Review

Strategies for Breeding Durable Resistance to Rice Blast Using *pi21*

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ABSTRACT

Rice blast caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) is a destructive disease of rice worldwide that poses a serious threat to rice production. Strengthening blast resistance is an important objective in rice breeding programs. Race-specific resistance genes (*R*-genes) confer complete resistance to blast, but new races of blast pathogen can overcome it. After the first report of breakdown of resistance conferred by the *R*-gene *Pik* in 1963, this type of resistance has frequently been broken 1–7 years after the release of resistant varieties to farmers in Japan and other countries. To overcome this genetic vulnerability, Japanese rice breeders have focused on the use of race-nonspecific resistance in Japanese upland rice varieties whose resistance has been maintained for a long time. However, linkage drag between genes controlling this type of blast resistance and undesired traits has hindered its use. Therefore, researchers genetically dissected race-nonspecific resistance to rice blast. Among detected QTLs, a single recessive resistance gene, *pi21*, was identified by map-based cloning. The use of *pi21* has improved durable resistance in rice breeding programs.

KEYWORDS: race-nonspecific resistance; quantitative resistance; durable resistance; multiline variety; gene pyramiding; marker-assisted selection; rice; blast disease

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ABBREVIATIONS

QTL, quantitative trait locus; NIL, near-isogenic line; *R*-gene, race-specific resistance gene

INTRODUCTION

Rice blast caused by the fungus *Magnaporthe grisea* (*Pyricularia oryzae*) is a major biotic constraint in rice cropping regions worldwide that threatens global rice production and productivity. Between 10% and 30% of the annual rice harvest is lost because of infection by rice blast fungus

[1]. Genetic improvement of resistance against rice blast is a significant and primary target in rice breeding programs. Varietal resistance has been explored for over a century for sustainable production [2]. As in other plant–pathogen interactions, resistance to blast is categorized into two types, race-specific (complete, qualitative or true) and race-nonspecific (partial, quantitative or field)[3,4]. Race-specific resistance (1) is based on the hypersensitive reaction, (2) is often complete, and (3) is characterized by a resistant infection type. Race-nonspecific resistance is a susceptible infection type that allows effective control of a pathogen under natural field conditions and is considered to be durable when plants are exposed to new races of the pathogen and maintain their previous degree of resistance.

Race-specific blast resistance is achieved through many race-specific resistance genes (*R*-genes) identified in a broad range of the world's rice germplasms [5]. *R*-genes dramatically enhance blast resistance, resulting in stable rice production, but their extensive use poses a serious risk of the generation of new races of the blast pathogen and the quick breakdown of resistance. In Japan, the first breakdown of resistance occurred only 2 years after the release of the resistant "Kusabue" with the *R*-gene *Pik* introgressed from a Chinese variety in 1963; similarly, resistance conferred by several different *R*-genes was broken in the following years (Table 1). Similar trends were reported in Korea and Colombia [6]. Hence, rice breeders started paying attention to the use of race-nonspecific resistance.

Table 1. Instances of breakdown of *R*-gene–mediated resistance to rice blast in Japan.

Variety	Resistance gene	Prefecture	Release	Breakdown	Duration (years)
Kusabue	*Pik*	Ibaraki	1961	1963	2
		Tochigi	1961	1963	2
		Fukushima	1960	1964	4
		Toyama	1961	1963	2
		Saitama	1961	1963	2
		Gunma	1961	1963	2
Yukara	*Pik*, *Pia*	Hokkaido	1962	1965	3
Teine	*Pik*, *Pia*	Hokkaido	1962	1964	2
Ugonishiki	*Pik*	Akita	1962	1964	2
Tachihonami	*Pik*	Yamagata	1966	1968	2
Shimokita	*Pita*, *Pia*	Aomori	1962	1969	7
Fukunishiki	*Piz*	Fukushima	1964	1969	5
Yamatenishiki	*Piz*	Yamagata	1976	1977	1

Adopted with modification from [7].

Japanese upland rice varieties are potential gene donors of race-nonspecific resistance [8]. Genetic studies indicate that their resistance is controlled by multiple genes or polygenes, two of which may be linked to the phenol reaction (*Ph*) locus on chromosome 4 or the lax

panicle (*lax*) locus on chromosome 11 [9,10]. However, conventional genetic approaches cannot determine the exact number of genes associated with the resistance or their chromosomal locations. Moreover, upland rice varieties have undesired characteristics, in particular poor grain and eating quality [8]. Before upland rice varieties can be used to improve durable resistance to rice blast, the genetic dissection of the resistance is required.

