DOI:10.13300/j.cnki.hnlkxb.2000.03.001
第19卷第3期 华中农业大学学报 Vol.19 No.3
2000年 6月 Journal of Huazhong Agricultural University June 2000, 183~189

Improvement of Bacterial Blight Resistance of Hybrid Rice by Molecular Marker-Assisted Selection *

Chen Sheng Zhang Qifa

(*National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan* 430070, *China*)

Abstract This paper described the practice of molecular marker-assisted selection (MAS) in the process of recurrent backcrossing to improve the bacterial blight (BB) resistance of two restorer lines of hybrid rice, 'Minghui 63' and '6078', by introgressing *Xa21*, a broad-spectrum BB resistance gene. A PCR-based MAS system for *Xa21* introgressing segment was established. It consisted of a marker representing the *Xa21* locus, a marker linked to the *Xa21* locus at a distance of 0.8 cM on one side, and a marker at distance of 3.0 cM from the gene on the other side. In the practice of the improvement of Minghui 63, the selection was carried out in tandem in which a recombinant was obtained between *Xa21* and one of the makers in BC_1F_1, and a subsequent recombinant was obtained between the gene and the marker at the other side in BC_2F_1. The genetic background of Minghui 63 was recovered in BC_3F_1 using a total of 128 RFLP markers evenly distributed on the 12 rice chromosomes. Thus the improved version of Minghui 63, tentatively referred to as 'Minghui 63(*Xa21*)', was exactly the same as the original Minghui 63 in the entire genome except for a fragment of less than 3.8 cM in length surrounding the *Xa21* locus. A slightly different strategy was adopted for introgressing *Xa21* to 6078, a new restorer line. The main difference is that the background selection was carried out mainly in BC_1F_1, and selections for recombinants were conducted in BC_2F_1 and BC_3F_1. Another difference is that AFLP marker was used for background selection. Both of the improved restorer lines, Minghui 63(*Xa21*) and '6078(*Xa21*)', showed the same level and spectrum of BB resistance as the donor parent 'IRBB21' with artificial inoculation. Minghui 63(*Xa21*) and '6078(*Xa21*)' also showed the same agronomic performance and combining ability as the original restorer lines when there is no disease stress. The agronomic performance of the improved restorer lines, Minghui 63(*Xa21*) and 6078(*Xa21*), and their hybrids was significantly better than the unimproved version under field conditions with artificial inoculation.

Key words hybrid rice, restorer line, bacterial blight, *Xa21*, molecular marker-assisted selection, molecular breeding

CLCN S511.035.3; S332.2

1 Introduction

Rapid advances in rice genome research and DNA marker technology have led to the application of molecular marker-assisted selection (MAS) in rice breeding. Over the past five years, there have been large numbers of published studies on MAS in plant breeding[1~4]. More than 400 papers containing the key words 'marker-assisted selection' or 'marker-assisted breeding' were published from 1995 to 2000. However, most of the studies attempted only to map the loci responsible for some agricultural important traits, others involved in computer simulations for exploring

Received date: 2000-04-21

*This study was supported in part by a grant from the National Natural Science Foundation of China and a grant from the Rockefeller Foundation.

Chen Sheng, male, born in 1966, Ph.D. Present address: College of Life Science, Hubei University, Wuhan 430062, China

MAS strategies in plant breeding, whereas the successful and practical examples leading to released germplasm or varieties are still rare[5~7].

Hybrid rice has made prominent contributions to rice production in China, which had great impact on food availability of this country. Hybrid rice has also now started to be used in several other Asian countries, and the areas planted under hybrid rice are projected to a considerable increase in a number of Asian countries in the next few years.

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most devastating diseases of rice worldwide[8]. BB is also a serious problem of hybrid rice production[9], because of susceptibility of the parental lines to the pathogen populations under field conditions. It has been reported that *Xa21*, identified from the wild rice *Oryza longistaminata*, is highly resistant to a broad spectrum of the *Xoo* races[10]. Artificial inoculation with multiple *Xoo* strains in our laboratory in the last several years has determined that *Xa21* would be very useful for improving BB resistance of the rice hybrids in China[4].

