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Blast resistance in rice: a review of conventional breeding to molecular approaches

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Abstract Blast disease caused by the fungal pathogen *Magnaporthe oryzae* is the most severe diseases of rice. Using classical plant breeding techniques, breeders have developed a number of blast resistant cultivars adapted to different rice growing regions worldwide. However, the rice industry remains threatened by blast disease due to the instability of blast fungus. Recent advances in rice genomics provide additional tools for plant breeders to improve rice production systems that would be environmentally friendly. This article outlines the application of conventional breeding, tissue culture and DNA-based markers that are used for accelerating the development of blast resistant rice cultivars. The best way for controlling the disease is to incorporate both qualitative and quantitative genes in resistant variety. Through conventional and molecular breeding many blast-resistant varieties have been developed. Conventional breeding for disease resistance is tedious, time consuming and mostly dependent on environment as compare to molecular breeding particularly marker assisted selection, which is easier, highly efficient and precise. For effective management of blast disease, breeding work should be focused on utilizing the broad spectrum of resistance genes and pyramiding genes and quantitative trait loci. Marker assisted selection provides potential solution to some of the problems that conventional breeding cannot resolve. In recent years, blast resistant genes have introgressed into Luhui 17, G46B, Zhenshan 97B, Jin 23B, CO39, IR50, Pusa1602 and Pusa1603 lines through marker assisted selection. Introduction of exotic genes for resistance induced the occurrence of new races of blast fungus, therefore breeding work should be concentrated in local resistance genes. This review focuses on the conventional breeding to the latest molecular progress in blast disease resistance in rice. This update information will be helpful guidance for rice breeders to develop durable blast resistant rice variety through marker assisted selection.

Keywords Blast disease · Conventional breeding · Tissue culture · Molecular approaches

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Introduction

Rice is the most valuable and primary food crop for more than 50 % of the world's population [1, 2]. The rice consumer is increasing and demand for rice is also moving up due to better living standards. Various studies have shown that to meet the increase demand for rice, production has to be increased more than 40 % by 2030 [1]. This challenge has to be overcome by the development of high yielding

rice varieties with tolerance to biotic and abiotic stress [3]. Though the yield potentiality of rice is 10 tons^{-ha} whereas farmers on an average harvesting about 5 tons ha^{-1} [4]. This yield difference is due to diseases in rice. Among the biotic stresses blast disease is the most harmful threat to high productivity of rice [5, 6], due to its wide distribution and ability to survive in wide range of environmental conditions. Due to this disease, yield loss ranged from 1 to 50 %, meaning each year destroys abundant rice to feed more than 60 million people and economic losses over $70 billion of dollar [7]. This loss in rice yield should be minimized in order to help the marginal and poor farmers of developing countries [8]. The fungus is able to develop resistance to both chemical treatments and genetic resistance which is continuous threat to the effectiveness of blast-resistant rice varieties. Hence, it is urgent to find out strategies for developing durable resistance varieties to the disease. In this perspective, major and minor genes can contribute to producing durable resistance [9].

In recent years, many techniques have been developed to control the fungal disease of rice. To control the spread of this disease chemical and biological method, disease forecasting and cultivation practices have been applied widely [10]. Unfortunately, these measures are not very effective. The use of pesticides is expensive as well as neither practical nor environmental-friendly. Some strategies i.e. multilines [11], mixtures [12] and pyramiding [13] are based on the use of complete and specific resistance genes and others are based on the accumulation of partial resistance [13] for breeding blast resistance. The molecular function of some blast resistance genes has been described [14], and many quantitative trait loci (QTL) for resistance to blast have been mapped. The attraction of QTL research can be explained by the fact that partial resistance implies more general mechanisms and, consequently, is thought to be more durable. Host resistance is the most economical and environmentally friendly way of disease control [15]. The use of resistant rice cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Using classical plant breeding techniques, plant breeders have developed a number of blast resistant cultivars adapted to different rice growing regions worldwide. Recent advances in rice genomics provide additional tools for plant breeders to develop rice production systems that could be environmentally beneficial. New information and knowledge gained from molecular biology research on disease resistance gene-mediated defense responses will undoubtedly provide new insights into the nature of rice disease resistance, which is convenience for developing new rice varieties with high blast disease resistance. So, understanding and application of molecular biology is a prerequisite for accelerating the development of blast resistance rice.

Many rice researchers and breeders [16–19] have developed new improved cultivated rice for resistance against blast through molecular breeding approach involving DNA markers, QTLs mapping, marker assisted selection (MAS) and genetic transformation. The PCR-based allele-specific markers provide an efficient marker system for MAS for blast resistance breeding [20, 21]. Conventional rice breeding is a slow process, typically requires 10–15 years from initiation to varietal release. Conventional breeding mostly depends on environmental conditions. Breeding for new varieties takes long time, the release of improved varieties cannot be guaranteed [22, 23]. In Indonesia some varieties like Gajah Mungkur, Way Rarem, Kalimutu, Jatilhur, Cirata, Limboto have developed and released as blast resistant varieties but they are not adopted by farmers as their resistance to blast has proven ineffective after several successive cultivation [24]. Moreover, MAS offer better selection strategies in rice breeding with a shorter period of time. MAS are more efficient, effective and reliable than phenotypic selection. Furthermore, MAS can shorten the development time of varieties significantly, so in some cases it will be more cost effective than selection based on phenotypes. MAS also allow the breeding of complex traits which not feasible through conventional methods. Certainly, MAS is not the silver bullet for all problems, but promising approach to conventional breeding. Considerable progress has been made in rice towards cloning and identification of disease resistance genes, characterization of defense responses, and elucidation of signal transduction leading to activation of defense responses. Several studies have shown that partial resistance can be specific [25, 26]. Recently, many rice varieties with complete resistance to *Magnaporthe grisea* have been developed [27]. Transferring blast resistance genes to different genetic backgrounds is difficult to identify using conventional approaches instead of MAS to facilitate at early stage selection with greater accuracy [28]. In this review, we focus on the progress towards the understanding of conventional breeding and molecular basis of blast disease resistance in rice. The main objectives of this review are to (i) highlight the conventional breeding, tissue culture and a set of biotechnological tools that are currently being used for the improvement of blast resistance (ii) explore a new promising concept using molecular data to breed for durable blast resistance in rice (iii) extensively review the information available on blast resistance genes, QTL and gene mapping, MAS and gene transformation.

