C. R. Biologies 338 (2015) 321–334

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Plant biology and pathology/Biologie et pathologie végétales

Current advance methods for the identification of blast resistance genes in rice

Fatah A. Tanweer [a,f], Mohd Y. Rafii [a,b,*], Kamaruzaman Sijam [c], Harun A. Rahim [d], Fahim Ahmed [a], Mohammad A. Latif [a,e]

[a] Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[b] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[c] Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[d] Bioscience and Agrotechnology Division, Malaysian Nuclear Agency, Bangi, 43000 Kajang, Selangor, Malaysia
[e] Bangladesh Rice Research Institute (BRRI), Gazipur, Dhaka, Bangladesh
[f] Department of Plant Breeding and Genetics, Faculty of Crop Production, Sindh Agriculture University Tandojam, Sindh, Pakistan

ARTICLE INFO

Article history:
Received 24 November 2014
Accepted after revision 3 March 2015
Available online 2 April 2015

Keywords:
Rice blast
Magnaporthe oryzae
Classical plant breeding
Marker-assisted selection
QTLs

ABSTRACT

Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases of rice around the world and crop losses due to blast are considerably high. Many blast resistant rice varieties have been developed by classical plant breeding and adopted by farmers in various rice-growing countries. However, the variability in the pathogenicity of the blast fungus according to environment made blast disease a major concern for farmers, which remains a threat to the rice industry. With the utilization of molecular techniques, plant breeders have improved rice production systems and minimized yield losses. In this article, we have summarized the current advanced molecular techniques used for controlling blast disease. With the advent of new technologies like marker-assisted selection, molecular mapping, map-based cloning, marker-assisted backcrossing and allele mining, breeders have identified more than 100 *Pi* loci and 350 QTL in rice genome responsible for blast disease. These *Pi* genes and QTLs can be introgressed into a blast-susceptible cultivar through marker-assisted backcross breeding. These molecular techniques provide timesaving, environment friendly and labour-cost-saving ways to control blast disease. The knowledge of host–plant interactions in the frame of blast disease will lead to develop resistant varieties in the future.

1. Introduction

Rice (*Oryza sativa*) is one of the most important cereal crop and staple diet throughout the world. It is grown everywhere, except in Antarctica, and has great economic value. The calorie intake from rice consumed by the world population is more than 23%. The necessity for rice production has increased due to the increasing world population [1]. The rapid growth of world population, the scarcity of cultivated land and water, all these factors made blast a serious issue. The average yield potential of rice is 10 tons/ha, but farmers only harvest about 5 tons/ha [2], and this yield loss is due to insect pest and diseases.

Among biotic stresses, blast is the most threatening disease for the productivity of rice, at a time when rice production has to be increased by more than 40% by 2030 to meet the demand of people [3]. This target can be achieved by developing highly resistant rice varieties against biotic and abiotic stresses [4].

Rice blast caused by *Magnaporthe oryzae* is a destructing disease of rice that poses serious threats to the world's food

* Corresponding author at: Universiti Putra Malaysia, Institute of Tropical Agriculture, Laboratory of Food Crops, 43400 Serdang, Selangor, Malaysia.
E-mail address: mrafii@upm.edu.my (M.Y. Rafii).

http://dx.doi.org/10.1016/j.crvi.2015.03.001
1631-0691/

security [5]. About 50 species of grass family are infected by *M. grisea*, including rice, wheat, and barley. The blast disease was first observed in India during 1919, with estimated yield losses of 4% [6], but yield losses were increased afterwards as losses only in Eastern India were estimated at around 50% [2], whereas in Japan, the crop losses varied from 20 to 100% due to various pathogens of blast disease [3]. The annual losses caused by blast disease in rice production can fulfil the annual requirement of 60 million people [7]. Rice diseases are the cause of yield loss up to 5% every year [8]. The use of resistant rice varieties has overcome the blast disease problem partially, as the pathogen have genetic variability depending on the conducive conditions. Hence, rice breeders are under a lot of pressure for developing durable resistant rice varieties.

Recently, various techniques have been used to control blast disease, including chemical and biological method, but these measures are not long lasting. Secondly, the use of fungicide is not only costly, but also hazardous to the environment. Introgression of resistant genes into rice cultivars is a very effective and safe option for controlling blast disease. Genetic studies related to blast resistance started a long time ago [9], but few cultivars have been developed with durable resistance [10] mainly due to the pathogen's variability towards virulence. Therefore, breeders recently focussed on combining genes with the help of molecular markers to get partial and complete resistance against blast [11]. The pyramiding of resistant genes or providing them with multigenic resistance is an efficient way to manage blast disease. Accordingly, different molecular techniques such as molecular mapping, gene cloning, QTL analysis, map-based cloning, marker-assisted selection have been introduced to analyse the blast-resistant genes in the rice genome [12].

The combination of a biotechnological approach and of molecular breeding can provide a high degree of resistance against *M. oryzae*. At present, the blast is spreading in major rice-growing areas of the world; therefore, there is an urgent need for rice varieties having high and durable resistance against blast to assure food security in the developing countries. Considering the importance of resistance development among rice cultivars against blast, this review highlights the current advances made in identifying the genes responsible for blast resistance in rice.

2. Molecular mapping of blast resistance genes

A complete rice genetic map was constructed by crossing a *japonica* variety (Nipponbare) with an *indica* variety (Kasalath) to get a population of 186 F_2 individuals [13]. Sasaki [14] was the first person who described the blast disease resistance gene, and later on Kiyosawa [15] identified and introduced the first blast resistance gene (*Pi-a*) into the *japonica* rice variety of Aichi Asahi. The construction of the rice genetic map started in 1997 with the use of Restriction Fragment Length Polymorphism (RFLP) markers [16–18]. Further saturation of RFLP mapping was done by using other DNA markers such as RAPD [19,20] and SSR markers [21].

Although the genetic linkage map of many crop species was constructed by conventional breeding using morphological markers, they are numerically few and very difficult to apply as compared to molecular markers. However, with the introduction of molecular mapping, confirming the presence or the absence of blast resistance genes and finding closely linked markers becomes effortless [22].

The molecular mapping of blast resistance genes is a most convenient and direct approach for the identification of blast resistance in the rice genome. More than 100 blast resistance genes have been identified by using this approach. In recent years, major blast resistance genes and Quantitative TRAIT Loci (QTL) linked to blast resistance have been localized through molecular mapping. Moroberekan, the traditional African variety with durable resistance against various pathotypes of *M. oryzae*, has been used as a donor for blast resistance in many breeding programmes. By using RFLP markers, Wang et al. [23] mapped *Pi5*(*t*), *Pi7*(*t*) blast resistance genes and nine QTLs linked with partial resistance in Moroberekan variety on different chromosomes.

The complete genome of rice has been sequenced by the International Rice Genome Sequencing Project (IRGSP) in 2005 [24]; it is helpful for identifying and locating a specific position of blast resistance genes through fine mapping. More than 30 blast resistance genes [*Pita-2*, *Pi39(t)*, and *Pi20(t)*, *Pi5(t)*, *Pi15*, *PiCO39(t)*, *Pi38*, *PBR*, *Pb1*, *Pi-kh*, *Pi1*, *Pik-m*, *Pik*, *Pik-p*, *Pik-s*, *Pi62(t)*, *Pi157, Pit, Pi27(t)*, *Pish*, *Pid1(t)*, *Pig(t)*, *Piy(t)*, *Piy2(t)*, *Pi39(t)*, *Pi10*, *Pi40(t)*, *Piz*, *Pigm(t)*, *Pi33*] have been identified through the molecular map-based approach. Accordingly, many blast resistance genes have been mapped on different chromosomes of rice using RFLP and SSR markers. After the sequencing of the whole rice genome, it becomes convenient to find the chromosomal location of genes linked with blast resistance through linkage to molecular markers.

The identification of molecular markers lying closely to blast resistance genes can be used for the indirect selection of genes called gene tagging [25]. Sharma et al. [26] mapped *Pi54* blast resistance gene allelic to *Pi-kh* in the Tetep cultivar. Tetep is a blast-resistant rice variety having a broad-spectrum resistance against different pathotypes of *M. oryzae*. Another two blast resistance genes, *Pi38* and *Pi42*, were identified in Tadukan and DHR9 cultivar by using different DNA markers [27,28]. The distribution of blast resistance genes is widely clustered on chromosome 6,11 and 12. Based on the mapping of blast resistance genes on different chromosomes, the genetic dissection showed that these genes are made up of nucleotide binding site-leucine-rich repeat (NBS-LRR) domain [29].

3. QTL mapping for blast resistance in rice

QTLs are regulated by more than one gene and are responsible for different characters such as yield, resistance against biotic (diseases) and abiotic factors (drought). Single marker analysis (SMA), standard interval mapping (SIM) and composite interval mapping (CIM) are three basic methods for QTL mapping [30].

The mapping population is divided into classes on the basis of the genotype at every marker locus in SMA method and demarcates QTL if significant differences in the overall

mean phenotypic score are found for every group [31]. In the case of SIM at each locus, a flanking marker is used between two marker intervals. QTL can be detected easily in this method as compared to SMA. The main drawback of SIM is the identification of sometimes false QTLs because of linked and unlinked QTLs [32]. The third approach, CIM, is often used; in this method, subsets of markers are used at both linked and unlinked QTLs. The interaction of QTL declares can be detected by Composite Interval Mapping (CIM) [32].

Knowledge about tightly linked markers increases the probability of QTL detection [33]. The mapping of QTLs for blast resistance gained in importance because of partial resistance, which is durable, polygenic, non-race specific, and controlled by QTLs [34]. The partial resistance is the most suitable blast control method because it is stable and remains for a long time in comparison to complete and incomplete resistance [35,36].

The QTLs strategies have been widely used for mapping major and minor genes linked with partial resistance in rice [37]. QTL associated with blast resistance was first identified in Moroberekan cultivar grown widely in Africa [23]. There are two types of resistance: true resistance and field resistance. True resistance is associated with major gene control, qualitative and quantitative characters controlled by minor genes [38]. Most of the QTLs associated with qualitative genes have been widely seen. Sometimes, on linkage maps, the regions are very rich in genes, providing resistance to more than one pathogen because quantitative and qualitative genes exist on same position. Generally, partial resistance is a quantitative resistance governed by multiple loci called QTLs in a specific cultivar [39]. Partial resistance reduces the development of disease and reproduction of pathogens when pathogen and plant are incompatible and cannot interact [35].

From different studies, it has been proven that partial resistance can be specific [40]. Four partial resistance genes *Pi34* [36,41], *Pif* [42], *pi21* [43] and *Pb1* [44] have been identified and considered as specific.