The Rice Genome Project was initiated in Japan in 1991 and has greatly contributed to rice genetics and breeding [11–14]. Molecular markers mapped over the 12 rice chromosomes and over the entire genome sequence are powerful tools to identify genes controlling quantitative traits. Many quantitative trait loci (QTLs) for agricultural traits, including race-nonspecific resistance of Japanese upland rice varieties, have been identified using these markers, and beneficial QTL alleles have been introduced into elite genetic backgrounds. This review focuses on the identification of *pi21*, a QTL allele conferring race-nonspecific resistance in a durably resistant variety, by genome-based analyses and its use in breeding programs. The current status of the identification of other genes found in durably resistant varieties, gene pyramiding and the use of multiline varieties are also discussed.

PARADIGM SHIFT IN RICE BLAST RESISTANCE

The breakdown of the resistance conferred by *R*-genes occurs 1–7 years after their release to farmers (Table 1). This so-called genetic vulnerability is explained by the emergence of new races of blast pathogen when varieties with the same resistance genotype are predominant in farmers' fields. Genetic studies on durably resistant varieties have accelerated a shift from race-specific to race-nonspecific resistance genes in rice breeding programs in Japan.

QTLs Underlying Race-Nonspecific Resistance to Rice Blast

QTLs for race-nonspecific resistance to rice blast were analyzed in progeny derived from crosses between Japanese upland and paddy rice varieties. In an "Owarihatamochi" (resistant, upland) × "Nipponbare" (moderately susceptible, paddy) cross, two resistance QTL alleles on chromosome 4 and one on chromosome 12 from "Owarihatamochi" were identified [15]. Each QTL explained from 13.7% to 45.7% of the total phenotypic variation. The results suggest that the resistance of "Owarihatamochi" is controlled by a small number of QTLs with different contributions. The QTL on chromosome 4 was inherited as a single recessive gene and was designated *pi21* [15]. In addition, one region on chromosome 11 was significant at a lower probability threshold [15]; it was later designated *Pi34* and was analyzed in lines derived from a Japanese upland variety [16,17]. Resistance QTL alleles from other upland varieties were detected in regions similar to those in "Owarihatamochi" on chromosomes 4, 11 and 12, although their relative contributions to

decreasing disease severity differed among cross combinations [18,19]. Resistance QTL alleles from other varieties were located mainly in a 30-Mb region of chromosome 4 and on chromosomes 1, 3, 6 and 11 [16,20–24]; these observations imply that genetic differentiation of disease resistance genes may cause variation in the magnitude of resistance conferred by respective QTLs. Accumulated evidence highlights the target regions for improving race-nonspecific resistance to rice blast.

Characterization of *pi21* Using a Near-Isogenic Line

The effect of the *pi21* resistance allele alone cannot be evaluated by inoculation tests using a donor variety that carries multiple resistance QTL alleles [15,25,26]. Near-isogenic lines (NILs; lines genetically identical except in one or a few loci) are useful for characterization of loci conferring complex agricultural traits owing to their homogeneous genetic background. A NIL for *pi21* in the genetic background of the susceptible variety "Aichiasahi" was used to test the response to 16 widely distributed blast races [26,27]; the quantitative and consistent effect of *pi21* against all of the races tested was found.

A transient increase in the expression of pathogenesis-related (*PR*) genes at 3–6 h after inoculation with a virulent race was observed in plants carrying *pi21* but not in plants carrying *R*-genes [27]. Inoculation of plants carrying *pi21* with elicitor solution mimicked this response, and removal of the elicitor from the inoculum decreased blast resistance in these plants [28]. These observations imply a role of *pi21* in the pre-penetration plant–pathogen interaction through elicitor-triggered immunity. Unlike in plants lacking *pi21*, inoculation tests after application of an antagonist of ethylene biosynthesis did not decrease blast resistance in plants carrying *pi21* in comparison with the corresponding untreated controls [26]. Since the inhibition of ethylene biosynthesis decreases resistance to a number of diseases [29], this distinctive response implies the involvement of ethylene signaling in *pi21*-mediated resistance. A recent study has suggested the complex control of signaling in *pi21*-mediated resistance [30].