Conventional breeding

Conventional approaches are important for producing novel genetic variants, conserving wild germplasm, sexual hybridization between contrasting parental lines and

mutation. Over the last 30 years, conventional breeding has given IRRI's elite cultivars with vast range of genes for resistance to diseases [13, 29]. In conventional breeding program, various methods namely—pedigree method, backcrossing, recurrent selection and mutation breeding are used. The pedigree method is the most widely used in rice improvement. The pedigree method is highly suitable to develop rice with resistance to insects and diseases if the resistance is governed by major genes. It is possible to combine genes for resistance to six or seven major diseases and insects in a short period [30]. The major disadvantage of pedigree breeding is that requires much time to evaluate lines periodically throughout the growing season and to keep records on which selection is based at maturity. Of all breeding methods, the pedigree method requires the greatest familiarity with the material and with the relative effects of genotype and environment on character expression. For traits governed by polygenes, this breeding is not the most effective approach. For example, resistance to stem borers and sheath blight appears to be under polygenic control. For these two traits, diallel selective mating system is suitable [30, 31]. Backcrossing is a widely most common technique in rice breeding for introgression or substitution of a target gene from donor parent to recipient. It provides a precise way to improve varieties that excel in a large number of attributes [32, 33]. The main purpose of backcrossing is to decline the donor genome content into the progenies [34]. Backcross breeding has been adopted in the South and Southeast Asia [35, 36] as breeding strategy to improve elite varieties such as KDML105, Basmati and Manawthukha for their resistances to blast [37].

Recurrent selection is another traditional breeding method used in rice for male sterility [38]. It allows defined and shorter breeding cycles, more precise follow-up of genetic gains, and provides opportunity to develop wide range genetic diversity breeding lines. Using this method, upland cultivar CG-91 was developed with resistance to rice blast [39, 40]. In order to assess the efficacy of this method in rice, an evaluation study observed 6.65 % genetic gain considering two cycles of recurrent selection in the irrigated rice population CNA-IRAT 4 [41]. On the other hand, 6.2 % gains was observed after selecting for rice blast resistance comparing cycles 1 and 2 of the upland rice population CNA-7 [42]. In almost all self-pollinated crops including rice, breeders chose to use pedigree selection which is alternative to recurrent selection.

Mutation breeding in rice is used to complement conventional breeding, since this technique is very effective for improving major traits, such as agronomic traits, resistance to pests and diseases and grain physical parameters and eating quality. In classical mutation breeding, induced mutations are used for developing a new variety, whereby it is difficult to trace the mutated genes in subsequent breeding. It is now possible to tag mutated genes, pyramid them into a single elite breeding line, and follow up them in subsequent breeding programs [43]. The advantages of mutation breeding include creation of new gene alleles that are not exist in germplasm pools, and the induction of new gene alleles into the new varieties that can be used directly as a commercial variety. The disadvantage of mutation breeding is limited power in generating the dominant alleles that might be desired; it is also less effective than cross breeding for a trait needs for a combination of multiple alleles. Many attempts have been made to improve disease resistance in rice through mutation breeding. Positive results, particularly for resistance to blast (*Pyricularia oryzae*) have been reported from several countries. The Department of Agriculture, Thailand, officially released the variety RD6, a radiation induced glutinous mutant of the popular non-glutinous variety Khao Dawk Mali 105 (KDML 105). Selections in the M_2 populations were made for glutinous and blast-resistant mutants [44]. An attempt was made to induce blast resistance in the high yielding variety Ratna (IR8/TKm 6) through chemomutagenesis with EMS 0.1 and 0.2 % concentrations. The mutagen treatment induced great variability; in different generators M_2 to M_5, higher resistance and higher susceptibility were found than the parent [45]. Blast resistance mutant R917 was derived from the F_1 progeny radiated by 10 krad 60Co γ-ray [46]. The Mtu 17 mutants possessing desirable agronomic characters through chemomutagenesis with dES; some of the mutants had blast resistance, whereas the parent Mtu 17 was susceptible [47]. Through mutation breeding, several mutant lines, such as Mahsuri Mutant [48], SPM 129, SPM 130 and SPM 142 [49] have produced successfully for blast resistance in Malaysia [50]. In China, the mutant rice variety 'Zhefu 802' deriving from var. 'Simei No. 2', induced by Gamma-rays, has a high resistance to rice blast [51, 52]. Today the technique became part of the tools kit breeders have to enhance specific rice characteristics in well-adapted varieties.

Through conventional breeding programs major genes *Pib*, *Pita*, *Pia*, *Pi1*, *Pikh*, *Pi2* and *Pi4* have been introduced into rice varieties for blast resistance [53, 54]. Identifying key genomic regions associated with blast resistance against a broad spectrum of isolates in backcross introgression lines have been developed through conventional breeding program [55]. By using conventional and molecular breeding many blast-resistant varieties have been developed [56]. Few examples of conventional breeding application for blast resistance in rice are shown in Table 2. Some components of breeding strategies suggest prolong durability of resistance which generally can be adopted for stabilization and control of blast disease in rice are discussed below:

Backcrossing for concentration of slow-blasting components

Breakdown of varietal resistance to rice blast disease attributed to the failure of varieties to capture the entire complement of genetic factors for disease resistance from the respective parent sources in their parentage [57]. Existence of slow-blasting characters, originally presented as horizontal resistance [58], mainly found only in tall, upland rice varieties [59, 60] and identified several slow blasting components in several varieties [60]. Identification of slow blasting sergeants in segregating populations is difficult particularly in bulk breeding systems. It might be somewhat easier in a pedigree system of breeding where discrete progeny rows can be evaluated for identification of lines with slow blasting components.

Combination of major genes with slow-blasting components

The combination of major genes (vertical resistance) with slow-blasting components (minor genes) is believed to provide increased stability of the resistance mechanism to blast, because the genes for vertical and horizontal resistance in combination increase the effectiveness of each other. Centre International de Agricultural Tropical (CIAT) rice breeding programme [61] attempted adopting this strategy, but found difficult to detect the combinations of the two types of resistance in a given pedigree line, as the lower level of horizontal resistance is masked by the presence of vertical genes. Under such circumstances, it is proposed to select for vertical resistance and hope for the best. Therefore, the practical outcome of using this strategy in a breeding programme is not predictable.

Mixtures of variety

Varietal mixtures are the way of reducing the development of blast races consisting of 80–90 % resistant plants and 10–20 % susceptible plants of similar varietal background. This strategy is easier to introduce but need to ensure their agronomical uniformity. In Yunan province of Southeast China, highly susceptible glutinous plants were mixed and planted with non-glutinous hybrid indica rice, reduced the development of blast in glutinous rice [62, 63]. But measuring panicle blast resistance is difficult because the panicle infection is influenced by weather, and even small differences in maturity period between lines can result inaccurate assessment of their level of resistance.