More than 350 QTLs conferring leaf blast resistance have been mapped on different chromosomes [45]. Most of these QTLs were derived from *japonica indica* crosses of 15 different populations. Ashkani et al. [46] found 13 QTLs, conferring partial resistance to rice leaf blast in the F3 population of local Malaysian cultivars Pongsu seribu × Mashuri. McCouch et al. [47] mapped 10 putative QTLs for blast resistance in 12 rice chromosomes, whereas Taiben et al. [48] mapped 9 QTL by using RFLP markers.

Various markers such as SSRs, RFLP and ISSR have been used to detect QTLs for blast resistance in different population and environments [49]. The 23 blast resistance loci within the QTL regions found are *Pi30(t)*, *Pi7(t)*, *Pi34*, *Pi24(t)*, *Pitq6*, *Pi31(t)*, *Pi32(t)*, *PiGD-3(t)*, *PiGD-1(t)*, *Pi28(t)*, *PiGD-2(t)*, *Pilm2*, *Pitq1*, *Pizh*, *Pi29(t)*, *Pi35(t)*, *Pitq5*, *Pi25(t)*, *pi21*, *Pi26(t)*, *Pi27(t)*, *Pi24(t)*, *Pi25(t)* [50]. The detail about the mapped blast resistance genes are listed in Table 1.

4. Marker-assisted selection of blast resistance genes

Marker-assisted selection is a promising and efficient way to select the desirable characters indirectly in a breeding program by using different markers such as SSR, ISSR, RFLP, RAPD [49]. The selection of genes of interest depends upon the location of the marker and distance lying within gene and marker. The identification of marker linked to the gene of interest is a basic prerequisite of marker-assisted selection through linkage analysis. The tagging of genes becomes possible with the advent of molecular markers responsible for resistance to different pathogens [92].

The application of marker-assisted selection is very powerful in case of blast resistance breeding because single or a few genes are involved in the resistance mechanism [32]. The development of durable blast-resistant rice varieties against the different strains of *M. oryzae* progressed through the availability of different molecular markers applied for marker-assisted selection [93]. A dominant marker specific for the particular blast resistance gene has been developed, it can be used in a marker-assisted selection programme for the development of an improved blast-resistant rice cultivar [94].

SSR markers are not only used in marker-assisted selection for blast resistance genes, but also in diversity analysis and inheritance studies. Three major blast resistance genes, *Pi-b*, *Pi-k*, and *Pi-ta*, have been extensively studied by different researchers and are molecularly characterized. The inheritances from one generation to another have enabled these genes to become suitable for marker-assisted selection because of their Co-segregation with respect to the specific markers [26,69].

The selection of blast resistance genes through marker-assisted selection is very precise because of the governing true interaction of the particular resistance (*R*) gene with the avirulence gene. Several blast resistance genes *Pi-z*, *Pi-ta* *Pi-b*, *Pi-k* and *Pi-I* have been introgressed through marker-assisted selection into susceptible varieties [95,96]. Rice breeding for blast resistance has gained an importance after the characterization and analysis of *R* genes because the dissection and identification of these genes within local varieties become effortless. Blast resistance genes, *Pita* [97], *Pi37* [98], *Piz* [99], *Pi35* [90], and *Pi9* [100] have been introgressed through the application of marker-assisted selection. The number of disease-resistant cultivars can increase with the continuous transfer of newly DNA markers to breeders.

To facilitate the incorporation of *R* genes into elite breeding lines, few blast resistance genes have been characterized molecularly and tightly linked markers such as SSR, SNP have been developed [69,101]. MAS may increase the genetic improvement if information about the molecular markers associated with the disease resistance QTL becomes available [102]. Successful examples of MAS for blast resistance in rice are displayed in Table 3 (Fig. 1).

5. Identification of molecular markers linked to blast resistance genes

The identification of tightly linked markers with genes of interest is the basic step for the map-based cloning, molecular mapping, marker-assisted selection and other molecular techniques involved in biotic stress resistance in rice.

Table 1
List of available mapped blast resistance genes.

Gene	Chromosomal position	Position (bp)	Position (cM)	Donor rice variety	Method of identification	References
Pi24(t)	12	5242654–5556378	20.97–22.22	Azuenca (J)	QTL mapping	[52]
Pi62(t)	12	2426648–18050026	9.7–77	Yashiro-mochi (J), Tsuyuake	Mapped within 1.9 cM	[53]
Pitq6	12	5758663–7731471	23.0–30.92	Tequing (I)	QTL mapping	[48]
Pi6(t)	12	1–6725831	1–1.68	Apura (I)	–	[47]
Pi12	12	6988220–15120464	27.95–60.48	Moroberekan (J)	Linkage analysis using RFLP markers	[54]
Pi21(t)	12	5242654–5556378	20.94–22.22	Owarihatamochi (J)	–	[55]
Pi31(t)	12	7731471–11915469	30.92–47.66	IR64 (I)	QTL mapping	[37]
Pi32(t)	12	13103039–18867450	52.41–75.46	IR64 (I)	QTL mapping	[37]
Pi157	12	12375000-15550000	49.5–62.2	Moroberekan	Mapped within 9.5 cM	[17]
Pita	12	10603772–10609330	42.41–42.43	Tadukan (I)	Cloned	[56]
Pita-2	12	10078620–13211331	40.31–52.84	Shimokita (J)	Mapped within 4.0 cM	[57]
Pi19(t)	12	8826555–13417088	35.30–53.67	Aichi Asahi (J)	Linkage analysis to other resistance genes	[58]
Pi39(t)	12	–	–	Chubu 111 (J),	Mapped within 37 kb	[59]
Pi20(t)	12	12875000-12950000	51.5–51.8	IR24 (I)	Mapped within 0.6 cM	[60]
PiGD-3(t)	12	13950000	55.8	Sanhuangzhan 2	QTL mapping	[61]
Pia	11	4073024–8078510	1.01–2.09	Aichi Asahi (J)	–	[62]
PiCO39(t)	11	6304007–6888870	25.21–27.55	CO39 (I)	Mapped within 1.2 cM	[62]
Pilm2	11	13635033–28377565	54.54–113.5	Lemont	QTL mapping	[48]
Pi30(t)	11	441392–6578785	1.76–26.31	IR64 (I)	QTL mapping	[37]
Pi7(t)	11	17850000-21075000	71.4–84.3	RIL29 (Moroberekan)	QTL mapping	[23]
Pi34	11	19423000–19490000	77.69–77.96	Chubu32 (J)	QTL mapping	[36]
Pi38	11	19137900–21979485	76.55–87.91	Tadukan (I)	Mapped within 20 cM	[27]
PBR	11	20125000-30075000	80.5–120.3	St. No. 1	Mapped within 22.9 cM	[44]
Pb1	11	21425000-22850000	85.7–91.4	Modan	Mapped within 12.4 cM	[63]
Pi44(t)	11	22850000-29475000	91.4–117.9	RIL29 (Moroberekan)	–	[64]
Pik-h	11	24761902–24762922	99.0–99.05	Tetep	Mapped within 1.2 cM	[26]
Pi1	11	26498854–28374448	105.99–113.49	LAC23 (J)	Mapped within 11.4 cM	[65]
Pik-m	11	27314916–27532928	109.25–110.13	Tsuyuake (J)	Mapped within 0.3 cM	[66]
Pi18(t)	11	26796917–28376959	107.18–113.50	Suweon365 (J)	Mapped using RFLP markers	[67]
Pik	11	27314916–27532928	109.25–110.13	Kusabue (I)	Mapped within 1.4 cM	[68]
Pik-p	11	27314916–27532928	109.25–110.14	HR22 (I)	Mapped within 2.8 cM	[56]
Pik-s	11	27314916–27532929	109.25–110.15	Shin 2 (J)	Mapped within 2.7 cM	[69]
Pik-g	11	27314916–27532930	109.25–110.16	GA20 (J)	Linkage analysis to other resistance genes	[70]
Pise1	11	5740642–16730739	22.96–66.92	Sensho	Linkage analysis using phenotypic marker	[71]
Pi f	11	24695583–28462103	98.78–113.84	Chugoku 31-1 (St. No. 1)	QTL mapping	[72]
Mpiz	11	4073024–16730739	16.29–66.92	Zenith (J)	Linkage analysis using phenotypic markers	[73]
Pikur2	11	2840211–18372685	11.36–73.49	Kuroka (J)	Linkage analysis using phenotypic markers	[74]

Table 1 (*Continued*)

