Genet. 96: 1044-1049 (1998)

Hirabayashi, H. and T. Ogawa, RFLP mapping of *Bph-1* (brown planthopper resistance gene) in rice. Breed. Sci. 45: 369-371 (1995)

Hittalmani,S., A.Parco, T.V.Mew, R.S. Zeigler, and N.Huang, Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Appl. Genet. 100:1121-1128 (2000)

Huang,N.,E.R.Angeles,J.Domingo,G.Magpantay S.Singh, G.Zhang, N. Kumaravadivel, J.Bennett and G.S. Khush, Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 95: 313-320 (1997)

Huang, Q., Y.He, R.Jin, H.Huang and Y.Zhu, Tagging of the restorer gene for rice HL-type CMS using microsatellite

markers. Rice Genet. Newsl. 16:75-77 (1999)

Ichikawa, N., N. Kishimoto, A. Inagaki, A. Nakamura, Y.Koshino, Y.Yokozeki, MOka, S. Samoto, H.Akagi, K.Higo, C.Shinjyo, T. Fujimura and H.Shimada, A rapid PCR-aided selection of a rice line containing the *Rf-I* gene, which is involved in restoration of the cyto-plasmic male sterility. Mol. Breed. 3: 195-202 (1997)

Imbe, T., S. Oba, M. J. T. Yanoria and H. Tsunematsu, A new gene for blast resistance in rice cultivar IR24. Rice Genet. Newsl. 14: 60-62 (1997)

Ishii, T., D.S.Brar, D.S.Multani and G.S. Khush, Molecular tagging of genes for brown plant-hopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa.* Genome 37:217-221 (1994)

Kadirvel, P., M. Maheswaran and K. Gunathilagaraj, Molecular mapping of quantitative trait loci (QTLs) associated with whitebacked planthopper in rice. Int. Rice Res. Notes 24: 12-14 (1999)

Kaji, R. and T.Ogawa, RFLP mapping of blast resistance gene *pik-m* in rice. Int. Rice Res. Notes 21: 47 (1996)

Kelly, J.D. and P.N.Miklas, The role of RAPD markers in breeding for disease resistance in common bean. Mol. Breed. 4: 1-11 (1998)

Koh, H.J., Y.H.Son, M.H.Heu, H.S.Lee, and S.R.McCouch, Molecular mapping of a new genic male-sterility gene causing chalky endosperm in rice *(Oryza sativa* L.). Euphytica 106: 57-62 (1999)

Kurata,N., Y.Nagamura, K.Yamamoto, Y. Harushima, N.Sue, J.Wu, B.A. Antonio, A.Shomura, T.Shimizu, S.Y.Lin, T. Inoue, A. Fukuda, T. Shimano, Y. Kuboki, T. Toyama, Y. Miyamoto, T. Kirihara, K. Hayasaka, A. Miyao, L. Monna, H.S. Zhong, Y.Tamura, Z. X. Wang, T. Momma, Y. Umehara, M. Yano, T.Sasaki and Y.Minobe, A 3000-Kilobase interval genetic map of rice including 883 expressed sequences. Nat. Genet. 8:365-372 (1994)

Lang, N.T., P.K.Subudhi, S.S.Virmani, D.S. Brar, G.S.Khush, Z.K.Li and N.Huang, Development of PCR-based markers for thermosensitive genetic male sterility gene *tms3(t)* in rice *(Oryza sativa* L.) Hereditas 131: 121-127 (1999)

Lange, D.A., S.Penuela, R.L.Denny, J. Mudge, V.C.Concibido, J.H.Orf and N.D.Young, A plant DNA isolation protocol suitable for polymerase chain reaction based marker assisted breeding. Crop Sci. 38: 217-220 (1998)

Liang, C.Z., M.H.Gu, X.B.Pan, G.H.Liang, and L.H.Zhu, RFLP tagging of a new semidwarfing gene in rice. Theor. Appl. Genet. 88:898-900 (1994)

Lin, X.H., D.P.Zhang, Y.F.Xie, H.P.Gao, and Q.F.Zhang, Identifying and mapping a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86: 1156-1159 (1996)

Liu, K.D., J.Wang, H.B.Li, C.G.Xu, A.M. Liu, X.H.Li and Q.F.Zhang, A genome-wide analysis of wide compatibility in rice and the precise location of the S_5 locus in the molecular map. Theor. Appl. Genet. 95:809-814 (1997)