Multiple lines

The durability resistance of multiline varieties depends upon the rate of blast races develop, the number of lines component in a mixture and the extent of planted area. Development of multiline varieties using blast resistant isogenic lines had been attempted for "Nipponbare" [64, 65], "Toyonishiki" [66] and "Sasanishiki" [67]. Blast control effects by the use of these multiple line varieties have been confirmed [68–72]. Actually multiple line variety of "Sasanishiki" has been commercially cultivated on a market scale since 1995. Moreover, new isogenic lines have been bred and elaborated variation analysis in the distribution of the races of the blast pathogen, which is essential for stable utilization [73, 74]. A cross combination of Koshihikari blast resistant isogenic lines (BLs) was made by [75]. The BLs were bred by crossing with Sasanishiki (*Pia*), Todorokiwase (*Pii*), *Pi4* (*Pita-2*), Niigatawase (*Piz*), Koshiminori (*Pik*), Tsuyuake (*Pik-m*), Toride 1 (*Piz-t*) and BL1 (*Pib*) as the donor parent respectively, and then repeated backcrossings with "Koshihikari" as the pollen parent were performed. Individuals used for backcrossing after the first filial generation of the first backcross generation (B_1F_1) were spray-inoculated with race 001 of the blast pathogen for the nursery test. About 30 heterozygotes showing resistance were selected for cultivation in the field. Individuals that resembled "Koshihikari" were selected prior to the backcrossing procedure. Selected individuals were transplanted to 1/5000 a Wagner pots and about 50 seeds were obtained per stump. Backcrossing was performed six times for BL No. 4 and five times for the other lines, following examples of BLs for Toyonishiki, Nipponbare and Sasanishiki, which have been bred so far. Seedlings of B_5F_2 or B_6F_2 after selfed generation were spray-inoculated with race 001 for selecting homozygotes with true resistance as well as for fixation. All the Koshihikari BLs was found to be identical with the original "Koshihikari" in terms of practical agronomic traits and clearly superior in suppressing blast.

Deployment of gene

This strategy involves the use of the distinct type of blast resistance mechanism in each variety and use of varieties in a predetermined pattern (temporal or spatial). Based on rice cultivation practices, seasonal and regional preferences for different location specific varieties are used. As a result, distinct varieties could be developed using diverse sources of blast resistance. Even among varieties used for a particular season, variety with different maturity period should consist of distinct sources of blast resistance. This situation will slow down the development of new virulent races, and improve the durability of blast resistance in present varieties. Among many strategies, distinct gene deployment in different maturity groups may help to improve the durability of blast resistance in newly developing rice varieties.

Nevertheless, the conventional resistance breeding has apparent weakness, such as long breeding cycle, low

selection efficiency and difficulty in distant crossing, leading to the lag between the development of new resistant cultivars and the emergence of virulent pathotypes of the causal pathogen.

Biotechnological and molecular approaches for blast resistance

Breeding work utilizing both phenotypic and genotypic markers are more reliable and fast. Conventional breeding are based on gene expression due to which many limitations e.g. epistatic effect exist. Conventional breeding methods may create a resistance variety which is time consuming and intensive task. On the contrary, biotechnological approaches are important in introducing genes which provide resistance against *M. grisea* [76]. Rice breeders are looking at basic bioscience and biotechnology for solving some important problems that conventional breeding methods have not satisfactorily solved. Therefore, future breeding strategies should focus at broadening the genetic and cytoplasmic background of new varieties that are being developed not only for blast resistance but also for other important pests and diseases as well. Currently, more than 95 genes have been identified in rice [77]. Some of these genes *Pi-1*(*t*), *Pi2*, *Pi9*, *Pi20*(*t*), *Pi27*(*t*), *Pi39*(*t*), *Pi40*(*t*) and *Pikh* are reported to have confers broad-spectrum resistance (BSR) [78–83] and some of them including *Pia*, *Pib*, *Pii*, *Pi-km*, *Pi-t*, *Pi12*(*t*) and *Pi19*(*t*) confers race-specific resistance (RSR) [83–85]. These information greatly advance our understanding of molecular mechanisms that govern race specificity. The application of advanced molecular technologies could speed up crop improvement. There are some biotechnological approaches that can be used for the development of blast resistance rice.

Tissue culture

The various tissue-culture methods and gene-transfer techniques now available, could significantly shorten the breeding process, and overcome some of the substantial agronomic and environmental problems that have not been solved using conventional methods [86]. Tissue culture is one of the fundamental tools of crop science research. Cell culture is one of the alternative methods of inducing resistance to diseases in susceptible cultivars which are well adapted to local soil and climatic conditions. The genetic variation produced in tissue culture, termed somaclonal variation has been reported in many crop species [87]. Fortunately during the last 30 years, extensive work has been done on selecting the disease resistant plants against different pathogens. The cell free culture filtrate (CF) or the pure toxins of the pathogens and direct infection by the pathogen or all of them together could be used for the selection of novel disease resistant plants [88–90].

However, the information on somaclonal variation for blast resistance is scanty. According to [91], there was no variation for blast resistance in somaclones. On the other hand, [92] obtained R2 lines resistant to blast from the calli derived from mature embryos. In Brazil, a high degree of partial resistance has been reported in progenies of regenerated plants derived from immature panicles of a susceptible upland rice cultivar IAC47 [93]. These discrepancies may be partly attributed to the test conditions and disease pressure under which the somaclones were assessed, and the nature of resistance. Also various other factors may affect somaclonal variation such as genotype, explants source, composition of the culture medium and time of cultivation [94]. The alterations in the genome have been attributed to expression of mutant cells in explants, meiotic crossing over, and cytological changes [94]. The studies on somaclonal variation may permit accomplishment of breeding objectives in relation to rapid development of blast resistant lines from existing commercial susceptible rice cultivars. An experiment was conducted by [95] on Basmati-370 rice which was susceptible to some pathotypes of *P. grisea* in Brazil, to assess the degree of blast resistance and some agronomic characteristics in the advanced generations of its somaclones. These evaluations were carried out in R_5 to R_9 generations, in field trials, in rice blast nursery and greenhouse. Significant variations in grain quality and other agronomic characteristics were not observed. However, some of the somaclones showed higher degree of blast resistance. Two somaclones, SCBAS04 and SBAS16 exhibited higher degree of partial resistance to blast. The degree of blast resistance of upland rice (*Oryza sativa* L.) cultivar Araguaia has decreased significantly due to yield loss obtained from resistant somaclones, adaptation to greenhouse and field selection procedures [96]. Greenhouse selection with two specific physiologic races yielded 44 somaclones with slow blasting resistance, similar plant type and yield potential as that of Aragaia. A step by step protocol followed for resistant calli selection via a tissue culture technique under stress of *P. oryzae* CFs [97]. The results reveal that the resistance in regenerated rice plantlets to *P. oryzae* pathogen segregated as 1 resistant: 2 moderate resistant: 1 susceptible to *P. oryzae* may be controlled by one pair of genes.