Gene	Chromosomal position	Position (bp)	Position (cM)	Donor rice variety	Method of identification	References
Piisi	11	2840211–19029573	11.36–76.11	Imochi Shirazu (J)	Linkage analysis using phenotypic markers	[71]
Pi28(t)	10	19565132–22667948	78.26–90.67	IR64 (I)	QTL mapping	[37]
Pii2(t)	9	1022662–7222779	4.09–28.89	Azucena	Linkage analysis using phenotypic markers	[75]
Pi5(t)	9	7825000-8250000	31.3–33.0	RIL125, RIL249, RIL260(Moroberekan)	Mapped within 170 kb	[76]
Pi3(t)	9	7825000-8250001	31.3–33.1	Kan-Tao	Linkage analysis using RFLP markers	[17]
Pi15	9	9641358–9685993	38.56–38.74	GA25 (J)	Mapped within 0.7 cM	[70]
Pii	9	2291804–28431560	9.16–113.72	Ishikari Shiroke (J)	Linkage analysis using phenotypic markers	[77]
Pi36	8	2870061–2884353	11.48–11.53	Q61 (I)	Cloned	[59]
Pi33	8	5915858–6152906	23.66–24.61	IR64 (I)	Mapped within 1.6 cM	[78]
Pizh	8	4372113–21012219	17.48–84.04	Zhai-Ya-Quing8 (I)	QTL mapping	[37]
Pi29(t)	8	9664057–16241105	38.65–64.96	IR64 (I)	Mapped within 0.7 cM	[37]
Pi17(t)	7	22250443–24995083	89.00–99.9	DJ 123	Mapped within 1.8 cM	[70]
Pi22(t)	6	4897048–6023472	19.5–24.09	Suweon365 (J)	QTL mapping	[55]
Pi26(t)	6	8751256–11676579	35.00–46.70	Azucena (J)	QTL mapping	[45]
Pi27(t)	6	5556378–744329	22.22–2.97	IR64 (I)	Mapped within 21.6 cM	[37]
Pi40(t)	6	16274830–17531111	65.09–70.12	O. australiensis(W)	Mapped within 1.8 cM	[79]
Piz	6	10155975–10517612	40.6–42.07	Zenith (J)	Mapped within 0.43 cM	[67]
Piz-t	6	14675000	58.7	Toride 1	Cloned	[56]
Pi9	6	10386510–10389466	41.5–41.55	O. minuta (W)	Cloned	[80]
Pi25(t)	6	18080056–19257588	72.32–77.03	Gumei 2	QTL mapping	[21]
Pid2	6	17159337–17163868	68.63–68.65	Digu	Cloned	[81]
Pigm(t)	6	10367751–10421545	41.47–41.68	Gumei 4	Mapped within 70 kb	[82]
Pi26(t)	5	8751256–11676579	35.00–46.70	Gumei 2 (I)	QTL mapping	[21]
Pi23(t)	5	10755867–19175845	43.02–76.70	Sweon 365	QTL mapping	[55]
Pi10	5	14521809–18854305	58.08–75.41	Tongil	Mapped within 6.7 cM	[83]
pi21	4	5242654–5556378	20.97–22.22	Owarihatamochi	QTL mapping	[55]
Pikur1	4	24611955–33558479	98.44–134.23	Kuroka (J)	Linkage analysis using phenotypic marker	[74]
Pi39(t)	4	26850000–27050000	107.4–108.2	Chubu 111 (J)	Mapped within 0.3 cM	[59]
Pi(t)	4	2270216–3043185	9.08–12.17	Tjahaja	Linkage analysis using phenotypic marker	[17]
Pid1(t)	2	21875000–22475000	87.5–89.9	Digu	Mapped within 11.8 cM	[84]
Pig(t)	2	34346727–35135783	137.38–140.54	Guangchangzhan (I)	Mapped within 11.8 cM	[85]
Pitq5	2	37625000–39475000	150.5–157.9	Teqing	QTL mapping	[48]
Piy1(t)	2	38300000–38525000	153.2–154.1	Yanxian No. 1	Mapped within 1.6 cM	[86]
Piy2(t)	2	38300000–38525001	153.2–154.1	Yanxian No. 1	Mapped within 3.0 cM	[86]
Pib	2	38300000–38525000	153.2–154.1	Tohoku IL9	Cloned	[87]
Pi25(t)	2	34360810–37725160	137.44–150.90	IR64 (I)	QTL mapping	[21]
Pi14(t)	2	1–6725831	1–26.90	Maowangu	Linkage analysis using isozyme markers	[70]
Pi16(t)	2	1–6725831	1–26.91	Aus373 (I)	Linkage analysis using isozyme markers	[88]

Table 1 (*Continued*)

Gene	Chromosomal position	Position (bp)	Position (cM)	Donor rice variety	Method of identification	References
Pit	1	2270216–3043185	9.08–12.17	Tjahaja	Mapped within 770 kb	[56]
Pi27(t)	1	6230045–6976491	24.29–27.90	IR64 (I)	Mapped within 21.6 cM	[37]
Pi24(t)	1	5242654–5556378	20.97–22.22	Azuenca (J)	QTL mapping	[35]
Pitp(t)	1	25135400–28667306	100.54–28667306	Tetep	Co-segregation marker was identified	[89]
Pi35(t)	1	33000000–34150000	132.0–136.6	Hokkai 188 (J)	QTL mapping	[90]
Pi37	1	33110281–33489931	132.44–133.95	St. No. 1 (J)	Cloned	[91]
Pish						
Pi67(t)	Map position still not identified					
Piss2						
Pise2						
Pise3						

Source: [12,26,51].
RFLP: restriction fragment length polymorphism; QTL: quantitative trait locus.

Recently, applications of molecular markers have been widely used in agriculture, especially in rice improvement research [16]. Molecular markers for blast resistance can be produced on different sites either on targeted or random sites to make more informative and dense genetic maps [103,104]. Accordingly, a large number of databases are available to select the molecular marker linked to *Pi* genes.

Molecular markers are powerful tools for monitoring the introgression of the gene of interest and for the detection of polymorphism within the different species [105]. The monitoring of an individual resistance gene and its pyramiding into a breeding line is very difficult; only a DNA marker tightly linked with the gene provides the straightforward way for the selection of multiple blast

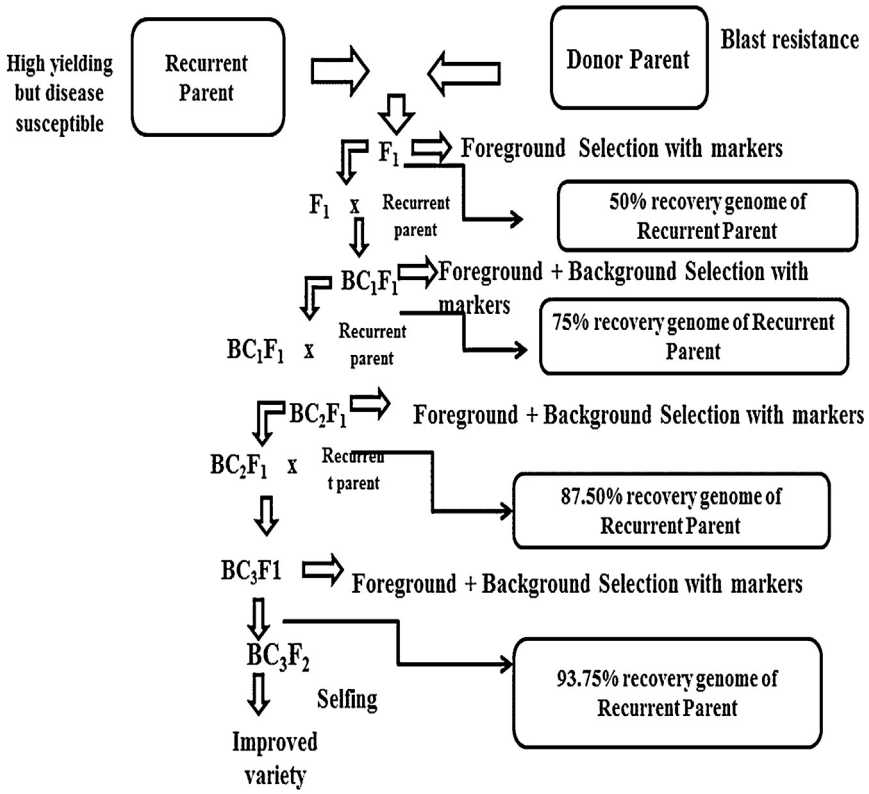

Fig. 1. Diagrammatic representation of general steps of marker-assisted backcross breeding.

resistance genes. The selection of the desired traits can be made at an early stage with the advent of molecular markers [106]. When a molecular marker is found very close to the gene of interest, it acts as a tag to be used for indirect selection in the molecular breeding program [25].

After the discovery of molecular markers, the selection of target traits becomes easier and many new cultivars have been developed accordingly. Nowadays, breeders are focusing on marker-assisted selection instead of using conventional breeding because it reduces the time for phenotypic selection, saves input costs, brings more reliability to select a desired trait with no influence of environmental factors [51].

Many DNA markers are directly linked with *Pi* genes in rice including simple sequence repeats (SSRs), amplified fragment length polymorphisms (AFLPs), and cleaved amplified polymorphic sequences (CAPS), random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), single-nucleotide polymorphisms (SNPs) and small insertions/deletions (InDels). SSRs and CAPS are PCR-based markers and require only a small amount of DNA for genotyping. These markers are very precise and cost effective and can be applied for the selection of plants containing blast resistance genes in rice at an early stage. Small InDels and SNPs markers are found in abundance and dispersed widely in the rice genome [107]. On the basis of information on these markers, Hayashi et al. [56] developed nine PCR-based markers linked with blast resistance in rice. These markers help in finding a gene within the desired target genome regions. Microsatellite markers, also called SSRs, are widely used for screening the blast-resistant and susceptible varieties. The difference between two varieties is based on polymorphism [105].

SSRs markers are the markers of choice for breeders because of their highly polymorphic nature; PCR-based and codominant SSR markers are widely used for studying genetic diversity among populations to estimate the proportion of the genome introgressed from the donor to the parents. More than 2000 SSR markers have been developed, linked with different blast resistance genes, which have thus accelerated the MAS and MABC for the improvement of rice cultivars [69]. The selection of allele with the advent of SSR markers is very precise, authentic and reliable because of the co-segregation of the marker. The target allele can segregate into homozygote, heterozygote and recessive allele with the application of SSR markers. The international Rice Microsatellite Initiative (IRMI) has developed a microsatellite map covering all 12 rice chromosomes, at least one microsatellite at the distance of 0.5 cM [108]. The multiline carrying more than one blast resistance gene can be developed with the help of SSRs markers, without screening against pathogens. Ashkani et al. [109] found six SSR markers, RM168, RM5961, RM413, RM1233, RM6836 and RM8225 directly associated with leaf blast resistance in a Malaysian rice variety. The major blast resistance genes *Pi-b*, *Pi-kh* and *Pi-ta* linked SSR markers have been developed and successfully introgressed into different susceptible rice cultivars through MAS [11,26,101,110].

Restriction Fragment Length Polymorphism (RFLP) is a non-PCR-based technique used to construct the genetic map of a species. The selection of the desired DNA sequence depends on different probes obtained after digestion with restriction enzymes. RFLPs markers are codominant; informative, reliable but very costly and time consuming. Several RFLPs markers linked with blast resistance genes conferring complete resistance have been mapped on different chromosomes in rice. A RFLP marker linked with *Pi-2(t)* was identified by Yu et al. [111], whereas Tohme et al. [112] also identified a RFLP linked with *Pi-4(t)*. The inheritance of complex traits such as blast resistance becomes possible with the advent of RFLP [113]. Several SNP, STS, InDel, and CAPS markers have been identified, linked with different blast resistance genes and are mentioned in Table 2.

6. Marker-assisted backcrossing for blast resistance

Marker-assisted backcross breeding has received attention for the introgression of blast resistance genes into the genomic background of the susceptible varieties of rice. The main objective of marker-assisted backcross breeding is to reduce the donor genome content into the target varieties.

Mainly, two steps i.e., foreground selection and background selection, are applied in backcrossing. Foreground selection is done by selecting a specific marker near the target genome, whereas background selection is applied by dispersing markers throughout the genome for the recovery of the recurrent parent [120].

Backcross breeding has been widely used for the introgression of qualitative characters such as resistance against specific pathotype disease controlled by a single dominant gene [121]. The main purpose of MABC is to transfer the desired character along with recovering the recurrent parent characters. Marker-assisted backcrossing is currently playing an important role in plant breeding and genetic engineering for the development of blast-resistant cultivars [122]. The implementation of marker-assisted backcross breeding is time saving and cost effective over conventional plant breeding [123].