In order to improve the BB resistance of rice hybrids, we developed a MAS backcross breeding program to transfer *Xa21* to 'Minghui 63' and '6078', two elite restorer lines of hybrid rice in China. In this paper, we describe the MAS practice and also discuss the optimal procedures for marker-assisted backcross breeding program for gene introgression.

2 The MAS scheme

2.1 The PCR-based MAS system for the *Xa21* locus

To establish a MAS system for transferring *Xa21*, a linkage map of the *Xa21* genomic region on chromosome 11 was constructed using 200 individuals of the F_2 population from the cross between 'Minghui 63' and 'IRBB21', a *Xa21*-containing isogenic line of 'IR24'[11]. Two markers, 21 and 248, cosegregated with the *Xa21* locus. These two markers were used for positive selection, i.e., selecting for the presence of the *Xa21* gene. Another two markers, C189 and AB9, flanked both sides of the *Xa21* locus at 0.8 cM and 3.0 cM, respectively. These two markers were chosen for negative selection, i.e., selecting for recombination between the *Xa21* locus and the flanking markers. Such negative selection would ensure the introgressed segment surrounding the *Xa21* locus to be less than the length between the two flanking markers (3.8 cM). In this MAS system, AB9 is a dominant type of marker with the PCR fragment from the donor parent IRBB21 detected in the breeding population, and the other three markers are co-dominant.

2.2 Strategies for genetic background selection

In order to eliminate the genetic background of the donor parent rapidly and efficiently to maintain as much as possible of the characteristics of the recipient parents, we designed two experimental schemes for background cleaning.

The first strategy, applied to the background selection in improving 'Minghui 63', was to identify restriction fragment length polymorphism (RFLP) markers distributed in the whole genome at regular intervals. By screening a total of 354 RFLP probes from the two published high-density maps[12, 13], we identified 158 markers that were polymorphic between Minghui 63 and IRBB21. One hundred and twenty-eight of the 158 polymorphic markers, representing all the 12 chromosomes with the largest interval less than 30 cM, were used for cleaning up the genetic background of the selections.

The other strategy for background selection involved the utilization of PCR-based markers. Four classes of PCR-based molecular markers, including random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), ran-

dom amplified microsatellite polymorphism (RAMP), and amplified fragment length polymorphism (AFLP), were compared for efficiency in background selection in backcross breeding (Table 1). The result showed that AFLP could provide more abundant information than other three classes of molecular markers because of its highest ratio of polymorphism on the basis of a primer pair. In addition, AFLP was highly repeatable compared to RAPD or RAMP[14~16], thus ensuring the accuracy of the results. Consequently, AFLP was used for selection of genetic background in backcross breeding.

Table 1 Polymorphism between Minghui 63 and IRBB21 screened using four types of PCR-based molecular markers

Marker	Pairs of primers	Polymorphic pairs of primers	Proportion of polymorphism /%	Polymorphic bands	Polymorphic bands per primer pair	Repeatability
RAPD	144	67	54.47	126	1.88	Poor
SSR	85	27	31.76	65	2.41	Good
RAMP	37	29	78.38	93	3.21	Fairly good
AFLP	37	37	100.00	492	13.30	Good

3 MAS practice

The *Xa21* gene was introgressed into the restorer lines to be improved following a recurrent backcrossing procedure, combined with tandem selection using molecular markers. The entire scheme took five generations: one cross between two parents, three generations of backcrosses to the recurrent parent, and one generation of self-fertilization.

3.1 Transferring of *Xa21* to Minghui 63

Minghui 63 is the restorer line for a number of widely used hybrids in rice production in China. The general characteristics of the hybrids produced using this restorer line include high yield and wide adaptability, which enabled these hybrids to occupy a total area of approximately 7 million ha per year in the late 1980's and early 1990's, accounting for more than 25% of the total rice production area in China during that period. It has been deduced that Minghui 63 carries the BB resistance gene *Xa4* (Y. F. Tan, unpublished data), and was considered to be BB resistant when it was first released. However, its resistance has largely become ineffective during the period of extensive cultivation as a result of evolution of the pathogen population.