Rice blast resistance genes and QTL

Blast resistance regulated by major genes or by QTLs [98]. Major genes prevent life cycle completion of *M. grisea* whereas QTLs reduce the sporulation of the pathogen within a compatible interaction. The deployment of major

gene resistance will minimize selection pressure and thereby prevent evolution of resistance in the pathogen population [13]. The difficulties associated with breeding for blast resistance can be attributed to two general problems. First, we are not entirely sure what genetic constitution results in durable resistance. Second, it is not necessarily possible to select for the presence of multiple genes, or for specific gene combinations, because of the epistatic interactions among genes. Molecular marker technology offers the opportunity to overcome both problems. First, molecular markers can improve the efficiency and resolution of genetic analysis, particularly when multiple resistance genes are exists in a single cultivar [99]. Second, markers linked to resistance genes provide tools to facilitate the selection of lines carrying resistance genes in desirable combinations [100]. Using this approach, new tools enable us to mimic ancient strategies for durable resistance breeding. QTLs are used for fine mapping of blast resistance genes and also for developing tightly linked markers related to blast resistance genes. Gene and individual QTL pyramiding should be considered for durable resistance to blast fungus.

The availability of molecular genetic map of rice [101, 102] provides the groundwork for identifying the chromosomal locations of blast resistance genes via linkage to molecular markers. The identification of a genes chromosomal location is known as "gene mapping". The identification of markers very closely linked to a gene, such markers can be used for indirect selection of gene, is known as "gene tagging" [103]. Information about the chromosomal location of a gene can be useful both for genetic analysis and for resistance breeding. The map location of a gene can indicate when genes are obviously non-allelic, or when rigorous complementation tests are needed to confirm allelism [104]. Information on allelism and linkage relationships can also be used to select donors and target loci for a gene pyramiding program, and can provide guidance about the population sizes needed to obtain recombinants carrying multiple genes. Markers tightly linked to a gene can be used for indirect selection (MAS) in a breeding program [98].

Two major categories of disease resistance in plants have been used such as vertical versus horizontal resistance [105], qualitative versus quantitative resistance [106], and complete versus partial resistance [107]. In most cases, qualitative resistance is modulated by interaction between the products of a major disease resistance (R) gene and an avirulence gene; this type of resistance is specific to pathogen race and is lifetime limited in a particular cultivar due to the strong selection pressure against the rapid evolution of the pathogen [108]. In contrast, quantitative resistance is conferred by QTLs and is presumably race non-specific and durable [109]. In many cases, qualitative and quantitative resistance genes are co-located on linkage maps and these regions are often rich in genes conferring resistance to multiple pathogens and/or to multiple specificities of the same pathogen [110]. On the integrated map of disease resistance genes in rice, for instance, blast resistance QTL are co-localized with *Pi* loci or QTL for resistance to other pathogens [111].

A greater number of genes with complete or partial resistance to blast in rice have been mapped and developed. Combining several genes and monitoring their presence is difficult by conventional methods because of their epistatic effects. Mapping blast resistance genes and locating closely linked markers have made it possible to confirm the presence of given gene in a variety with multiple genes [112]. The *Pi-b* and *Pi-ta* genes are two major blast resistance (R) genes that have been characterized molecularly [113]. Mostly the genes resistance to blast fungus is monogenic dominant. Structural and functional analyses of many major R genes have shown that they encode proteins with similar structural motifs nucleotide binding site, kinase domains, leucine-rich repeats- that are responsible for ligand recognition and signal transduction [114].

True resistance is governed by qualitative gene also called major gene and field resistance by quantitative genes also called minor genes. Approximately 96 rice blast resistance genes have been identified (Table 1) and among these 74 has been mapped [77]. Nine blast resistance genes- *Pi-b* [114], *Pi-ta* [115] and *Pi-kh* [116], *Pi37* [117], *Piz-5* and *Piz-t* [118], *Pi9* [119], *Pid2* [120] and *Pi36* [121] have been cloned. The wide genetic variation available in blast fungus may be the main reason why many resistance genes in rice have evolved. Majority of the QTLs are associated with qualitative genes. Quantitative resistance is generally considered as partial resistance in a particular cultivar [107] which is controlled by multiple loci, known as QTLs. Approximately 350 leaf blast resistances QTL have been mapped. These QTL were identified in 15 different populations, most of which are derived from indica japonica crosses [25, 127, 166, 186–189].

Partial resistance is characterized by compatibility between the pathogen and the plant with reduced development of disease compared to plants with no partial resistance [190, 191]. Several researchers mentioned that there are minor genes that play an important role in maintaining an acceptable level of disease under field conditions [192–194]. Such genes (minor) are difficult to identify and characterize in the presence of major genes due to epistatic interactions among themselves. Their presence could also affect the accuracy of classification of lines for complete resistance to blast [9]. Moreover, four partial-resistance genes have been identified and described as specific, *Pif* [195], *Pi21* [196], *Pb1* [143] and *Pi34* [197]. All these results suggest that partial resistance is

Table 1 List of available blast resistance genes and tightly linked markers in rice