Recently, blast resistance genes *Piz5* and *Pi54* have been introgressed into the genetic background of PRR78 rice variety from donor parent C101A51 and Tetep, and blast-resistant lines have been developed with the application of marker-assisted backcross breeding [124]. The selection was based on foreground markers RM287 and RM206 by following repetitive backcrossing. *Pi1* leaf blast resistance gene has been introgressed into D521 line derived from donor line BL122 [125].

With the application of marker-assisted backcrossing elite, 304 parental lines of hybrid rice have also been improved with bacterial blight and blast resistance [126]. Marker-assisted backcross breeding has been applied for both biotic and abiotic stresses. Recently from IR64 cultivar submergence, tolerant gene *Sub1* has been introgressed into OM1490 variety [127]. The QTL saltol derived from a salt-tolerant variety has also been introgressed into popular cultivars of Vietnam [128]. The

Table 2
Blast resistance genes and their respective tightly linked DNA markers.

Type of marker							
Gene	Chromosome	SSR	SNP	STS	InDel	CAP	References
Pi27(t)	1	RM151,RM259					[114]
Pi37	1	RM302,RM212 FPSM1, FPSM2,FPSM4					[98]
Pi35(t)	1	RM1216,RM1003					[90]
Pitp(t)	1	RM246					[89]
Pid1(t)	2	RM262					[84]
Pig(t)	2	RM166,RM208					[114]
Piy1	2	RM3248,RM20					[86]
Piy2	2	RM20,RM3248					[86]
Pib	2	RM138,RM166,RM208, RM266,RM138,RM166	b213,b28,b2, b3989,Pibdom				[86]
pi21	4			P702D03	OPF62700 OPF62700		[84]
Pi39	4	RM3843,RM5473					[115]
Pi10	5				OPF62700		[116]
Pi40(t)	6	RM3330,RM527				S2539	[79]
Piz	6		z60510,z5765, z56592,z565962				[56]
Piz-t	6		z60510,z5765, zt56591 zt5659		z4794		[56]
Pigm(t)	6				z4794	C26348	[82]
Pi36	8	RM5647			S47656	CRG2,CRG3, CRG4	[61]
Pi33	8	RM72,RM44					[78]
Pi5(t)	9		JJ817			94A20r,76B14f 40N23r	[117]
Pia	11					yca72	[76]
PiCO39	11					RGA8, RZ141 yca72, RGACO39	[62]
Pi38	11	RM206,RM21					[27]
Pik	11		K6438, K6415K6415, K8823,K39512		K6816 K2167		[56]
Pik-m	11				K6816 K2167		[56]
Pik-p	11						[56]
Pi-kh	11	RM206,TRS26,TRS33, RM144,RM224,RM1233, RM224					[118]
Pik-s	11	RM144, RM224RM1233RM144, RM224					[69]
Pita	12	OSM89,RM155, RM7102RM7102	ta642,ta801ta3, ta577,ta5,Pi-ta 440, Pita-1042,Pi-ta 403				[56]
Pita-2	12		ta801,ta642, ta3 ta577,ta5				[56]
Pi20(t)	12	RM1337,RM5364, RM7102					[119]
Pi39(t)	12					39M6, 39M7	[59]

CAP: cleaved amplified polymorphic; InDel: insertion/deletion.

Table 3
Some successful examples of MAS for blast resistance in rice cultivars.

S.no	Trait	Genes	Type of markers	Method applied for transferring target gene	References
1	Blast resistance	*Pi1*, *Piz-5 Pita*	RFLP	Marker-assisted-selection applied for combining these genes into a single cultivar Co-39	[65]
2	Blast resistance	*Pi54*	SSR	Marker-assisted-selection applied to improve Pusa Basmati 1 from Tetep	[124]
3	Blast resistance	*Pid1*, *Pib* and *Pita*	SSR	Introduced into G46B cultivar through MAS	[84]
4	Blast resistance	*Pi2*	SSR	Introduced into Zhenshan97B through MAS	[84]
5	Blast resistance	*Pi1*, *Pi2*, *Pi33*	SSR	Introgressed into Jin23B cultivar through marker-assisted backcrossing	[129]
6	Blast resistance	*Pi1*, *Piz-5*, *Pi2*, *Pita*	RFLP, SSR, ISSR	Introduced into C039 cultivar through MAS	[65]
7	Blast resistance	*Pi-9 (t)*	pB8 (gene specific marker)	MABC applied to introgress into Luhui17 cultivar	[130]
8	Blast resistance	*Pi-ta*	Gene specific marker	MAS applied	[131]

RFLP: restriction fragment length polymorphism; MAS: marker-assisted-selection.

successful example of MAS is mentioned in Table 3 and general steps of marker assisted backcross breeding have been described in Fig 1.

7. Cloning of blast resistance genes

This is the most effective and direct approach for the characterization and identification of the blast resistance gene. The first step for cloning is the fine mapping of genes closely linked with the DNA marker, followed by molecular cloning of the desired gene.

So far, more than 100 blast resistance genes have been identified in both *indica* and *japonica* rice cultivars. Out of them, only 19 blast resistance genes have been cloned and characterized molecularly [5]. Map-based cloning is also called positional cloning; it sometimes reverses genetics because it does not require information about the gene or its gene product; however, the knowledge of the

Table 4
List of cloned blast resistance gene.

S.no	Name of gene	Year of cloning	Chromosome	Cultivar name	Type of rice (I – *indica*; J – *japonica*)	Characteristics	References
1	*Pib*	1999	2	Tohoku IL9	J	NBS–LRR	[87]
2	*pi-ta*	2000	12	Tadukan	I	NBS–LRR	[110]
3	*pi54*	2005	11	Tetep	I	NBS–LRR	[26]
4	*pi2*	2006	6	Jefferson	J	Lectin receptor	[133]
5	*pi-zt*	2006	6	Zenith	I	NBS–LRR	[134]
6	*pi9*	2006	6	75-1-127 (101141)	*Oryza minuta*	NBS–LRR	[80]
7	*pid2*	2006	6	Digu	I	NBS–LRR	[81]
8	*pi36*	2007	8	Q61	I	CC–NBS–LRR	[61]
9	*pik-m*	2008	1	Tsuyuake	I	NBS–LRR	[135]
10	*pi37*	2008	11	St. No. 1	J	NBS–LRR	[98]
11	*pi21*	2009	9	Owarihatamochi	J	CC–NBS–LRR	[44]
12	*pid3*	2009	1	Digu	I	CC–NBS–LRR	[136]
13	*pit*	2009	6	Tjahaja	J	NBS–LRR	[56]
14	*pi5*	2009	4	Moroberekan	J	NBS–LRR	[117]
15	*pb1*	2010	1	Modan	I	CC–NBS–LRR	[137]
16	*pish*	2010	11	Shin 2	J	CC–NBS–LRR	[51]
17	*pik*	2011	11	Koshiminori	I	CC–NBS–LRR	[138]
18	*pikp*	2011	11	HR22	I	CC–NBS–LRR	[56]
19	*pia*	2011	11	Aichi Asahi	J	CC–NBS–LRR	[139]

NBS-LRR: nucleotide binding site-Leucine-rich repeat; CC: coiled-coil.

chromosomal location of the desired gene is essential [132].

The interest in cloning and characterizing blast resistance genes among researchers increased when Wang et al. [87] successfully cloned *Pi-b* gene from cultivar Tohoku IL9. *Pi-ta* was another major blast resistance gene cloned after *Pi-b* [110]. After the successful cloning of these genes, Sharma et al. [26] isolated and characterized the *Pi-kh* gene from cultivar Tetep. Afterwards *Pi-d2*, *Pi9*, *Pi-2*, *Piz-t*, *Pi36*, *Pi37*, *Pik-m*, *Pi5*, *Pit*, *Pid3*, *Pi21*, *Pis-h*, *PB1*, *Pi-k*, *Pik-p*, *Pia* have been cloned from different *indica* and *japonica* cultivars (see Table 4 for references).

The genetic dissection of cloned genes revealed that they contain the NBS-LRR domain, except *Pid-2*, which contains the receptor kinase domain. The maximum distributions of cloned genes have been found on chromosomes 6, 11 and 12 (Fig. 2).

NBS-LRR is one of major protein family, providing disease resistance. NBS-LRR is further divided into two subfamilies based on the presence of Toll/interleukin-1 receptor (TIR) or coiled-coil (CC) motifs in the amino-terminal domain [140]. Among 19 cloned blast resistance genes, 10 genes fall into a category of NBS-LRR proteins, eight into CC-NBS-LRR type domain, while *Pid-2* possesses a unique type of protein called B-lectin receptor a having serine threonine kinase gene (Fig. 3).

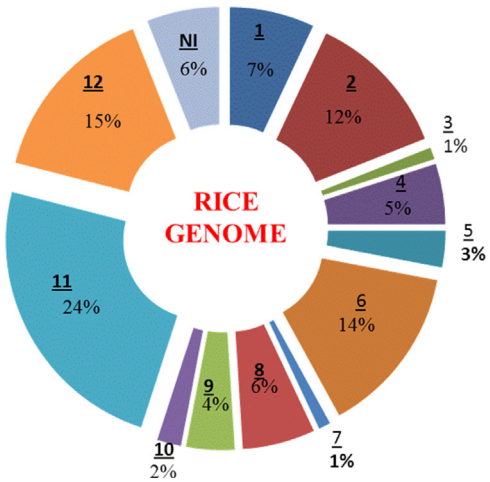

Fig. 2. (Colour online.) Distribution of blast resistance genes among 12 chromosomes of rice. Underlined numbers represent the chromosome position. NI indicates that no information available.
The figure was modified from Sharma et al. [26].

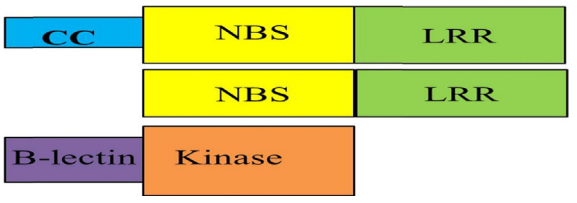

Fig. 3. (Colour online.) Different types of domain combinations found among blast resistance genes present in rice.