Unlike other defense genes such as *WRKY45* and *BSR1*, which alter resistance to multiple plant pathogens [31,32], *pi21* does not affect resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* or the fungal pathogen *Rhizoctonia solani* [28]. Therefore, it might inhibit the hyphal growth of the blast pathogen, and identification of protein(s) interacting with *Pi21* would provide further insight into mechanism of *pi21*-mediated resistance.

CLONING AND CHARACTERIZATION OF GENES FOR RACE-NONSPECIFIC RESISTANCE

More than 100 loci for resistance to blast have been identified and more than 30 of them have been cloned [33–35]. Most of them encode nucleotide-binding site (NBS) leucine-rich repeat (LRR) proteins that interact with pathogen effectors and trigger defense reactions according

to the gene-for-gene model of recognition [36,37]; the exceptions are *Pid2*, which encodes a receptor-like kinase [38], and *Ptr*, which encodes an Armadillo repeat protein [39]. Recent studies in rice and *Arabidopsis* showed that different genes for NBS–LRR proteins, such as a pair of tightly linked NBS–LRR genes, cooperate in pathogen recognition and resistance [40–45]. In contrast, the number of genes that have been cloned for race-nonspecific resistance is limited.

Map-Based Cloning of *pi21*

Linkage analysis and progeny testing narrowed down the *Pi21* locus to a small region carrying a single gene locus, *Os04g0401000* [27]. This gene encodes a protein with a putative heavy-metal-binding domain and a proline-rich region. Comparison of sequences between resistant and susceptible varieties identified 21- and 48-bp deletions in the resistant variety, suggesting that one or both of these deletions confer resistance. Transgenic complementation testing confirmed that a loss-of-function mutation in the gene improves resistance to blast. Suppression of the expression of the gene in a susceptible variety resulted in a level of resistance similar to that of plants carrying *pi21* [27]. Conversely, transgenic plants with higher expression levels of the susceptibility-conferring *Pi21* allele were more susceptible to blast than those with lower expression. Increased expression of *Pi21* did not alter the strength of the *Pia*-dependent hypersensitive reaction against an avirulent race. These results demonstrated that *Pi21* is a negative component of defense that belongs to a pathway different from *R*-gene–mediated resistance.

Information on the variation of QTL alleles allows the use of a wide range of germplasm. In the case of the *pi21* gene, Asian cultivated rice has 12 haplotypes determined by insertion/deletion variations at three sites in the proline-rich region, which is presumed to be involved in protein–protein interactions in multicellular organisms [46,47]. Each of the 12 haplotypes carries one of the two deletions or two smaller deletions compared with “Owarihatamochi” haplotype, but it is difficult to predict the resistance/susceptibility phenotype from DNA sequences. Inoculation testing using a series of backcrossed lines carrying each of the *Pi21* haplotypes in the same genetic background indicated that only the line carrying the “Owarihatamochi” haplotype showed improved resistance to blast; the rest were susceptible, similar to the recipient variety [27]. The results suggest that the two deletions in the resistance *pi21* allele are optimal to cause the loss of function, which increases resistance to blast. Rice varieties carry susceptibility alleles that can be replaced with the resistance *pi21* allele.

Genes associated with disease susceptibility are considered essential for plant growth, and their loss frequently has deleterious effects [48]. Hence, for practical use, resistance alleles that show a partial loss of function and are mildly pleiotropic are desirable. In the *xa13* allele for resistance to *X. oryzae* pv. *oryzae*, a mutation prevents pathogen

propagation, but the mutated protein retains certain functions in normal pollen development [49]. DNA variations that cause amino acid changes in *Pi21* in Asian cultivated rice were found only in the proline-rich motif sequences encoded in the middle of the gene, whereas the C-terminal region and the putative heavy-metal-binding domain in the N-terminal region are free from variations [27]. Such observations imply that the resistance *pi21* allele maintains certain functions important for plant growth, as in the case of *xa13*. The lower survival rate of plants whose *Pi21* expression is strongly suppressed by RNAi is in line with this hypothesis (S. Fukuoka, unpublished data). A recent study reported increased blast resistance conferred by a CRISPR/Cas9-edited *Pi21* gene [50]. Characterization of the agronomic traits of lines having diverse *Pi21* variants will provide further evidence on this topic.