Chromosome	Gene	Tightly linked marker		Map position (cM)	Donor rice		Resistance type	References
		Marker type	Marker name		Variety (Original donor)	Rice type		
12	*Pi24*(t)	SNP	–	10.3	Zhong 156	Indica	Complete	[54, 122]
	Pi62(t)	–	–	12.2–26.0	Yashiromochi	Japonica	–	[123]
	Pitq6	–	–	29.2–47.5	Teqing	Indica	Complete	[124]
	Pi6(t)	–	–	32.6–63.2	Apura	–	Complete	[98]
	Pi12(t)	–	–	42.8–53	RIL10 (Moroberekan)	Japonica	Complete	[125]
	Pi21(t)	–	–	43.4–59.6	Sweon 365	Japonica	–	[126]
	Pi31(t)	–	–	44.3	IR64	Indica	–	[127]
	Pi32(t)	–	–	47.5	IR64	Indica	–	[127]
	Pi12(t)	–	–	47.6–48.2	K80 (Hong-jiaozhan)	Indica	–	[127]
	Ipi(t)	–	–	47.6–58.3	–	–	–	[128]
	IPi3(t)	–	–	47.6–58.3	–	–	–	[102]
	Pi157	–	–	49.5–62.2	Moroberecan	Japonica	–	[102]
	Pita	SNP	Ta642, ta801, ta3, ta577, ta5, Pi-ta 440, pi-ta 1042, Pi-ta403	50.4	Taducan	Indica	Complete	[20, 115, 129]
	Pita-2	SNP	Ta642, ta801, ta3, ta577, ta5	50.4	Shimokita	Japonica	Complete	[20, 130, 131]
		Microsatellite	OSM89, RM155, OSM89, RM155,RM7102, OSM89, RM7102					
	Pi19(t)	–	–	–	Aichi Asahi	Japonica	Complete	[132–134]
	Pi39(t)	CAPS	39M6, 39M7	50.4	Q15	–	–	[121]
	Pi20(t)	Microsatellite	RM1337, RM5364, RM7102	51.5–51.8	IR24	Indica	Complete	[82, 135]
	PiGD-3(t)	–	–	55.8	Sanhuangzhan 2	–	–	[136]
11	*Pia*	CAPS	yca72	36	Aichi Asahi	Japonica	Complete	[137, 138]
	PiCO39(t)	CAPS	RGA8, RZ141, RGACO39	49.1	CO39	Indica	Complete	[139]
	Pilm2	–	–	56.2–117.9	Lemont	Japonica	Complete	[124, 140]
	Pi30(t)	–	–	59.4–60.4	IR64	Indica	–	[127]
	Pi7(t)	–	–	71.4–84.3	RIL29 (Moroberekan)	Japonica	Complete	[9]
	Pi34	–	–	79.1–91.4	Chubu 32	Japonica	Partial	[141]
	Pi38	Microsatellite	RM206, RM21	79.1–88.7	Tadukan	Indica	–	[142]
	PBR	–	–	80.5–120.3	St No. 1	Indica	–	[143]
	Pb1	–	–	85.7–91.4	Modan	Indica	Partial	[144]
	Pi44(t)	–	–	91.4–117.9	RIL29 (Moroberekan)	–	Complete	[145]
	Pik-h	Microsatellite	RM206, TRS26, TRS33, RM144, RM224, RM1233, RM144, RM224, RM1233, RM144, RM224	101.9	Tetep	Indica	Complete	[116, 131, 146]
	Pi54	–	–	–	Taipei 309 (TP)	Indica	–	[147]
	Pi1	–	–	112.1–117.9	C101LAC (Lac23)	–	Complete	[148]
	Pik-m	InDel	k6816, k2167	115.1–117.0	Tsuyuake	Japonica	Complete	[6, 20, 149]
		SNP	k641, k6441, k4731, k7237					
	Pi18(t)	–	–	117.9	Sweon 365	Japonica	Complete	[150]
	Pik	InDel	k6816, k2167	119.9–120.3	Kusabue	Japonica	Complete	[151]
		SNP	k6438, k6415, k8823, k8824, k3951, k39512					
	Pik-p	SNP	k641, k39575, k403, k3957	119.9–120.3	HR22	–	Complete	[20]
	Pik-s	Microsatellite	RM144, RM224, RM1233, RM144, RM224, RM1233	115.1–117.3	Shin 2	Japonica	Complete	[131]
	Pik-g	–	–	–	GA20	–	Complete	[152]
	Pise1	–	–	–	Sensho	Japonica	–	[153]
	Pi f	–	–	–	Chugoku 31-1 (St. No.1)	Japonica	Partial	[154]
	Mpiz	–	–	–	Zenith	–	–	[155]
	Pikur2	–	–	–	Kuroka	Japonica	–	[156]
	Piisi	–	–	–	Imochi shirazu	Japonica	–	[153]
10	*Pi28*(t)	–	–	114.7	Azucena	Japonica	–	[127]
	PiGD-2(t)	–	–	–	Sanhuangzhan 2	–	–	[157]

Table 1 continued

Chromosome	Gene	Tightly linked marker		Map position (cM)	Donor rice		Resistance type	References
		Marker type	Marker name		Variety (Original donor)	Rice type		
9	*Pii2*(t)	–	–	–	Ishikari shiroke	Japonica	–	[158]
	Pi5(t)	CAPS	94A20r, 76B14f, 40N23r	31.3–33.0	RIL125, RIL249, RIL260(Moroberekan)	Japonica	Complete	[138, 159]
		SNP	JJ817*					
	Pi3(t) Pai-	–	–	31.3–33.0	Kan-Tao	Japonica	Complete	[160]
	Pi15	–	–	31.3–34.9	GA25	–	Complete	[152, 161]
	Pii	–	–	–	Ishikari shiroke	Japonica	Complete	[162]
8	*Pi36*	Microsatellite	RM5647	21.6–25.2	Q61	Indica	–	[121, 136]
		CAPS	CRG2, CRG3, CRG4					
	Pi33	Microsatellite	RM72, RM44	45.4	IR64, Bala	Indica	Complete	[163]
	Pizh	–	–	53.2–84.8	Zhai-Ye-Quing	Indica	Complete	[102]
	Pi29(t)	–	–	69	IR64	Indica	–	[127]
	PiGD-1(t)	–	–	–	Sanhuangzhan 2	–	–	[163]
7	*Pi17*(t)	–	–	94.0–104.0	DJ 123	–	Complete	[164, 165]
6	*Pi22*(t)	–	–	38.7–41.9	Sweon 365	Japonica	–	[126]
	Pi26(t)	–	–	51.0–63.2	Gumei 2	Indica	–	[166]
	Pi27(t)	–	–	51.9	IR64	Indica	–	[127]
	Pi40(t)	Microsatellite	RM3330, RM527	54.1–61.6	IR65482-4-136-2-2 (Acc100882)	*Oryza Australiensis*	–	[80]
		CAPS	S2539					
	Piz-5	–	–	58.7	Tadukan	Indica	Complete	[167]
	Piz	InDel	z4794	58.7	Zenith	–	Complete	[20, 137, 155, 168]
		SNP	z60510, z5765, z56592, z565962					
	Piz-t	InDel	z4794	58.7	Toride 1	Japonica	Complete	[20, 167]
		SNP	z60510, z5765, zt56591, zt5659					
	Pi9	–	–	58.7	75-1-127 (101141)	*Oryza minuta*	Complete	[119]
	Pi25(t)	–	–	63.2–64.6	Gumei 2	Indica	–	[166, 169]
	Pid2	–	–	65.8	Digu	Indica	Complete	[120]
	Pigm(t)	CAPS	C26348	65.8	Gumei 4	Indica	–	[170]
		InDel	S47656					
	Pitq1	–	–	103.0–124.4	Teqing	Indica	Complete	[124]
	Pi8	–	–	–	Kasalath	Indica	Complete	[152, 164]
	Pi13(t)	–	–	–	Maowang	–	Complete	[152]
	Pi13	–	–	–	Kasalath	Indica	–	[171, 172]
5	*Pi26*(t)	–	–	22.5–24.7	Azucena	Japonica	–	[127]
	Pi23(t)			59.3–99.5	Sweon 365	Japonica	–	[126]
	Pi10	InDel	OPF62700	88.5–102.8	Tongil	Indica	Complete	[173, 174]
4	*pi21*	STS	P702D03_#79	58.6	Owarihatamochi	Japonica	Partial	[175, 176]
	Pikur1	–	–	86	Kuroka	Japonica	–	[156]
	Pi39(t)	Microsatellite	RM3843, RM5473	107.4–108.2	Chubu 111 (Haonaihuan)	Japonica	–	[177]
	Pi(t)			–	–	–	–	[102]
2	*Pid1*(t)	Microsatellite	RM262	87.5–89.9	Digu	Indica	–	[178]
	Pig(t)	Microsatellite	RM166, RM208	142.0–154.1	Guangchangzhan	Indica	–	[179]
	Pitq5	–	–	150.5–157.9	Teqing	Indica	Complete	[124]
	Piy1(t)	Microsatellite	RM3248, RM20	153.2–154.1	Yanxian No.1	Indica	–	[180]
	Piy2(t)	Microsatellite	RM3248, RM20	153.2–154.1	Yanxian No.1	Indica	–	[180]
	Pib	SNP	b213**,b28, b2**, b3989, Pibdom***	154.1	Tohoku IL9	Japonica	Complete	[20, 113, 131, 171]
		Microsatellite	RM138, RM166, RM208, RM266, RM138, RM166, RM208, RM266					
	Pi25(t)	–	–	157.9	IR64	Indica	–	[127]
	Pi14(t)	–	–	–	Maowang	–	Complete	[152]
	Pi16(t)	–	–	–	AUS373	–	Complete	[181]