8. Allele mining strategies for blast resistance genes

The major goal of plant breeding is to find the superior/novel agronomic characters. The amount of variation found within the plant germplasm contributes significantly to achieve this goal. All the plants have the potential to collect a beneficial allele for certain agronomic traits, whereas the evolution of novel or new alleles depends heavily upon the natural mutations [141]. There are many factors involved in mutation in plants such as tranvertions, InDels, and transitions. Allele mining is an advanced and newly developed technique to determine the allelic variation at candidate gene regulating important agronomic traits. With the passage of time, allele mining opened a new avenue for the validation of blast resistance genes and mining of favourable alleles present at *Pi* loci [142]. The identification of alleles of identified gene/locus responsible for controlling any given trait or identification of superior allele from the natural population is called allele mining. Usually two approaches are used for allele mining in the naturally developed populations, i.e. Targeting Induced Local Lesions in Genomes (TILLING) [143], called EcoTilling and Re-sequencing [144], or sequencing-based allele mining. In TILLING, heteroduplex analysis is carried out to directly identify the induced point mutation in the gene. TILLING enables the identification of single-base-pair (bp) allelic variation in a target gene in a high throughput manner. This technique employs the mismatch-specific endonuclease in order to detect induced or natural DNA polymorphisms [145]. The fragments produced are separated and the site of mutation can be identified by the fragment size analysis. Sequence- and sequencing-based allele mining consists in a PCR-based amplification of alleles of a gene in varied genotypes, then in DNA sequencing to recognize the nucleotide variance in alleles. The sequences are further analysed for the presence of SNPs, and InDels. The SNPs and InDels help to construct the haplotyps. Haplotypes are the basis for understanding the effect of mutation on organization and gene structure. Both above-described approaches have been widely used for the dissection of important blast resistance genes from wild rice species. From the identified rice blast resistance genes, *Pi1* [146], *Pik* [147] *Pi-ta* [148], *Pi1* [111], *Pi2* [149], *Pib* [87], *Pi9* [133], *Pi5* [117], and *Pik-p* [150] are considered as very well defined. The identification of blast resistance genes *Pi9*, *Pi2* and *Piz-t* from different rice cultivars showed that these genes tend to be an allele. The physical locations of these genes are on the same locus, but express different levels of resistance spectra in different cultivars

Table 5
Current status of allele mining for blast resistance in rice.

Name of gene	Trait control	Allelic gene	Rice variety	References
Pid3	Blast resistance	*Pid3-A4*	Digu	[154]
Pi-ta	Blast resistance	*Pita (Konibora)*	(Konibora)	[155]
Pi-kh	Blast resistance	*Pi54*	Tetep	[26]
Pi-2	Blast resistance	*Pi2-2*	Jefferson	[156]

[106,151,152]. Allele mining for *Pi-kh*, *Pi-ta* and *Pi-zt* genes has been also reported in Indian land races of rice [153]. Allele mining can be essential for determining and utilizing novel alleles, hidden in genetic diversity. The identified blast resistance genes through allele mining strategy have been described in Table 5.

9. Conclusion

The demand for rice is increasing day by day due to the increase in the population globally. To fulfil the dietary requirements, high yielding varieties of rice are required. The high yield can be obtained by reducing the yield losses due to pest and disease damage. To overcome these losses because of diseases, molecular breeding techniques have expedited the rice research at the DNA level to explore the mechanism of host–pathogen interaction. Rice breeding has gained many direct and indirect advantages from rice genomic research, but further research is still required to exploit tools, knowledge, and research in breeding programs.

The comparison between the sequences of different blast resistance genes with different spectrum of resistance will lead to identify the specific DNA region responsible for the resistance specificity. The application of molecular techniques to produce the broad-spectrum resistance in rice is increasing consistently to reduce the cost of inputs.

Although many blast resistance genes have been mapped and cloned, they provide resistance to specific pathotypes. Due to the genetic stability and pathogenic variability, the rice varieties have rapidly been broken down. The need for mapping and cloning has increased so as to identify more specific and suitable blast resistance genes that confer resistance to different pathotypes of *M. oryzae*.

The QTL identification, fine mapping, marker-assisted selection, cloning of blast resistance genes are valuable tools to understand the genetic mechanism and function of genes. The QTL mapping requires a large population; however, it is not possible to identify the precise position of the desired genes until cloning and fine mapping is done. Hundreds of QTLs has been cloned that control different traits in rice, but still more QTLs needs to be cloned and identified in the future. Map-based cloning methods have investigated many QTLs for specific resistance genes, which can be applied in rice breeding programmes.

This article will be helpful to study the current advanced methods of gene identification that could be utilized to study the structure, function and regulation of genes involved for providing resistance against blast disease. The different molecular techniques will accelerate the development of resistant cultivars to meet the demand for rice at the global level.

10. Future prospects

The majority of world countries consume rice as a basic food because of its high carbohydrate value and also because it is financially affordable. Rice production methods have been improved in the last decades due to rice breeding and improved cultural practice, but blast diseases have caused heavy loses to rice and are considered as major constraints to sustainable rice production. The instability of pathogens in the field subjected rice breeders to pressure for producing more resistant varieties having durable resistance.

There will be more blast resistance genes introduced in the future by dissecting the interaction between the *R* genes and AVR protein. To date, few blast resistance genes have been characterized; in the future, more blast resistance genes will be characterized.

A differential system for gene characterization is necessary. Currently, monogenic lines and nearly isogenic lines have been released with the collaborative research project of IRRI-Japan. These lines will be useful as gene sources/donor parents for developing blast-resistant varieties and international standard differential varieties used for the characterization of blast resistance genes. Breeders have to explore more selection markers applicable for MAS on the basis of an increasing number of blast resistance genes.

Till to date, blast genes identified by different researchers provide resistance against the leaf blast. Most of the blast-resistant rice varieties produced yet provide monogenic resistance; sometimes, because of monogenic resistance, the cultivar loses its resistance. The tagging blast resistance genes with molecular markers have already been achieved. It will be more feasible if breeders work with several genes and pyramid into elite rice cultivars. The IR64 variety is the best example: it contains at least five blast resistance genes that provide them with durable resistance.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgments

The authors would like to acknowledge Long-Term Research Grant Scheme (LRGS), Food Security Project, Ministry of Education, Malaysia, for the financial support for conducting research on rice breeding. The author would also like to acknowledge Sindh Agriculture University Tandojam, Sindh, Pakistan, for providing financial support.

References

[1] Food and Agricultural Organization of the United Nations (FAO). Food and Population: FAO Looks ahead, 2004.
[2] G.S. Khush, K. Jena, Current status and future prospects for research on blast resistance in rice (*Oryza sativa* L.), in: X. Wang, B. Valent (Eds.), Advances in Genetics, Genomics and Control of Rice Blast Disease, Springer, Dordrecht, 2009, pp. 1–10.
[3] G.S. Khush, What it will take to feed 5.0 billion rice consumers in 2030, Plant Mol. Biol. 59 (1) (2005) 1–6.
[4] K. Selvaraj, S. Chander, M. Sujithra, Determination of multiple-species economic injury levels for rice insect pests, Crop Prot. 32 (2012) 150–160.
[5] T. Sharma, A. Rai, S. Gupta, J. Vijayan, B. Devanna, S. Ray, Rice blast management through host-plant resistance: retrospect and prospects, Agric. Res. 1 (1) (2012) 37–52.
[6] S.Y. Padmanabhan, Estimating losses from rice blast in India, in: The Rice Blast Diseases, John Hopkins Press, Baltimore, MA, USA, 1965 pp. 203–221.
[7] D. Parker, M. Beckmann, P. Enot, D.P. Overy, Z.C. Rios, M. Gilbert, J. Draper, Rice blast infection of *Brachypodium distachyon* as a model system to study dynamic host/pathogen interactions, Nat. Protoc. 3 (3) (2008) 435–445.