The molecular marker-assisted backcross breeding was conducted using the above MAS system. Among a total of 49 plants in BC_1F_1 that contained *Xa21* as determined by both disease inoculation and PCR selection, one individual was found to be a recombinant between *Xa21* and the marker locus AB9 and was subsequently backcrossed to Minghui 63. In the same way, one of the 180 *Xa21*-containing plants in BC_2F_1 was found to be a recombinant between *Xa21* and the marker locus C189. Thus an individual containing an introgressed segment of less than 3.8 cM in length (0.21% of the rice genome, assuming a total length of 1800 cM) was obtained in BC_2F_1, and was further backcrossed to Minghui 63 to obtain the BC_3F_1.

Among a total of 250 plants that carried *Xa21* in BC_3F_1, two plants were found to be homozygous for the Minghui 63 genotypes at all marker loci except the RG103 locus residing in the *Xa21* gene region. These plants were self-pollinated to produce BC_3F_2, from which progenies homozygous for the *Xa21* allele were obtained and

designated as 'Minghui 63(*Xa21*)'. Minghui 63 (*Xa21*) was crossed with Zhenshan 97A and Maxie A to produce rice hybrids, tentatively designated as 'Shanyou 63(*Xa21*)' and 'Maxie 63 (*Xa21*)', respectively.

3.2 Transferring of *Xa21* to '6078'

6078 is a newly bred restorer line of hybrid rice with large panicles and high yielding potential. However, it is susceptible to BB. MAS was conducted in the process of transferring *Xa21* to 6078. By both PCR selection and disease inoculation, 204 plants were identified to be *Xa21* positive in BC_1F_1. All these plants were then assayed using 10 pairs of AFLP primers. One plant showed only 10 bands from the donor parent IRBB21 among a total of 129 polymorphic bands detected between the two parents. Assuming random distribution of the AFLP markers in the genome, this indicated 92.25% recovery of the 6078 genotype in this individual. This individual was subsequently backcrossed to 6078.

Among a total of 160 *Xa21* containing plants in BC_2F_1, four recombinant plants were found to be recombinants between *Xa21* and the marker locus AB9. AFLP assay of these recombinants identified one recombinant carried only three polymorphic bands from the donor IRBB21, indicating 97.67% recovery of the recurrent parent genome in this plant. This plant was further backcrossed to 6078 to obtain BC_3F_1.

A total of 120 *Xa21* positive plants was obtained in BC_3F_1, and one plant was found to be a recombinant between *Xa21* and the marker locus C189. This plant was self-pollinated to produce BC_3F_2, from which individuals homozygous for the *Xa*21 allele were obtained, and designated as '6078(*Xa21*)'. Thus 98.8% of the genetic background of 6078(*Xa21*) was expected to be from the recurrent parent. 6078(*Xa21*) was crossed with II-32A to obtain rice hybrid 'IIY6078(*Xa21*)'.

4 Evaluation of the improved restorer lines and their hybrids

4.1 Resistance of the improved lines and their hybrids to *Xoo* strains

Two improved versions of restorer lines and their hybrids were challenged with a mixture of 12 *Xoo* strains to examine the resistance. The results showed that the resistance of the improved restorer lines Minghui 63(*Xa21*) and 6078(*Xa21*) as well as hybrid Maxie 63(*Xa21*) was at the same level as the donor parent IRBB21, and the resistance of the hybrids Shanyou 63(*Xa21*) and IIY6078(*Xa21*) was also significantly improved. In contrast, all the original restorer lines and their hybrids were highly susceptible to the mixture of the *Xoo* strains (Table 2).