Table 1 continued

Chromosome	Gene	Tightly linked marker		Map position (cM)	Donor rice		Resistance type	References
		Marker type	Marker name		Variety (Original donor)	Rice type		
1	*Pit*	SNP	t311, t256, t8042	12.2	Tjahaja	Japonica	Complete	[20]
	Pi27(t)	Microsatellite	RM151, RN259	28.4–38.8	Q14	–	Complete	[79]
	Pi24(t)	–	–	64.4	Azucena	Japonica	–	[127]
	Pitp(t)	Microsatellite	RM246	114.1	Tetep	Indica	–	[182]
	Pi35(t)	Microsatellite	RM1216, RM1003	132.0–136.6	Hokkai 188	Japonica	Partial	[183]
	Pi37	Microsatellite	RM302, RM212, FPSM1, FPSM2, FPSM4	136.1	St. No. 1	Japonica	–	[117, 178]
		STS	S15628, FSTS1, FSTS2, FSTS3, FSTS4					
	Pish	–	–	148.7–154.8	Shin 2	Japonica	Complete	[184, 185]
Unidentified map position	*Pi67*(t)	–	–	–	Tsuyuake	Japonica	–	[123]
	Piis2	–	–	–	Imochi shirazu	Japonica	–	[153]
	Pise2	–	–	–	Sensho	Japonica	–	[153]
	Pise3	–	–	–	Sensho	Japonica	–	[153]

Source: [77]

sometimes specific and does not necessarily have a broader resistance spectrum than complete resistance. However, QTL mapping can be used to efficiently detect complete resistance against blast [127], in contrary QTL mapping strategy does not guarantee the identification of partial resistance. Many rice blast researchers have mentioned that conducive environmental factors have led to the development of several blasts endemic. However, selection for partial or quantitative resistance to rice blast is difficult because of its genetic nature that is controlled by several minor genes. Rice lines selection with complete resistance are more stable than rice lines selection as partially resistant [198]. They also concluded that developing high levels of multigenic resistance for disease-prone environments is an attractive and achievable alternative to partial resistance. Field resistance rice blast was detected using QTL in Japanese upland rice and mapped using RFLP and SSR markers [175]. QTL analysis was carried out in F_4 progeny lines from the cross between Nipponbare (moderately susceptible, lowland) and Owarihatamochi (resistant, upland). Two QTLs were detected on chromosome 4 and one QTL was detected on each of chromosomes 9 and 12. The phenotypic variation explained by each QTL ranged from 7.9 to 45.7 % and the four QTLs explained 66.3 % of the total phenotypic variation. Backcrossed progeny lines were developed to transfer the QTL with largest effect using the susceptible cultivar Aichiasahi as a recurrent parent. The average score for blast resistance measured in the field was 4.2 ± 0.67, 7.5 ± 0.51 and 8.2 ± 0.66, for resistant, heterozygous and susceptible groups, respectively. Marker-assisted selection in relation to major genes and QTLs conferring improve field resistance to blasts in rice.

Marker assisted selection and blast resistance in rice

Conventional breeding for disease resistance is laborious, time consuming and highly dependent on environmental conditions in comparison to molecular breeding particularly MAS, which is simpler, highly efficient and precise [54]. Furthermore, conventional breeding generally depend upon the phenotype of artificial identification and the performance of field resistance, is too long to catch up with the frequent emergences of new virulent races of the pathogen and the release of improved varieties cannot be guaranteed [22, 23]. Marker assisted selection (MAS) is extremely powerful in blast resistance breeding because resistance phenotypes are often simple or encoded by single or few genes [199]. MAS have the advantage for the blast control by governing definite interaction of a particular resistance (R) gene with a particular avirulence gene in the pathogen [200]. Molecular markers have greater opportunity to improve the efficiency of conventional breeding by carrying out selection not directly on the trait of interest but also on linked molecular markers of that particular trait. Some SSR markers (RM168, RM8225, RM1233, RM6836, RM5961 and RM413) have been found by Ashkani et al. [201] that could be used in MAS programs. Availability of molecular markers along with marker-assisted selection strategies are essential to develop durable blast resistant variety against different races of *M. oryzae* [202]. MAS is of vast use in gene pyramiding confirming the presence of more than one gene [203, 204]. Now-a-days, MAS is used for screening of selected populations to track introgression of resistance genes *Pi-b*, *Pi-k*, *Pi-i*, *Pi-z*, and *Pi-ta*. Also, it is possible to pyramid *Pi-ta* with either of these major resistance genes to achieve broad-spectrum resistance in

the improved germplasm. Two *Pi*-genes i.e. *Pi-b* and *Pi-ta* have been molecularly characterized [113, 115]. These conserved DNA sequence variation have been used to develop *Pi-ta* and *Pi-b* dominant markers for MAS [129, 205]. Newly developed DNA markers also include SSR markers for *Pi-b, Pi-k* and *Pi-ta2* [205]. DNA markers for *Pi-ta* have been used to follow its introgression into advanced breeding lines [206]. Determination of *Pi20*(*t*) gene confers a broad-spectrum resistance against diverse blast pathotypes (races) in China based on inoculation experiments utilizing 160 Chinese *Magnaporthe oryzae* isolates [82], among which isolate 98095 can specifically differentiate the *Pi20*(*t*) gene present in cv. IR24. Pyramiding three blast R genes, *Pi1*, *Piz-5* and *Pita-2*, into cultivars to provide a broadspectrum resistance to many isolates of *M. oryzae* [148].