[8] F. Song, R.M. Goodman, Molecular biology of disease resistance in rice, Physiol. Mol. Plant Pathol. 59 (1) (2001) 1–11.
[9] Y. Takahashi, Studies on the mechanism of the resistance of rice plant to *Piricularia oryzae*, in: The Rice Blast Diseases, John Hopkins Press, Baltimore, MA, 1965, pp. 303–329.
[10] S.H. Ou, Rice Diseases, Second ed., Commonwealth Mycological Institute, Kew, Surrey, England, 1985.
[11] M. Wang, S. Van Bergen, B. Van Duijn, Insights into a key developmental switch and its importance for efficient plant breeding, Plant Physiol. 124 (2) (2000) 523–530.
[12] G. Miah, M. Rafii, M. Ismail, A. Puteh, H. Rahim, R. Asfaliza, M. Latif, Blast resistance in rice: a review of conventional breeding to molecular approaches, Mol. Biol. Rep. 40 (3) (2013) 2369–2388.
[13] B. Antonio, A. Shomura, T. Shimano, Y. Kuboki, S. Lin, T. Inoue, Y. Nagamura, High-density linkage map of rice with expressed sequence tags, Rice Genet. 3 (1996) 34–56.
[14] R. Sasaki, Existence of strains in rice blast fungus, Jpn. J. Plant Protect. 9 (1922) 631–644.
[15] S. Kiyosawa, Gene analysis for blast resistance, Oryza 18 (1982) 196–203.
[16] S. McCouch, G. Kochert, Z. Yu, Z. Wang, G. Khush, W. Coffman, S. Tanksley, Molecular mapping of rice chromosomes, Theor. Appl. Genet. 76 (6) (1988) 815–829.
[17] M.A. Causse, M. Fulton, Y.G. Cho, S.N. Ahn, J. Chunwongse, K. Wu, S.E. Harrington, Saturated molecular map of the rice genome based on an interspecific backcross population, Genetics 138 (4) (1994) 1251.
[18] A. Saito, M. Yano, N. Kishimoto, M. Nakagahra, A. Yoshimura, K. Saito, S. Kuhara, Y. Ukai, M. Kawase, T. Nagamine, S. Yoshimura, O. Ideta, R. Ohsawa, Y. Hayano, N. Iwata, M. Sugiura, Linkage map of restriction fragment length polymorphism loci in rice, Jpn. J. Breed. 41 (1991) 665–670.
[19] J. Welsh, M. McClelland, Fingerprinting genomes using PCR with arbitrary primers, Nucleic Acids Res. 18 (24) (1990) 7213–7218.
[20] J.G. Williams, A.R. Kubelik, K.J. Livak, J.A. Rafalski, S.V. Tingey, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, Nucleic Acids Res. 18 (22) (1990) 6531–6535.
[21] K.S. Wu, S.D. Tanksley, Abundance, polymorphism and genetic mapping of microsatellites in rice, Mol. Gen. Genet. 241 (1–2) (1993) 225–235.
[22] N. Huang, E. Angeles, J. Domingo, G. Magpantay, S. Singh, G. Zhang, G. Khush, Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR, Theor. Appl. Genet. 95 (3) (1997) 313–320.
[23] G.L. Wang, D.J. Mackill, J.M. Bonman, S.R. McCouch, M.C. Champoux, R.J. Nelson, RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar, Genetics 136 (4) (1994) 1421–1434.
[24] I.R.G.S. Project, The map-based sequence of the rice genome, Nature 436 (7052) (2005) 793–800.
[25] D. Mackill, M. Salam, Z. Wang, S. Tanksley, A major photoperiod-sensitivity gene tagged with RFLP and isozyme markers in rice, Theor. Appl. Genet. 85 (5) (1993) 536–540.
[26] T. Sharma, M. Madhav, B. Singh, P. Shanker, T. Jana, V. Dalal, H. Upreti, High-resolution mapping, cloning and molecular characterization of the *Pi-kh* gene of rice, which confers resistance to *Magnaporthe grisea*, Mol. Genet. Genomics 274 (6) (2005) 569–578.
[27] M. Gowda, S. Roy-Barman, B. Chattoo, Molecular mapping of a novel blast resistance gene Pi38 in rice using SSLP and AFLP markers, Plant Breed. 125 (6) (2006) 596–599.
[28] P. Kumar, S. Pathania, P. Katoch, T. Sharma, P. Plaha, R. Rathour, Genetic and physical mapping of blast resistance gene Pi-42 (t) on the short arm of rice chromosome 12, Mol. Breed. 25 (2) (2010) 217–228.
[29] B. Monosi, R. Wisser, L. Pennill, S. Hulbert, Full-genome analysis of resistance gene homologues in rice, Theor. Appl. Genet. 109 (7) (2004) 1434–1447.
[30] M.J. Kearsey, H.S. Pooni, The Genetical Analysis of Quantitative Traits, Stanley Thornes Publishers Ltd., UK, 1998.
[31] A.E. Melchinger, H.F. Utz, C.C. Schön, Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects, Genetics 149 (1) (1998) 383–403.
[32] N. Young, QTL mapping and quantitative disease resistance in plants, Annu. Rev. Phytopathol. 34 (1) (1996) 479–501.
[33] M. Mohan, S. Nair, A. Bhagwat, T. Krishna, M. Yano, C. Bhatia, T. Sasaki, Genome mapping, molecular markers and marker-assisted selection in crop plants, Mol. Breed. 3 (2) (1997) 87–103.
[34] J. Lopez-Gerena, Mapping QTL Controlling Durable Resistance to Rice Blast in the Cultivar Oryzica Llanos 5, (Ph.D. thesis), Universidad del Valle, Plant Pathology College of Agriculture, Cali, Colombia and Kansas State University, Manhattan, KS, USA, 2006.
[35] S. Koizumi, Durability of resistance to rice blast disease, JIRCAS Working Rep. 53 (2007) 1–10.
[36] K. Zenbayashi, T. Ashizawa, T. Tani, S. Koizumi, Mapping of the QTL, Theor. Appl. Genet. 104 (4) (2002) 547–552.
[37] C. Sallaud, M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P. Svestasrani, J.L. Notteghem, Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy, Theor. Appl. Genet. 106 (5) (2003) 794–803.
[38] P. Kongprakhon, A. Cuesta-Marcos, P.M. Hayes, V. Hongtrakul, P. Sirithunya, T. Toojinda, N. Sangduen, Four QTL in rice associated with broad-spectrum resistance to blast isolates from rice and barley, J. Phytopathol. 158 (2) (2010) 125–131.
[39] J.E. Parlevliet, Components of resistance that reduce the rate of epidemic development, Annu. Rev. Phytopathol. 17 (1) (1979) 203–222.
[40] J.M. Bonman, D. Mackill, Durable resistance to rice blast disease, Oryza 25 (2) (1988) 103–110.
[41] K. Zenbayashi-Sawata, T. Ashizawa, S. Koizumi, Pi34-AVRPi34: a new gene-for-gene interaction for partial resistance in rice to blast caused by *Magnaporthe grisea*, J. Gen. Plant Pathol. 71 (6) (2005) 395–401.
[42] T. Yunoki, A. Ezuka, T. Morinaka, Y. Sakurai, Studies on the varietal resistance to rice blast. 4. Variation of field resistance due to fungus strains, Bull. Chugoku Agric. Exp. Stn. Ser. A (6) (1970) 21–41.
[43] S. Fukuoka, K. Okuno, QTL analysis and mapping of pi21, a recessive gene for field resistance to rice blast in Japanese upland rice, Theor. Appl. Genet. 103 (2–3) (2001) 185–190.
[44] K. Fujii, Y. Hayano-Saito, A. Shumiya, M. Inoue, Genetical mapping based on the RFLP analysis for the panicle blast resistance derived from a rice parental line St. No. 1, Breed Sci. 45 (Suppl. 1) (1995) 209.
[45] J.L. Wu, Y.Y. Fan, D.B. Li, K.L. Zheng, H. Leung, J.Y. Zhuang, Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates, Theor. Appl. Genet. 111 (1) (2005) 50–56.
[46] S. Ashkani, M. Rafii, H. Rahim, M. Latif, Genetic dissection of rice blast resistance by QTL mapping approach using an F3 population, Mol. Biol. Rep. 40 (3) (2013) 2503–2515.
[47] S. McCouch, R. Nelson, J. Tohme, R. Zeigler, Mapping of blast resistance genes in rice, in: R.S. Zeigler, S.A. Leong, P.S. Teng (Eds.), Rice blast disease, CAB International, Wallingford, UK, 1994, pp. 167–186.
[48] R. Tabien, Z. Li, A. Paterson, M. Marchetti, J. Stansel, S. Pinson, Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties, Theor. Appl. Genet. 105 (2–3) (2002) 313–324.
[49] M.A. Latif, M.Y. Rafii, M.M. Rahman, M.R.B. Talukdar, Microsatellite and minisatellite markers based DNA fingerprinting and genetic diversity of blast and ufra resistant genotypes, C. R. Biologies 334 (4) (2011) 282–289.
[50] S. Fukuoka, S.I. Yamamoto, R. Mizobuchi, U. Yamanouchi, K. Ono, N. Kitazawa, S. Koizumi, Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast, Sci. Rep. 4 (2014) 4550, http://dx.doi.org/10.1038/srep04550.
[51] Y. Koide, N. Kobayashi, D. Xu, Y. Fukuta, Resistance genes and selection DNA markers for blast disease in rice, *Oryza sativa*, Jpn. Agric. Res. Quart. (JARQ) 43 (4) (2010) 255–280.
[52] J.Y. Zhuang, W.B. Ma, J.L. Wu, R.Y. Chai, J. Lu, Y.Y. Fan, K.L. Zheng, Mapping of leaf and neck blast resistance genes with resistance gene analog, RAPD and RFLP in rice, Euphytica 128 (3) (2002) 363–370.
[53] K. Wu, C. Martinez, Z. Lentini, J. Tohme, F. Chumley, P. Scolnik, B. Valent, Cloning a blast resistance gene by chromosome walking, in: G.S. Khush (Ed.), Rice Genetics III. Proceedings of the Third International Rice Genetics Symposium, IRRI: International Rice Research Institute, Manila, Philippines, 1996, pp. 669–674.
[54] T. Inukai, R. Nelson, R. Zeigler, S. Sarkarung, D. Mackill, J. Bonman, T. Kinoshita, Genetic analysis of blast resistance in tropical rice cultivars using near isogenic lines, in: G.S. Khush (Ed.), Rice Genetics III. Proc 3rd Int Rice Genet Symp, Oct. 16–20 1995, Manila, The Philippines, 1996, pp. 447–450.
[55] S.N. Ahn, Y.K.H.C. Hong, S.S. Han, H.C. Choi, S.R. McCouch, H.P. Moon, Mapping of genes conferring resistance to Korean isolates of rice blast fungus using DNA markers, Kor. J. Breed. 29 (4) (1997) 416–423.
[56] K. Hayashi, H. Yoshida, I. Ashikawa, Development of PCR-based allele-specific and InDel marker sets for nine rice blast resistance genes, Theor. Appl. Genet. 113 (2) (2006) 251–260.
[57] S. Nakamura, S. Asakawa, N. Ohmido, K. Fukui, N. Shimizu, S. Kawasaki, 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, Mol. Gen. Genet. 254 (6) (1997) 611–620.
[58] N. Iwata, Registration of new gene symbols, Rice Genet. News 13 (1996) 12–18.

[59] X. Liu, Q. Yang, F. Lin, L. Hua, C. Wang, L. Wang, Q. Pan, Identification and fine mapping of *Pi39(t)*, a major gene conferring the broad-spectrum resistance to *Magnaporthe oryzae*, Mol. Genet. Genomics 278 (4) (2007) 403–410.

[60] W.G. Liu, S.J. Jin, X.Y. Zhu, F. Wang, J.H. Li, Z.R. Liu, Y.B. Liu, Improving blast resistance of a thermo-sensitive genic male sterile rice line GD-8S by molecular marker-assisted selection, Rice Sci. 15 (3) (2008) 179–185.

[61] X. Liu, L. Wang, S. Chen, F. Lin, Q. Pan, Genetic and physical mapping of *Pi36(t)*, a novel rice blast resistance gene located on rice chromosome 8, Mol. Genet. Genomics 274 (4) (2005) 394–401.

[62] R. Chauhan, M. Farman, H.B. Zhang, S. Leong, Genetic and physical mapping of a rice blast resistance locus, *PiCO39 (t)* that corresponds to the avirulence gene AVR1-CO39 of *Magnaporthe grisea*, Mol. Genet. Genom. 267 (5) (2002) 603–612.

[63] H.Y. Fujii, K. Saito, N. Sugiura, N. Hayashi, T. Tsuji, T.I.M. Izawa, Identification of a RFLP marker tightly linked to the panicle blast resistance gene, *Pb1*, in rice, Breed. Sci. 50 (3) (2000) 183–188.

[64] D.H. Chen, M. Dela Vina, T. Inukai, D. Mackill, P. Ronald, R. Nelson, Molecular mapping of the blast resistance gene, Pi44 (t), in a line derived from a durably resistant rice cultivar, Theor. Appl. Genet. 98 (6–7) (1999) 1046–1053.

[65] S. Hittalmani, A. Parco, T. Mew, R. Zeigler, N. Huang, Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice, Theor. Appl. Genet. 100 (7) (2000) 1121–1128.