Table 2 Reactions of rice varieties and combinations tested in this study to 12 BB strains mixture

Rice Variety/Combination	Lesion Length/cm
Minghui 63	19.80±3.06[1)]
Minghui 63 (*Xa21*)	2.69±0.44
6078	22.76±1.04
6078 (*Xa21*)	2.60±1.21
Shanyou 63	23.01±2.01
Shanyou 63 (*Xa21*)	7.94±2.18
Maxie 63	16.41±1.15
Maxie 63 (*Xa21*)	3.79±0.64
II Y 6078	22.64±1.35
II Y 6078 (*Xa21*)	7.99±1.25
IRBB21	1.35±0.60
Zhenzhu Ai	22.72±1.12

1) Data in this table are shown as average±standard deviation among blocks

4.2 The agronomic performance of the improved lines and their hybrids

The agronomic performance of the improved version of restorer lines and the hybrids we compared with the originals. Under field conditions without artificial inoculation, Minghui 63 and Minghui 63(*Xa21*) were identical for all the agronomic traits examined, as were Shanyou 63 and

Shanyou 63(*Xa21*). No significant difference was detected in yield and the three traits that were components of yield (tillers per plant, grains per panicle and grain weight), either between 6078 and 6078 (*Xa21*), or between IIY6078 and IIY6078(*Xa21*). However, plant stature of 6078 (*Xa21*) seemed to be slightly shorter than the original 6078, and the panicle length of IIY6078 (*Xa21*) also appeared to be slightly shorter than IIY6078.

Table 3　Yield and its components of Minghui 63 (*Xa21*) and Shanyou 63 (*Xa21*) inoculated with strains Pxo99, ZJ173 and LN44 (Hainan, 1999)

Variety/ Combination	Tillers per plant	Grains per panicle	1000 grain weight/g	Yield per plant/g
Minghui 63	5.3	68.6	26.12	9.52
Minghui 63 (*Xa21*)	4.8	73.2	27.97	9.81
IRBB21	5.4	73.1	20.14	7.96
Shanyou 63	4.8	74.6	26.89	9.52
Shanyou 63 (*Xa21*)	5.2	91.1	28.80	13.56
$LSD_{0.05}$[1)]		12.2	1.21	1.09
$LSD_{0.01}$		20.2	2.01	1.80

1) $LSD_{0.05}$ and $LSD_{0.01}$ mean least significant differences at 0.05 and 0.01 probability levels, respectively

Table 4　Yield and its components of 6078 (*Xa21*) and IIYou 6078 (*Xa21*) inoculated with mixture of 12 BB strains (Wuhan, 1999)

Variety/ Combination	Tillers per plant	Grains per panicle	1000 grains weight/g	Yield per plant/g
6078	10.5	78.9	21.88	17.81
6078 (*Xa21*)	10.7	99.5	22.89	24.10
IRBB21	13.0	97.5	20.86	26.74
II Y 6078	10.2	122.4	23.11	28.92
II Y 6078 (*Xa21*)	9.9	127.8	24.65	31.68
$LSD_{0.05}$[1)]		20.17	0.70	4.21
$LSD_{0.01}$		29.35	1.02	6.13

1) $LSD_{0.05}$ and $LSD_{0.01}$ mean least significant differences at 0.05 and 0.01 probability levels, respectively.

Under heavily diseased conditions, Minghui 63 (*Xa21*) showed significantly higher grain weight and spikelet fertility than Minghui 63. The differences of yield related traits were even more pronounced between Shanyou 63 and Shanyou 63 (*Xa21*). Yield and all three yield component traits were higher for Shanyou 63 (*Xa21*) than Shanyou 63 (Table 3). Similarly, under heavily diseased conditions simulated by artificial inoculation with a mixture of 12 *Xoo* strains, 6078 (*Xa21*) showed significantly higher performance than the original 6078 in grains per panicle, grain weight and yield per plant. Grain weight of IIY6078 (*Xa21*) was also highly significantly higher than IIY6078 (Table 4).

4.3　Comparison of the performance of hybrids with four male sterile lines

To evaluate the effects on the combining ability of the restorer lines, we also examined the agronomic performance of the hybrids produced by crossing both of the original and the improved restorer lines with each of the four male sterile lines widely used in rice production in China. The results showed that the agronomic performance was almost identical between the two hybrids of Minghui 63 and Minghui 63(*Xa21*) crossed with all the four male sterile lines, Jin 23A, V20A, II-32A or Zhenshan 97A, except a small but statistically significant difference in grains per panicle and grain yield between the hybrids of Minghui 63 and Minghui 63 (*Xa21*) crossed with V20A. Likewise, no significant difference was detected in all the six traits examined between the two hybrids of 6078 and 6078(*Xa21*) crossed with all the four male sterile lines except in two cases. The first case was a small but statistically significant difference in grain weight between the hybrids of 6078 and 6078(*Xa21*) crossed with Jin 23A. The second case was also a small but significant difference in tillers per plant between the hybrids of 6078 and 6078(*Xa21*) crossed with V20A. Thus, the performance of the hybrids of these male sterile