The application of R genes in rice breeding programs is considered an effective, economical and environmentally friendly strategy for controlling the disease. One approach that has been applied to increase the precision of introgressions is the tagging of molecular markers to a particular R gene in a cultivar and the subsequent use of that cultivar in MAS [207, 208]. In the previous years, few blast R genes have been molecularly characterized and their tightly linked SSR markers, SNP markers, and the perfect markers derived from R genes have been developed to facilitate their incorporation into elite breeding lines [20, 129, 131, 209]. Characterization and analyses of these R genes have provided transgenic tools or tightly linked markers in marker-assisted selection for rice breeding programs. In following years MAS has been engaged for transferring *Pita* [210], *Piz* [207], *Pi37* [211], *Pi35* [183] and *Pi1*, *Pi9* [212] to new varieties. Without phenotypic selection of plants, more than one gene can be performed by DNA markers. On the basis of closely linked DNA markers rice plants with two and three known resistance genes can be selected to exemplify the power of marker-aided selection. Continuous discovery and transfer of new DNA markers to breeders will accelerate the development of disease resistant cultivars. For a particular trait, information on molecular markers associated with QTL may increase the rate of genetic improvement through MAS because marker information permits selection accuracy, reduce generation intervals, or an increase selection intensity [213]. MAS [36, 214] and empirical phenotype selection [215] showed tremendous success in rice breeding program by improving and stabilizing grain yield from increasing levels of resistance to biotic and abiotic stresses. Comparing conventional breeding, MAS may greatly increase the efficiency and effectiveness of breeding.

The process of accumulating more genes together from more than two parents into a single genotype is known as pyramiding. Pyramiding may be possible through conventional breeding but it is usually not easy to identify the plants containing more than one gene. However, many researchers have postulated a pyramid of major genes to constitute a stable basis for blast resistance [57]. In this approach, the first step has to be the identification of functionally different major genes in parental sources. However, successful pyramiding of these genes is severely limited to the lack of suitable screening techniques. The present blast screening technique cannot be used to identify and differentiate advanced breeding lines pyramided with identical genes or functionally different genes. However, Centre International de Agriculture Tropical (CIAT) has successfully developed improved rice breeding lines with pyramided genes for blast resistance [61]. Pyramiding has been applied widely for combining multiple disease resistance genes. Some evidence suggests that the accumulation of multiple genes can provide durable resistance [216–218]. Strategies for MAS pyramiding of linked genes have been evaluated [219]. Pyramiding is the most common application of MAS in rice for resistance to diseases. Unfortunately, pyramiding genes is difficult using traditional greenhouse screens because plants that contain one resistance gene are generally fully resistant to the particular races of blast corresponding to that gene. The use of DNA markers linked to resistance genes is one way to overcome the ambiguity of greenhouse screens, and has already been used to successfully pyramid blast resistance genes [148]. Some examples of MAS application for blast resistance in rice are shown in Table 2.

Hindrance for the adoption of marker assisted selection

Now-a-days one of the most important constraints for MAS in rice is the prohibitive cost. The high cost of MAS will be a major obstacle for it adoption in the developing countries in the nearest future. There are few reports on the economics of MAS compare to conventional breeding but observed differences considerable among the studies. The initial cost of using markers is more expensive compared to conventional breeding; however time savings could lead to accelerate variety release which could considerable lead to greater profit. The low reliability of markers to determine phenotype is another important factor obstructing the successful application of markers for line development. This is often attributed to the 'thoroughness' of the primary QTL mapping study. In small population, detected QTLs and greater proportion of the phenotype may be influenced by sampling bias, and therefore may not be useful for MAS. Optimization of marker genotyping methods in terms of cost-effectiveness and greater proportion of integration between molecular and conventional breeding represent one of the main challenges for the greater adoption of MAS in rice breeding in near future.

Table 2 Successful examples of conventional breeding and MAS applications for blast resistance in rice

Sl. no.	Target trait	Gene(s)/QTL(s)	Type/name of marker(s) used	Remarks	References
Conventional breeding application for blast resistance in rice					
1.	Blast resistance (Basmati, Type-3)	–	–	Dwarfism (*sd-1*) from Pusa-1121 and aroma and blast resistant from Khalsa-7 introduced in basmati (Type-3) using traditional breeding supplemented with molecular markers	[220]
2.	Blast resistance	–	–	KDML105 was improved for blast resistance by conventional breeding programs in Thailand. IRRI shuttle breeding program developed blast resistant KDML105 by conventional backcross breeding	[55]
3.	Blast resistance (male sterile line, Rongfeng 3A)	*Pi1, Pi2*	–	A new cytoplasmic male sterile line, Rongfeng 3A, with *Pi1, Pi2* was successfully developed through successive backcross breeding	[221]
4.	Blast resistance (Minghui 63)	–	–	The most famous hybrid rice "Minghui 63" developed by conventional crossbreeding in China	[222]
5.	Blast resistance	*Pib* and *Pita*	–	IR 5, IR 8, IR 20, IR 22, IR 24, IR 26, IR 28, IR 29, IR 30, IR 32, IR 34, IR 36, IR 38, IR 40, IR 42, IR 43, IR 44, IR 45, IR 46, IR 48, IR 50 IR 52, IR 54, IR 56, IR 58, IR 60, IR 62, IR 64, IR 65, IR 66, IR 68, IR 70, IR 72, IR 74 have developed through conventional breeding	[223, 224]
6.	Blast resistance	*Pi-1*, *Pi-2* and *Pi-33*	–	Three resistance genes (*Pi-1,Pi-2* and *Pi-33*) were introgressed into an elite variety Jin 23B by crossing, backcrossing and multi-crossing combined with MAS	[225]
7.	Resistant to neck blast and susceptible to leaf blast	–	–	Norin 6 (Joshu X Senichi) was developed in 1935 and	[226]
	Resistant to leaf blast and susceptible to neck blast			Norin 8 (Ginbozu X Asahi) in 1936 by systematic breeding	
8.	Resistant to both leaf and neck blast	–	–	Norin 22 and Norin 23 were produced by hybridization	[226]
MAS application for blast resistance in rice					
1.	Bacterial blight (BB) resistance + Blast resistance	*Xa21 & Piz*	STS for *Piz,* transgene specific marker for *Xa21*	MAS applied for pyramiding of target traits. Xa21 gene originally introduced into donor lines through genetic engineering (target variety: IR50)	[16]
2.	Blast resistance	*Pi1, Piz-5, Pi2, Pita*	RFLP markers for *Pi1, Pi2* and *Pita* and a PCR based SAP marker for *Piz-5*	MAS applied for gene pyramiding (target variety: C039)	[148]
3.	Blast resistance	*Pi1*	SSR and ISSR markers	MAS applied for backcross breeding (target variety: Zhenshan 97A)	[227]
4.	Submergence Tolerance + BPH resistance + Bacterial blight resistance + Blast resistance + quality	*Subchr9 QTL, Xa21,* Bph and blast QTLs and quality loci	SSR and STS	MAS applied for backcross breeding	[36]
5.	Blast resistance	*Pid1*, *Pib*, *Pita*	–	Target genes were pyramided to G46B variety	[228]
6.	Blast resistance	*Pi2*	–	*Pi2* gene was introduced into Zhenshan 97B through MAS	[228]