[66] L.Y. Li, L. Wang, J.X. Jing, Z.Q. Li, F. Lin, L.F. Huang, Q.H. Pan, The *Pik-m* gene, conferring stable resistance to isolates of *Magnaporthe oryzae*, was finely mapped in a crossover-cold region on rice chromosome 11, Mol. Breed. 20 (2) (2007) 179–188.

[67] S. Ahn, Y. Kim, S. Han, H. Choi, H. Moon, S. McCouch, Molecular mapping of a gene for resistance to a Korean isolate of rice blast, Rice Genet. Newslett. 13 (1996) 74–76.

[68] H. Hayasaka, RFLP mapping of a rice blast resistance gene Pi-k, Breed. Sci. 46 (Suppl. 2) (1996) 68 (in Japanese).

[69] R. Fjellstrom, C.A. Conaway-Bormans, A.M. McClung, M.A. Marchetti, A.R. Shank, W.D. Park, Development of DNA markers suitable for marker-assisted-selection of three genes conferring resistance to multiple pathotypes, Crop Sci. 44 (5) (2004) 1790–1798.

[70] Q. Pan, L. Wang, H. Ikehashi, T. Tanisaka, Identification of a new blast resistance gene in the *indica* rice cultivar Kasalath using Japanese differential cultivars and isozyme markers, Phytopathology 86 (10) (1996) 1071–1075.

[71] I. Goto, Genetic studies on the resistance of rice plant to the blast fungus I. Inheritance of resistance in crosses Sensho x H-79 and Imochishirazu x H-79, Ann. Phytopathol. Soc. Jpn. 36 (1970) 304–312.

[72] H. Shinoda, Studies on the varietal resistance of rice to blast. 6. Linkage relationship of blast resistance genes, Bull. Chugoku Agric. Exp. St. Ser. A 20 (1971) 1–25 (in Japanese).

[73] I. Goto, Genetic studies on resistance of rice plant to blast fungus, difference in resistance to the blast disease between Fukunishiki and its parental cultivar, Zenith, Ann. Phytopathol. Soc. Jpn. 42 (3) (1976) 253–260.

[74] I. Goto, Genetic studies on resistance of rice plant to blast fungus, 7: blast resistance genes of Kuroka, Ann. Phytopathol. Soc. Jpn. 54 (1988) 460–465.

[75] T. Kinoshita, S. Kiyosawa, Some considerations on linkage relationships between *Pii* and Piz in the blast resistance of rice, Rice Genet. Newslett. 14 (1997) 57–59.

[76] S.W. Kwon, Y.C. Cho, Y.G. Kim, J.P. Suh, J.U. Jeung, J.H. Roh, Y.T. Lee, Development of near isogenic *japonica* rice lines with enhanced resistance to *Magnaporthe grisea*, Mol. Cells 25 (3) (2008) 407–416.

[77] K. Ise, Linkage analysis of some blast resistance gene in rice, *Oryza sativa* L., Jpn. J. Breed. 42 (Suppl. 2) (1991) 388–389 (in Japanese).

[78] R. Berruyer, H. Adreit, J. Milazzo, S. Gaillard, A. Berger, W. Dioh, D. Tharreau, Identification and fine mapping of *Pi33*, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene ACE1, Theor. Appl. Genet. 107 (6) (2003) 1139–1147.

[79] J. Jeung, B. Kim, Y. Cho, S. Han, H. Moon, Y. Lee, K. Jena, A novel gene, Pi40 (t), linked to the DNA markers derived from NBS-LRR motifs confers broad-spectrum of blast resistance in rice, Theor. Appl. Genet. 115 (8) (2007) 1163–1177.

[80] S. Qu, G. Liu, B. Zhou, M. Bellizzi, L. Zeng, L. Dai, G.L. Wang, 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) (2006) 1901–1914.

[81] X. Chen, J. Shang, D. Chen, C. Lei, Y. Zou, W. Zhai, G. Cao, AB-lectin receptor kinase gene conferring rice blast resistance, Plant J. 46 (5) (2006) 794–804.

[82] Y. Deng, X. Zhu, Y. Shen, Z. He, 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, Theor. Appl. Genet. 113 (4) (2006) 705–713.

[83] N.I. Naqvi, J.M. Bonman, D.J. Mackill, R.J. Nelson, B.B. Chattoo, Identification of RAPD markers linked to a major blast resistance gene in rice, Mol. Breed. 1 (4) (1995) 341–348.

[84] X. Chen, S. Li, J. Xu, W. Zhai, Z. Ling, B. Ma, Y. Ma, Identification of two blast resistance genes in a rice variety, Digu, J. Phytopathol. 152 (2) (2004) 77–85.

[85] X. Zhu, Q. Yang, J. Yang, C. Lei, J. Wang, Z. Ling, Differentiation ability of monogenic lines to *Magnaporthe grisea* in *indica* rice, Acta Phytopathol. Sin. 34 (4) (2004) 361–368.

[86] C. Lei, D. Huang, W. Li, J. Wang, Z. Liu, X. Wang, Z. Ling, Molecular mapping of a blast resistance gene in an *indica* rice cultivar Yanxian No. 1, Rice Genet. Newslett. 22 (2005) 76–77.

[87] Z.X. Wang, M. Yano, U. Yamanouchi, M. Iwamoto, L. Monna, H. Hayasaka, T. Sasaki, The Pib gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes, Plant J. 19 (1) (1999) 55–64.

[88] Q. Pan, T. Tanisaka, H. Ikehashi, Studies on the genetics and breeding of blast resistance in rice VII. Gene analysis for the blast resistance of Indian native cultivar, Aus 373, Breed Sci. 47 (Suppl. 1) (1997) 35.

[89] S. Barman, M. Gowda, R. Venu, B. Chattoo, Identification of a major blast resistance gene in the rice cultivar 'Tetep', Plant Breed. 123 (3) (2004) 300–302.

[90] T. Nguyen, S. Koizumi, T. La, K. Zenbayashi, T. Ashizawa, N. Yasuda, A. Miyasaka, *Pi35 (t)*, a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188, Theor, Appl. Genet. 113 (4) (2006) 697–704.

[91] F. Lin, S. Chen, Z. Que, L. Wang, X. Liu, Q. Pan, 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) (2007) 1871–1880.

[92] J.S. Jeon, G. An, Gene tagging in rice: a high throughput system for functional genomics, Plant Sci. 161 (2) (2001) 211–219.

[93] S. Ashkani, M.Y. Rafii, I. Rusli, M. Sariah, S.N.A. Abdullah, H.A. Rahim, M. Latif, SSRs for marker-assisted selection for blast resistance in rice (*Oryza sativa L.*), Plant Mol. Biol. Rep. 30 (1) (2012) 79–86.

[94] K. Jena, D. Mackill, Molecular markers and their use in marker-assisted selection in rice, Crop Sci. 48 (4) (2008) 1266–1276.

[95] S.J. Stavely, D.P. Coyne, D.T. Lindgren, Belneb rust resistant-1 and -2 great northern dry bean germplasm, Hortic. Sci. 24 (1989) 400–401.

[96] J.D. Kelly, Use of RAPD markers in breeding for major gene resistance to plant pathogens, Hortic. Sci. 30 (1995) 461–465.

[97] K. Rybka, M. Miyamoto, I. Ando, A. Saito, S. Kawasaki, High-resolution mapping of the *indica*-derived rice blast resistance genes II. *Pi-ta2* and *Pi-ta* and a consideration of their origin, Mol. Plant Microbe Interact. 10 (4) (1997) 517–524.

[98] S. Chen, L. Wang, Z. Que, R. Pan, Q. Pan, Genetic and physical mapping of *Pi37(t)*, a new gene conferring resistance to rice blast in the famous cultivar St. No. 1, Theor. Appl. Genet. 111 (8) (2005) 1563–1570.

[99] C. Conaway-Bormans, M. Marchetti, C. Johnson, A. McClung, W. Park, Molecular markers linked to the blast resistance gene Pi-z in rice for use in marker-assisted selection, Theor. Appl. Genet. 107 (6) (2003) 1014–1020.

[100] P. Du, L. Loan, N. Sang, Blast research in Mekong river delta of Vietnam, Jpn. Int. Res. Cent. Agric. Sci. (JIRCAS) 53 (2007) 53–63.

[101] Y. Jia, S.A. McAdams, G.T. Bryan, H.P. Hershey, B. Valent, Direct interaction of resistance gene and avirulence gene products confers rice blast resistance, EMBO J. 19 (15) (2000) 4004–4014.

[102] M. Soller, J. Beckmann, Genetic polymorphism in varietal identification and genetic improvement, Theor. Appl. Genet. 67 (1) (1983) 25–33.

[103] L. Monna, A. Miyao, H.S. Zhong, T. Sasaki, Y. Minobe, Screening of RAPD markers linked to the photoperiod-sensitivity gene in rice chromosome 6 using bulked segregant analysis, DNA Res. 2 (3) (1995) 101–106.

[104] L. Monna, A. Miyao, H. Zhong, M. Yano, M. Iwamoto, Y. Umehara, T. Sasaki, Saturation mapping with subclones of YACs: DNA marker production targeting the rice blast disease resistance gene, *Pi-b*, Theor. Appl. Genet. 94 (2) (1997) 170–176.

[105] G. Miah, M.Y. Rafii, M.R. Ismail, A.B. Puteh, H.A. Rahim, K.N. Islam, M.A. Latif, A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance, Int. J. Mol. Sci. 14 (11) (2013) 22499–22528.

[106] X. Zhu, S. Chen, J. Yang, S. Zhou, L. Zeng, J. Han, Q. Pan, The identification of Pi50 (t), a new member of the rice blast resistance *Pi2/Pi9* multigene family, Theor. Appl. Genet. 124 (7) (2012) 1295–1304.

[107] J. Yu, S. Hu, J. Wang, G.K.S. Wong, S. Li, B. Liu, X. Zhang, A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*), Science 296 (5565) (2002) 79–92.

[108] S.R. McCouch, L. Teytelman, Y. Xu, K.B. Lobos, K.M. ClareWalton, Y. Xing, Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.), DNA Res. 9 (6) (2002) 199–207.