Liu,G., G.Lu, L.Zeng and G.L.Wang, Two broad-specttrum blast resistance genes, Pi9(t), and Pi2(t), are physically linked on rice chromosome 6. Mol. Genet. Genomics 267: 472-480 (2002)

Mackill, D.J., Genome analysis and breeding In: Molecular biology of rice. New York (USA) Springer-Verlag. Pp. 17-14 (1999)

Mackill, D.J., H.T.Nguyen and J.X.Zhang, Use of molecular markers in plant improvement programs for rainfed lowland rice. Field Crops Res. 64: 177-185 (1999)

Mackill, D.J., M.A.Salam, Z.Y.Wang, and S.D.Tanksley, A major photoperiod-sensitivity gene tagged with RFLP

and isozyme markers in rice. Theor. Appl. Genet. 85: 536-540 (1993)

McCouch, S. R. and R. W. Doerge, QTL mapping in rice. Trends Genet. 11: 482-487 (1995)

McCouch, S.R., G.Kochert, Z.H.Yu, Z.Y.Wang, G. S. Khush, W. R. Coffman and S.D. Tanksley, Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76: 815-829 (1998)

Mei, M.H., L.Chen, Z.H.Zhang, Z.Y.Li, C.G.Xu, and Q.F.Zhang, *pms3* is the locus causing the original photoperiod-sensitive male sterility mutation of 'Nongken 58S'. Sci. China Ser. C-Life Sci. 42: 316-322 (1999)

Mei,M., C.Zhuang, R.Wan, J.Wu, W.Hu and G. Kochert, Genetic analysis and tagging of genes for brown planthopper resistance in indica rice. In: Khush GS, (editor). Rice genetics III. Proceedings of the Third International Rice Genetics Symposium, 16-20 Oct 1995, Manila, Philippines. Manila (Philippines): International Rice Research Institute, Pp 590-595 (1996)

Miyamoto, M., I. Ando, K. Rybka, O. Kodama and S.Kawasaki, High resolution mapping of the indica-derived rice blast resistance genes. 1. *Pi-b*. Mol. Plant-Microbe Interact. 9: 6-13 (1996)

Mohan, M., S.Nair, J.S.Bentur, U.P.Rao and J. Bennett, RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge *(Orseolia oryzae*). Theor. Appl. Genet. 87: 782-788 (1994)

Mohan,M., S.Nair, A.Bhagwat, T.G. Kirshna, M. Yano, C.R.Bhatia and T. Sasaki, Genome mapping, molecular markers and marker-assisted selection in crop plants. Mol. Breed. 3:87-103 (1997a)

Mohan,M.,P.V.Sathyanarayanan, A.Kumar, M.N.Srivastava and S.Nair, Molecular mapping of a resistance-specific PCR-based marker linked to a gall midge resistance gene (*Gm4t*) in rice. Theor. Appl. Genet. 95: 777-782 (1997b)

Naqvi, N. I., J. M. Bonman, D. J. Mackill, R. J. Nelson and B. B Chattoo, Identification of RAPD markers linked to a major blast resistance gene in rice. Mol. Breed. 1: 341-348 (1995)

Naqvi,N.I. and B.B.Chattoo, Molecular genetic analysis and sequence characterized amplified region-assisted selection of blast resistance in rice. In: Khush GS, (editor). Rice genetics III. Proceedings of the Third International Rice Genetics Symposium 16-20th Oct. 1995 Manila, Philippines. International Rice Research Institute. Pp.570-576 (1996)

Ni, J., P.M.Colowit, J.J.Oster, K.Xu and D.J. Mackill, Molecular markers linked to stem rot resistance in rice. Theor. Appl. Genet. 102: 511-516 (2001)

Ogi,Y., H.Kato, K.Maruyama, A.Saito and F.Kikuchi, Identification of RFLP markers closely linked to the semi-dwarfing gene at the *sd-1* locus in rice. Jpn. J. Breed. 43:141-146 (1993)

Reddy,O.U.K., E.A.Siddiq, N.P.Sarma, J. Ali, A.J.Hussain, P.Nimmakayala, P. Ramasamy, S.Pammi and A.S.Reddy, Genetic analysis of temperature-sensitive male sterility in rice. Theor. Appl. Genet. 100: 794-801 (2000)

Ronald, P.C., B. Albano, R. Tabien, L. Abenes, K.S.Wu, S.McCouch and S.D. Tanksley, Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. Mol. Gen. Genet. 236:113-120 (1992)