Table 2 continued

Sl. no.	Target trait	Gene(s)/QTL(s)	Type/name of marker(s) used	Remarks	References
7.	Blast resistance +BB	*Pi1* and *Pi2* for blast resistance and *Xa23* for BB	SSR	MAS applied for backcross breeding Target variety: Rongfeng B	[221]
8.	Blast resistance	*Pi-9*(*t*)	marker pB8	Target gene was introgressed into hybrid restorer Luhui17 by using backcross approach and molecular marker-assisted selection (MAS) technique	[229]

Fortune of marker assisted selection in rice breeding

We are optimistic that despite the relatively small adoption of markers in rice breeding to date, there will be a greater level of adoption within the next few years. For accelerating greater adoption of MAS in rice we need to consider the following factors i.e. builtup facilities for marker genotyping and personnel training within/among many rice breeding institutes in home and abroad, continuous update of available data on genes/QTLs controlling traits and find out tightly linked markers, setup of effective strategies for using markers in breeding, establishment and updating of public databases for QTL/marker data, plentiful resource for generating new markers from rice genome sequencing and research on functional genomics. In future new marker technology can potentially minimize the cost of MAS. If the effectiveness of the new methods is legitimate and the necessary equipment made available, this will make MAS to be more widely applicable for rice breeding programmes.

Gene transformation

Transgenic plants can acquire a single desired trait without any alteration of the original genetic make up and can overcome the limitation of traditional breeding. Transgenic technologies allow multiple genes insertion simultaneously into genome to obtain broad-spectrum resistant lines. Genetic engineering are important approaches in the management of fungi diseases by introducing and over-expressing of genes that encode proteins involved in the synthesis of compounds toxic to fungi and with direct inhibitory effect on the growth of fungi [230]. Some transgenic strategies for improving blast resistance based on the host–pathogen gene-for-gene interaction system and antifungal protein genes have been developed [231, 232]. Up till now, there have been reports on increasing rice blast resistance through transformation of chitinase gene [233], plant antitoxin gene [234], chitinase–glucanase gene [235], trichosanthin gene [236], wasabi phytoalexin gene [237] and rice blast resistance genes *Pi-ta*, *Pi-9*, *Pi-2* etc. [113, 115, 119, 120, 167]. Transgenic approach has been used as an attractive alternative to conventional techniques for the genetic improvement of Basmati rice [238]. During the last 10 years, a rapid progress has been made towards the development of transformation methods in rice. Several transformation methods including Agrobacterium, biolistic, and DNA uptake by protoplasts, have been employed to produce transgenic rice. The application and future prospects of transformation technology to engineer the resistance against blast diseases and improved nutritional quality (accumulation of provitamin A and essential amino acids in endosperm) in Basmati rice, have been addressed. We hope that the results would provide useful information for molecular breeding.

Up till now, five types of toxin have been purified from the crude toxin of rice blast fungi, namely, piricularin, picolinic acid, tenuazonic acid, piriculol and coumarin. The crude toxin has strong inhibition on seed germination and extension of plumule and radicle of rice, and can serve as a selection pressure for disease-resistant mutant selection at the callus level [239]. The transgenic rice blast resistant gene improves the resistance to rice blast crude toxin, the transgenic *Pi-d2* stable rice line TP-Zh01-62-5 was used under different selection pressures of rice blast crude toxin. The results showed that as the concentration of rice blast crude toxin increased, the embryonic callus induction rate decreased obviously. When the concentration of crude toxin reached 50 %, the embryonic callus induction rate of the control decreased to zero, and the growth of embryos was completely suppressed, whereas that of the transgenic plants was still at 30.1 %, suggesting that the resistance of transgenic plants to the rice blast crude toxin was increased compared with the control. This provided another evidence for the feasibility of rice blast crude toxin as measures for resistance to rice blast.

Especially for *M. grisea* fungal transformation, chlorimuronethyl resistance gene is increasingly used as a selectable marker [240]. However, rice blast strains

collected from infectious rice fields have highly conserved resistance to chlorimuronethyl, even comparable to transformants which carry chlorimuronethyl resistance genes as selectable marker in laboratory conditions. Chlorimuronethyl selectable markers were used for *Neurospora crassa* [241], Cercospora nicotianae [242] and other fungal transformations. Although *M. grisea* transformation has many available selectable markers, in some special cases, other selectable markers such as the marker bearing the chlorimuronethyl resistance gene has been developed.

Conclusion

Considering ever-increasing population, urgent need to increase rice production globally. The use of resistant rice cultivars is a powerful tool to reduce the use of environmentally destructive pesticides. Using classical plant breeding techniques, plant breeders have developed a number of blast resistant cultivars adapted to different rice growing regions worldwide. However, the rice industry remains threatened by blast disease due to the instability of the rice blast fungus. Recent advances in rice genomics provide additional tools for plant breeders to develop rice production systems that could be sustainable and environmentally favorable. It is very important to the rice breeders to find the easiness of molecular techniques and their cost-effectiveness to integrate these techniques to conventional breeding. In my opinion, this article outlines will be provided about the principal application of conventional breeding to different molecular approaches that can be used for accelerating the development of blast resistant rice cultivars by sustaining rice yields to meet up the demand and food security in the coming years and decades globally.

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