[109] S. Ashkani, M. Rafii, M. Sariah, N.A.A. Siti, I. Rusli, A. Harun, M. Latif, Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*), Gen. Mol. Res. 10 (2011) 1345–1355.
[110] G.T. Bryan, K.S. Wu, L. Farrall, Y. Jia, H.P. Hershey, S.A. McAdams, B. Valent, A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*, Plant Cell Online 12 (11) (2000) 2033–2045.
[111] Z. Yu, D. Mackill, J. Bonman, S. Tanksley, Tagging genes for blast resistance in rice via linkage to RFLP markers, Theor. Appl. Genet. 81 (4) (1991) 471–476.
[112] J.M.J. Tohme, M.V. Montenegro, F. Correa, C. Martinez, R. Zeigler, W. Roca, Tagging resistance genes to Rice Hoja Blanca virus and Colombian isolates of rice blast with RFLP and RAPD markers, in: Abstract presented at the Fifth Annual Meeting of the International Programme on Rice Biotechnology, Tuczon, AZ, USA, 2–5 October, 1991.
[113] S.D. Tanksley, M.W. Ganal, G.B. Martin, Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes, Trends Genet. 11 (2) (1995) 63–68.
[114] J. Zhou, J. Wang, J. Xu, C. Lei, Z. Ling, Identification and mapping of a rice blast resistance gene *Pi-g (t)* in the cultivar Guangchangzhan, Plant Pathol. 53 (2) (2004) 191–196.
[115] T. Terashima, S. Fukuoka, N. Saka, S. Kudo, Mapping of a blast field resistance gene *Pi39 (t)* of elite rice strain Chubu 111, Plant Breed. 127 (5) (2008) 485–489.
[116] N.I. Naqvi, B.B. Chattoo, Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast resistance gene in rice, Genome 39 (1) (1996) 26–30.
[117] J.S. Jeon, D. Chen, G.H. Yi, G. Wang, P. Ronald, Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast, Mol, Genet. Genomics 269 (2) (2003) 280–289.
[118] H. Sharma, J. Crouch, K. Sharma, N. Seetharama, C. Hash, Applications of biotechnology for crop improvement: prospects and constraints, Plant Sci. 163 (3) (2002) 381–395.
[119] W. Li, C. Lei, Z. Cheng, Y. Jia, D. Huang, J. Wang, X. Guo, Identification of SSR markers for a broad-spectrum blast resistance gene *Pi20 (t)* for marker-assisted breeding, Mol. Breed. 22 (1) (2008) 141–149.
[120] A. Charcosset, Marker-assisted introgression of quantitative trait loci, Genetics 147 (3) (1997) 1469–1485.
[121] I. Allard, Principles of Plant Breeding, John Wiley & Sons, New York, 1960 (Chapters 6–9).
[122] R.M. Sundaram, M. Vishnupriya, G.S. Laha, N.S. Rani, P.S. Rao, S.M. Balachandran, R.V. Sonti, Introduction of bacterial blight resistance into Triguna, a high yielding, mid-early duration rice variety, Biotechnol. J. 4 (3) (2009) 400–407.
[123] S. Gopalakrishnan, R. Sharma, K. Anand Rajkumar, M. Joseph, V. Singh, A. Singh, T. Mohapatra, Integrating marker-assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice, Plant Breed. 127 (2) (2008) 131–139.
[124] V.K. Singh, A. Singh, S. Singh, R.K. Ellur, V. Choudhary, S. Sarkel, K. Vinod, Incorporation of blast resistance into "PRR78", an elite Basmati rice restorer line, through marker-assisted backcross breeding, Field Crops Res. 128 (2012) 8–16.
[125] C. Fu, T. Wu, W. Liu, F. Wang, J. Li, X. Zhu, M. Zhu, 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, Afr. J. Biotechnol. 11 (67) (2012) 13104–13114.
[126] P. Zhou, Y. Tan, Y. He, C. Xu, Q. Zhang, Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection, Theor. Appl. Genet. 106 (2) (2003) 326–331.
[127] N.T. Lang, N. Tao, B.C. Buu, Marker-assisted backcrossing (MAB) for rice submergence tolerance in Mekong delta, Omonrice 18 (2011) 11–21.
[128] L.T.N. Huyen, L.M. Cuc, T.D.K. Le Huy Ham, Introgression the SALTOL QTL into Q5DB, the elite variety of Vietnam using marker-assisted-selection (MAS), Am. J. BioSci. 1 (4) (2013) 80–84.
[129] H.C. Chen, Z.X. Ni, S. Zuo, S.M. Pan, X.B. Zhu, XD, pyramiding three genes with resistance to blast by marker-assisted selection to improve rice blast resistance of Jin 23B, application, Zhongguo Shuidao Kexue (Chinese Journal of Rice Science) 22 (1) (2008) 23–27.
[130] S. Wen, B. Gao, Introgressing blast-resistant gene *Pi-9 (t)* into elite rice restorer Luhui17 by marker-assisted selection, Rice Genomics Genet. 2 (4) (2012) 31–36.
[131] Z. Wang, Y. Jia, J. Rutger, Y. Xia, Rapid survey for presence of a blast resistance gene *Pi-ta* in rice cultivars using the dominant DNA markers derived from portions of the *Pi-ta* gene, Plant Breed. 126 (1) (2007) 36–42.
[132] F.S. Collins, Positional cloning: let's not call it reverse anymore, Nat. Genet. 1 (1) (1992) 3–6.
[133] G. Liu, G. Lu, L. Zeng, G.L. Wang, Two broad-spectrum blast resistance genes, *Pi9 (t)* and *Pi2 (t)*, are physically linked on rice chromosome 6, Mol. Genet. Genomics 267 (4) (2002) 472–480.
[134] M. Yokoo, H. Fujimaki, Tight linkage of blast resistance with late maturity observed in different indica varieties of rice, Jpn. J. Breed 21 (1) (1971) 35–39.
[135] I. Ashikawa, N. Hayashi, H. Yamane, H. Kanamori, J. Wu, T. Matsumoto, M. Yano, Two adjacent nucleotide binding site–leucine-rich repeat class genes are required to confer Pik-m specific rice blast resistance, Genetics 180 (4) (2008) 2267–2276.
[136] J. Shang, Y. Tao, X. Chen, Y. Zou, C. Lei, J. Wang, Z. Lu, Identification of a new rice blast resistance gene, Pid3, by genome wide comparison of paired nucleotide binding site–leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes, Genetics 182 (4) (2009) 1303–1311.
[137] N. Hayashi, H. Inoue, T. Kato, T. Funao, M. Shirota, T. Shimizu, T. Matsumoto, Durable panicle blast resistance gene *Pb1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication, Plant J. 64 (3) (2010) 498–510.
[138] J. Wang, Y. Jia, J. Wen, W. Liu, X. Liu, L. Li, J. Ren, Identification of rice blast resistance genes using international monogenic differentials, Crop Prot. 45 (2013) 109–116.
[139] Y. Okuyama, H. Kanzaki, A. Abe, K. Yoshida, M. Tamiru, H. Saitoh, D.C. Galam, A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes, Plant J. 66 (3) (2011) 467–479.
[140] L. McHale, X. Tan, P. Koehl, R.W. Michelmore, Plant NBS-LRR proteins: adaptable guards, Genome Biol. 7 (4) (2006) 212.
[141] R. Latha, L. Rubia, J. Bennett, M. Swaminathan, Allele mining for stress tolerance genes in *Oryza* species and related germplasm, Mol. Biotechnol. 27 (2) (2004) 101–108.
[142] B.K. Joshi, H.P. Bimb, G. Parajuli, B. Chaudhary, Molecular tagging, allele mining and marker aided breeding for blast resistance in rice, BSN e-Bull. 1 (2009) 1–23.
[143] L. Comai, K. Young, B.J. Till, S.H. Reynolds, E.A. Greene, C.A. Codomo, A.R. Odden, Efficient discovery of DNA polymorphisms in natural populations by Ecotilling, Plant J. 37 (5) (2004) 778–786.
[144] C.L. Huang, S.Y. Hwang, Y.C. Chiang, T.P. Lin, Molecular evolution of the Pi-ta gene resistant to rice blast in wild rice (*Oryza rufipogon*), Genetics 179 (3) (2008) 1527–1538.
[145] B.J. Till, S.H. Reynolds, E.A. Greene, C.A. Codomo, L.C. Enns, J.E. Johnson, N.E. Taylor, Large-scale discovery of induced point mutations with high throughput TILLING, Genome Res. 13 (3) (2003) 524–530.
[146] B. Yuan, C. Zhai, W. Wang, X. Zeng, X. Xu, H. Hu, Q. Pan, The *Pik-p* resistance to *Magnaporthe oryzae* in rice is mediated by a pair of closely linked CC-NBS-LRR genes, Theor. Appl. Genet. 122 (5) (2011) 1017–1028.
[147] C. Zhai, F. Lin, Z. Dong, X. He, B. Yuan, X. Zeng, Q. Pan, The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication, New Phytol. 189 (1) (2011) 321–334.
[148] K. Yoshida, N.T. Miyashita, DNA polymorphism in the blast disease resistance gene *Pita* of the wild rice *Oryza rufipogon* and its related species, Genes Genet. Syst. 84 (2) (2009) 121–136.
[149] D. Chen, R. Zeigler, S. Ahn, R. Nelson, Phenotypic characterization of the rice blast resistance gene *Pi-2 (t)*, Plant Dis. 80 (1) (1996) 52–56.
[150] L. Wang, X. Xu, F. Lin, Q. Pan, Characterization of rice blast resistance genes in the *Pik* cluster and fine mapping of the *Pik-p* locus, Phytopathology 99 (8) (2009) 900–905.
[151] B. Zhou, S. Qu, G. Liu, M. Dolan, H. Sakai, G. Lu, G.L. Wang, The eight amino acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*, Mol. Plant Microbe Interact. 19 (11) (2006) 1216–1228.
[152] J. Nan, W. Suhua, L. Zhiqiang, Analysis of the antimicrobial spectrum of three rice blast resistance genes at *Pi2/9* locus and genetic diversity of rice blast strains, J. Hunan Agric. Univ. 38 (2012) 506–510.
[153] T. Sharma, Y. Gupta, S. Thakur, P. Singh, H. Upreti, N. Singh, R. Kaushik, Allele mining for important blast resistance genes from Indian land races of rice, in: Paper presented at the 6th International Rice Genetics Symposium, Manila, Philippines, 2009.
[154] Q. Lv, X. Xu, J. Shang, G. Jiang, Z. Pang, Z. Zhou, X. Li, Functional analysis of *Pid3-A4*, an ortholog of rice blast resistance gene *Pid3* revealed by allele mining in common wild rice, Phytopathology 103 (6) (2013) 594–599.
[155] G. Ramkumar, M. Madhav, S. Rama Devi, P. Manimaran, K. Mohan, M. Prasad, B. Viraktamath, Nucleotide diversity of *Pi-ta* a major blast resistance gene and identification of its minimal promoter, Gene 546 (2014) 250–256.
[156] N. Jiang, Z. Li, J. Wu, Y. Wang, L. Wu, S. Wang, P. Sun, 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) (2012) 1–7.