Map-Based Cloning of Other QTLs for Race-Nonspecific Resistance

Pi35 is a major resistance QTL on chromosome 1; its resistance allele was found in a Japanese breeding line, “Hokkai 188”, that has maintained resistance under natural field conditions since 1961 [51]. Linkage analysis and complementation testing revealed that *Pi35* is allelic to *Pish*, a typical *R*-gene that encodes an NBS–LRR protein [52]. Among 6 differences in the deduced amino acid sequences between *Pi35* and *Pish*, one of the four residues in the LRR region is significantly associated with race-nonspecific resistance [53]. However, the analysis of chimeras between *Pish* and *Pi35* confirmed that the three other residues in the LRR region and two residues in the NBS domain are also associated with the resistance, suggesting that a combination of multiple functional polymorphisms in the gene confers race-nonspecific resistance [53]. Plants with *Pish* are completely resistant to a single blast isolate but susceptible to other isolates tested under natural field conditions in Japan. Plants with *Pi35* were less resistant to the isolate avirulent to *Pish* plants; but were consistently resistant against the other blast isolates tested [53]. This example implies that the quantitative nature of resistance governed by an NBS–LRR protein gene may decrease selection pressure against the pathogen.

Pi63 is a major resistance QTL in a 30-Mb region of chromosome 4; its resistance alleles were found in a Japanese upland rice variety, “Kahei” [19]. Linkage analysis and complementation testing demonstrated that this gene encodes an NBS–LRR protein and is located within an *R*-gene cluster [54,55]. Not only the difference in amino acid sequences, but also different expression levels of *Pi63* and its counterpart allele in a susceptible variety could lead to the resistant phenotype [54]. Interestingly, resistance conferred by *Pi63* is isolate-specific, as demonstrated using a NIL for *Pi63*. Such characteristic has not been identified in the genetic background of the donor variety because of the effect of the race-nonspecific *pi21* allele and alleles of other resistance QTLs. Increased expression of *Pi63* in transgenic lines led to moderate resistance against pathogen isolates that produce a highly susceptible phenotype in the NIL for *Pi63*. Therefore,

variations of the expression levels of genes for NBS–LRR proteins could be part of the genetic mechanism of race-nonspecific resistance in rice.

Panicle blast 1 (*Pb1*) on chromosome 11 is a gene derived from the *indica* variety "Modan" [56]. Plants carrying this gene are blast susceptible during young vegetative stages, but the resistance level increases as the plants grow, and persists even after heading [57]. This gene is useful for conferring resistance to panicle blast because the varieties that have this gene maintain resistance over several decades [57]. Map-based cloning of *Pb1* revealed that it encodes an atypical NBS–LRR protein that has no P-loop and some motifs in the NBS domain are degenerated [58]. *Pb1* transcript levels have increased during the development of *Pb1*-resistant varieties and effectively control panicle blast [58]. *Pb1*-mediated resistance seems to be mediated by a signaling pathway distinct from that involving typical NBS–LRR proteins [59].

PRE-BREEDING AND BREEDING OF RICE RESISTANT TO BLAST USING *pi21*

Most Japanese upland rice varieties are donors of *pi21*; their morphological and physiological characteristics are distinct from those of elite genotypes. Breeding efforts to introduce resistance alleles of major QTLs from upland rice started in the 1920s. But the trials were unsuccessful because of the co-introduction of undesirable characteristics from the donors [7,60]. This co-introduction could be explained by tight linkage of genes controlling independent traits (linkage drag) and/or by the effect of the target gene on other traits (pleiotropic effect). Therefore, the development of NILs for *pi21* in a desirable genetic background is a possible strategy for determination of the cause of this association and for enhancing the use of resistance QTL alleles from unimproved genetic resources.

Development of NILs for *pi21*

Agronomic traits of NILs for *pi21* in the genetic background of an elite rice variety ("Mineasahi") were evaluated [27]. Despite the presence of less than 5% of donor chromosome sequences, plants carrying *pi21* had poor grain and eating quality, which were not observed in reference lines carrying *Pi21* [27]. The results strongly support the idea that *pi21* or a gene(s) tightly linked with it controls grain characteristics. During NIL development, DNA markers tightly linked with the target QTL are used for foreground selection, and background selection around that QTL is not intensive. When the precise map position of the target is not determined, the size of the selected introgression will be larger so as not to miss the gene. Such situation could be the reason for the difficulty in the use of beneficial traits of unimproved genetic resources. The two cases, linkage drag and pleiotropy, cannot be discriminated unless the linkage can be broken.