Rybka,K., M.Miyamoto,I.Ando, A.Saito and S. Kawasaki, High resolution mapping of the indica-derived rice blast resis-tance genes. 2. *Pi-ta*(2) and *Pi-ta* and a consideration of their origin. Mol. Plant Microbe Interact.10: 517-524 (1997)

Sanchez,A.C., D.S.Brar, N.Huang, Z.Li and G.S.Khush, Sequence tagged site marker-assisted selection for three

bacterial blight resistance genes in rice. Crop Sci. 40: 792-797 (2000)

Sebastian,L.S., R.Ikeda, N.Huang, T. Imbe, W.R.Coffman and S.R.McCouch, Mole-cular mapping of resistance to rice tungro spherical virus and green leaf hopper. Phytopathol. 86:25-30 (1996)

Shen,Y.W., Z.Q.Guan, J.Lu, J.Y.Zhuang, K.L.Zheng, M.W.Gao and X.M.Wang, Linkage analysis of a fertility restoring mutant generated from CMS rice. Theor. Appl. Genet. 97:261-266 (1998)

Sobrizal, K.Ikeda, P.L.Sanchez and A. Yoshimura, RFLP mapping of a seed shattering gene on chromosome 4 in rice. Rice Genet. News L. 16: 74-75 (1999)

Stam,P. and A.C.Zeven, The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica 30: 227-238 (1981)

Subudhi,P.K., R.P.Borkakati, S.S.Virmani and N.Huang, Molecular mapping of a thermosensitive genetic male sterility gene in rice using bulked segregant analysis. Genome 40 :181-194 (1997)

Tamura,K., Y.Fukuta, M.Hirae, S.Oya, I. Ashikawa and T.Yagi, Mapping of the *Grh1* locus for green rice leafhopper resistance in rice using RFLP markers. Breed. Sci. 49:11-14 (1999)

Tan,X.L., A.Vanavichit, S.Amornsilpa and S.Trangoonrung, Genetic analysis of rice CMS-WA fertility restoration based on QTL mapping. Theor. Appl. Genet. 97: 994-999 (1998)

Wang, A.Y., W.P.Yu, R.H.Juang, J.W. Huang, H.Y.Sung and J.C.Su, Presence of three rice sucrose synthase genes as revealed by cloning and sequencing of cDNA. Plant Mol. Biol. 18:1191-1194 (1992)

Wang, B., W.W.Xu, J.Z.Wang, W.Wu, H.G. Zheng, Z.Y.Yang, J.D.Ray and B.T.Nguyen, Tagging and mapping the thermo-sensitive genic male-sterile gene in rice *(Oryza sativa* L.) with molecular markers. Theor. Appl. Genet. 91: 1111-1114 (1995)

Wang,G.L., D.J.Mackill, J.M.Bonman S.R. McCouch, M.C.Champoux and R.J.Nelsnon, RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421-1434 (1994)

Williams, C.E. and P.C.Ronald, PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. Nucleic Acids Res. 22: 1917-1918 (1994)

Wu,K.S., C.Martinez, Z.Lentini, J.Tohme, F.G. Chumley, P.A.Scolnik and B.Valent, Cloning a blast resistance gene by chromosome walking In: Khush GS, (editor). Rice genetics III. Proceedings of the Third International Rice Genetics Symposium, 16-20 Oct 1995. Manila, Philippines. Los Banos (Philippines): International Rice Research Institute. Pp. 669-674 (1996)

Xu, K. and D. J. Mackill, A major locus for submergence tolerance mapped on rice chromosome 9. Mol. Breed. 2:219-224 (1996)

Yamaguchi, Y., R. Ikeda, H. Hirasawa, M. Minami and A.Ujihara, Linkage analysis of thermosensitive genic male sterility gene, *tms-2*, in rice (*Oryza sativa* L.).Breed. Sci. 47:371-373 (1997)

Yamasaki,M.,H.Tsunematsu, A.Yoshimura, N. Iwata and H.Yasui, Quantitative trait locus mapping of ovicidal response in rice *(Oryza sativa* L.) against white-backed planthopper *(Sogatella furcifera* Horvath).Crop Sci.39:1178-1183 (1999)

Yanagihara,S.,S.R.McCouch, K.Ishikawa, Y.Ogi, K.Maruyama and H.Ikehashi, Molecular analysis of the inheritance of the *S-5* locus, conferring wide compatibility in indica/japonica hybrids of rice (*O. sativa* L.). Theor. Appl. Genet. 90: 182-188 (1995)