lines with two improved restorer lines was essentially the same as those crossed with the originals. This indicates that the combining ability of the original restorer line Minghui 63 and 6078 was well maintained in the improved versions of restorer line Minghui 63 (*Xa21*) and 6078 (*Xa21*) obtained by molecular marker-assisted backcross breeding.

5 Discussion

The strategies used in transferring *Xa21* into Minghui 63 and 6078 differed in the selection procedure and background cleaning. In the process of transferring *Xa21* into Minghui 63, the background selection took place in the BC_3F_1 generation, whereas in the process of transferring *Xa21* into 6078, background selection was practiced in the BC_1F_1 population and also in the BC_2F_1 population. In the former case, 250 BC_3F_1 plants were surveyed for RFLP with 128 polymorphic probes detecting 128 polymorphic loci distributed at regular intervals along the 12 chromosomes, following a parental survey using 354 probes. Based on the results, it can be determined with certainty that the genetic background of the improved version is entirely from the recurrent parent except the fragment less than 3.8 cM surrounding the *Xa21* locus. In the latter case, a total of 209 plants (204 in BC_1F_1 and 5 in BC_2F_1) was surveyed for AFLP with 10 pairs of primers, which detected 129 polymorphic bands. It is not certain exactly how much of the genetic background of the recurrent parent was recovered in the individual obtained in the final selection, although the agronomic performance and combining ability of the selected line is very similar to the original. This is because of the unknown distribution of the 129 polymorphic bands on the 12 chromosomes.

The advantage of using AFLP technique in background selection is its high efficiency and reduced cost compared with RFLP analysis. We previously discussed the advantage and feasibility of practicing background selection in the BC_1F_1 generation[4], and suggested conducting background selection in BC_1F_1 among individuals with recombination on either side of the targeted gene.

Based on the above discussions, we recommend the selection procedure to involve the following: (1) using AFLP for background selection; (2) performing a linkage analysis in BC_1F_1 based on the AFLP data of the targeted gene-positive individuals to insure a good coverage of the markers in the genome; (3) conducting background selection in BC_1F_1 to identify the individual with the highest proportion of recurrent parent background and carrying recombination on at least one side of the gene; (4) identifying individuals in BC_3F_1 with the genetic background coming entirely from the recurrent parent except a very small segment carrying the targeted gene.

As a strategy to improve the restorer line of a certain hybrid variety, the most important aspects include the following two: (1) While introgressing the target allele, other unfavorable alleles that tightly linked with the target allele in the donor parent should be avoided so as not to deviate the expected breeding objective; (2) While the target trait improved, many excellent characters of the original restorer lines such as high combining ability and wide adaptability should be maintained in the improved version of restorer line. Therefore, an efficient and reliable marker-assisted backcross breeding strategy should detect the length of the introgressing segment containing the target allele, but also select the whole genetic background efficiently and quickly. Consequently, this procedure described above is aiming at the genetic improvement of the restorer line of rice hybrids. Anyway, it can also be applied to the genetic improvement of the restorer line of other flowering plants. Moreover, to the improvement of many conventional varieties, the MAS system aiming at the introgressing segment containing the target allele is also suitable. Sometimes, the background selec-

tion is also necessary only if not so strictly as described above.

参考文献

1 Mohan M, Nair S, Bhagwat A et al. Genome mapping, molecular marker and marker-assisted selection in crop plants. Mol Breed, 1997, 3: 87~103

2 Yoshimura S, Yoshimura A, Iwata N et al. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol Breed, 1995, 1: 375~387

3 Huang N, Angeles E R, Domingo J et al. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. Theor Appl Genet, 1997, 95: 313~320

4 Chen S, Lin X H, Xu C G et al. Improvement of bacterial blight resistance of 'Minghui 63', an elite restorer line of hybrid rice, by molecular marker assisted selection. Crop Sci, 2000, 40: 239~244

5 Ribaut J M, Hoisington D. Marker-assisted selection: new tools and strategies. Trends in Plant Sci, 1998, 3: 236~239

6 Knapp S J. Marker-assisted selection as a strategy for increasing the probability of selecting superior genotypes. Crop Sci, 1998, 38: 1164~1174

7 Young N D. A cautiously optimistic vision for marker-assisted breeding. Mol Breed, 1999, 5: 505~510

8 Mew T W. Current status and future prospects of research on bacterial blight of rice. Annu Rev Phytopathol, 1987, 25: 359~382

9 章　琦. 我国水稻抗白叶枯病基因的利用及策略. 植物病理学报, 1995, 22: 241~246

10 Khush G S, Bacalangco E, Ogawa T. A new gene for resistance to bacterial blight from *O. longistaminata*. Rice Genet Newsl, 1990, 7: 121~122

11 Ikeda R, Tabien R E, Khush G S. Chromosomal location of *Xa-21*. Rice Genet Newsl, 1991, 8: 102~103

12 Causse M A, Fulton T M, Cho Y G et al. Saturated molecular maps of the rice genome based on an interspecific backcross population. Genetics, 1994, 138: 1251~1274

13 Kurata N, Nagamura Y, Yamamoto K et al. A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genetics, 1994, 8: 365~372

14 Devos K M, Gale M D. The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet, 1992, 84: 567~572

15 Hallden C, Hansen M, Nisson N O et al. Competition as a source of errors in RAPD analysis. Theor Appl Genet, 1996, 93: 1185~1192

16 Lowe A J, Hanctte O, Guarino L. A standard molecular genetic technique used in identity of germplasm resource: random amplified polymorphic DNA (RAPD). Plant Genet Resource Newsl, 1996, 107: 50~54

分子标记辅助选择改良杂交水稻的白叶枯病抗性

陈　升　张启发

(华中农业大学作物遗传改良国家重点实验室, 中国武汉 430070)

摘要　分子标记辅助选择技术以其对目标基因快速而精确的选择为回交育种提供了非常有效的工具。华中农业大学作物遗传改良实验室运用分子标记辅助的回交育种方法, 将广谱高抗白叶枯病基因 *Xa21* 迅速导入到杂交水稻的两个优良恢复系明恢 63 和 6078 之中, 成功地完成了其抗性改良, 并通过配组而达到了改良杂交水稻抗性的目的。本研究所运用的分子标记辅助的水稻回交育种程序是: 通过一次杂交、三次回交和一次自交的育种程序, 将目标基因导入到待改良的杂交稻恢复系中; 采用以 PCR 为基础的对目标基因片段实施选择的MAS 体系检测并选择与目标基因共分离和/或紧密连锁的分子标记基因型, 使含有目标基因的转移片段小于 3.8 cM; 应用 RFLP 或 AFLP 标记技术对遗传背景实施选择, 使除开目标基因区段之外, 改良型恢复系基因组与原恢复系相同。多菌系接种鉴定试验结果表明: 改良型恢复系明恢 63(*Xa21*)和 6078(*Xa21*)及其所配杂交组合的抗性水平较原恢复系及其杂交组合均显著提高。农艺性状比较试验结果表明: 在无病害发生的自然生长区, 改良型恢复系及其所配组合的各种农艺性状表现与原恢复系及其所配组合基本相同; 而在严重病害条件下, 改良型恢复系及其所配组合的农艺表现, 包括产量及其三个构成因子, 均较原恢复系及其所配组合显著提高。改良型恢复系与 5 个在我国应用很广的籼型三系雄性不育系所配组合的农艺性状考查结果亦表明, 经过分子标记辅助的回交育种程序所培育的改良型恢复系, 较好地保持了原恢复系的配合力水平。

关键词　杂交稻; 恢复系; 白叶枯病; *Xa21*; 分子标记辅助选择; 分子育种

(责任编辑: 张志钰)