Yang,D.C., G.B.Magpantay, M.Mendoza, N. Huang and D.S.Brar, Construction of

a contig for a fertility restorer gene, *Rf2*, in rice using BAC library and its sequence with *Rf2* gene of maize. Rice Genet. Newsl. 14:116-117 (1997)

Yano, M. and T.Sasaki, Genetic and molecular dissection of quantitative traits in rice. Plant Mol. Bio. 35:145-153 (1997)

Yao,F.Y., C.G.Xu, S.B.Yu, J.X.Li, Y.J. Gao, X.H.Li and Q.F.Zhang, Mapping and genetic analysis of two fertility restorer loci in the wild-abortive cytoplas-mic male sterility system of rice *(Oryza sativa* L.). Euphytica 98:183 - 187 (1997)

Yoshimura,S., A.Yoshimura, N.Iwata, S.R.McCouch,M.L.Abenes,M.R.Baraoidan T.W.Mew and R.J.Nelson, Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol. Breed. 1: 375-387 (1995)

Yoshimura,S., A.Yoshimura, A.Saito, N. Kishimoto, M.Kawase, M.Yano, M. Nakagahra, T.Ogawa and N.Iwata, RFLP analysis of introgressed chromo-somal segments in three near-isogenic lines of rice for bacterial blight resistance genes, *Xa-1, Sa-3,* and *Xa-4*. Jpn. J. Genet. 67:29-37 (1992)

Young,N.D. and S.D.Tanksley, Restriction fragment length polymorphism maps and the concept of graphical genotypes. Theor. Appl. Genet. 77:95-101 (1989)

Yu,Z.H., D.J.Mackill, J.M.Bonman, S.R. McCouch, E.Guiderdoni, J.L.Nottegherm and S.D.Tanksley, Molecular mapping of genes for resistance to rice blast *(Pyricularia grisea* Sacc.). Theor. Appl. Genet. 93:859-863 (1996)

Yu,Z.H., D.J.Mackill, J.M.Bonman and S.D. Tanksley, Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor. Appl. Genet. 81:471-476 (1991)

Zhang, G., E.R.Angeles, M.L.P.Abenes, G.S.Khush and N.Huang, RAPD and RFLP mapping of the bacterial blight resistance gene *xa-13* in rice . Theor. Appl. Genet. 93:65-70 (1996)

Zhang, G., T.S.Bharaj, Y.Lu, S.S.Virmani and N.Huang, Mapping of the *Rf-3* nuclear fertility restoring gene for WA cytoplasmic male sterility in rice using RAPD and RFLP markers. Theor. Appl. Genet. 94:27-33 (1997)

Zhang,G.Y., Y.Guo, S.L.Chen and S.Y. Chen, RFLP tagging of a salt tolerance gene in rice. Plant Sci. 110:227-234 (1995)

Zhang, J.S., C.Xie, Z.Y.Li and S.Y.Chen, Expression of the plasma membrane H^+-ATPase gene in response to salt stress in a rice salt-tolerant mutant and its original variety. Theor. Appl. Genet. 99:1006-1011 (1999)

Zhang, Q., B.Z.Shen, X.K.Dai, M.H.Mei, M.A. Saghai Maroof, Z.B.Li, Using bulked extremes -46 and recessive class to map genes for photoperiod-sensitive genic male sterility in rice. Proc. Natl. Acad. Sci. USA 91:8675-8679 (1994)

Zheng,K.,P.K.Subudhi,J.Domingo,G.Mag pantay and N.Huang, Rapid DNA isolation for marker assisted selection in rice breeding. Rice Genet. Newsl. 12:255-258 (1995)

Zheng, K.L., J.Y. Zhuang, J. Lu, H.R. Qian and H.X.Lin, Identification of DNA markers tightly linked to blast resistance genes in rice. In: Khush GS, (editor) Rice genetics III. Proceedings of the Third International Rice Genetics Symposium, 16-20 Oct 1995, Manila, Philippines Los Banos (Philippines): International Rice Research Institute. Pp. 565-569 (1996)

Zhu, L.H., Y.Chen, Y.B.Xu, J.C.Xu, H.M. Cai and Z.Z.Ling, Construction of a mole-cular map of rice and gene mapping using a double haploid population of a cross between Indica and japonica varieties. Rice Genet. Newsl. 10: 132 -134 (1993)