Removal of Linkage Drag and Development of Varieties Carrying *pi21*

To remove donor chromosome segments around the *Pi21* locus and elsewhere in the genome, a line carrying the *pi21* allele was backcrossed with an elite paddy rice variety ("Koshihikari"), and progeny carrying a single 1.8-Mb fragment around the *Pi21* locus from the donor was selected. Plants with recombination events within a 40-kb interval containing the *Pi21* locus were selected from approximately 6000 progeny. The eating quality of a progeny line carrying the "Koshihikari" chromosomal sequence from a point less than 2.4 kb downstream of the *Pi21* locus was equivalent to that of the elite variety, and the line was highly resistant to blast. In contrast, a progeny line carrying the donor chromosomal sequence up to 37 kb downstream of the *Pi21* locus showed inferior eating quality [27]. These results clearly show that the resistance *pi21* allele does not penalize agronomic traits, and the cause of the association is tight linkage with genes causing undesirable traits. The recessive nature of the resistance allele also made it difficult to select this locus by conventional procedures.

The promising line with improved blast resistance and desirable grain characteristics was released as "Tomohonami" ("Chubu 125") in 2009 [61]. "Tomohonami" has been used as an excellent donor of *pi21* at more than 15 prefectural breeding stations and 6 research centers of the National Agriculture and Food Research Organization, Japan. More than 15 breeding lines carrying *pi21* have been developed. Recently, "Fufufu", a line derived from "Tomohonami" carrying *pi21* and *Apq1* for high-temperature tolerance during ripening, was released, and its area of cultivation is increasing [62,63].

Mutant allele of negative regulators of defense such as *pi21* may reduce yield because of constitutive activation of defense responses and have other secondary effects, as barley *Mlo* does [64,65]. However, slow induction of defense by *pi21* contributes to pathogen control without penalty on yield, as confirmed by field tests at several locations [66]. The *pi21* alleles are effective against diverse fungus races, so the use of *pi21* might not be a strong driving force for changes in the structure of pathogen populations. The durability of resistance conferred by a gene needs to be proved by prolonged resistance of varieties carrying that gene alone under natural field conditions [67]. Monitoring of newly released varieties carrying *pi21* will provide further evidence to confirm or disprove the durability of resistance conferred by *pi21*.

BREEDING STRATEGIES FOR DURABILITY OF BLAST RESISTANCE

Developing varieties that are resistant in a disease-prone area is a challenge in crop breeding. Despite the largest effect of the *pi21* allele in comparison with other resistance alleles in "Owarihatamochi", a durably resistant variety, this allele alone may not be sufficient to control the

disease under high disease pressure. Two breeding approaches are proposed to increase the durability of resistance to pathogens in crop plants [68]: (i) the use of multiline varieties carrying different resistance genes and (ii) combining multiple resistance genes in the same genotype. This section overviews these two approaches and explains technical issues that need to be considered for sustainable use of durable resistance to blast in rice.

Multiline Varieties for Blast Control in Rice

A multiline variety is a mixture of pure lines carrying different resistance genes. This concept was originally proposed in 1952 for controlling disease in oat [69], and its usefulness was confirmed [70,71]. Mixing varieties with different characteristics contributes to disease control in rice [72], but NILs in elite genetic backgrounds are more desirable components of multiline varieties to ensure the uniformity of agricultural traits. Case studies of various crop–pathogen combinations have shown differences in resistance among multiline varieties [73]. Hence, guidelines for the management of multiline varieties should be based on the evidence for particular crop–pathogen combinations.

As discussed above, the resistance conferred by a single *R*-gene is vulnerable, while in a field of heterogeneous plants with different *R*-genes, the damage by the pathogen is decreased. This phenomenon may be explained by (i) the dilution of inoculum owing to a decrease in the density of infected plants [73,74], (ii) barrier effect of resistant plants [75,76], and (iii) induced resistance because of pre-inoculation with avirulent pathogen isolates [77,78]. Under appropriate management, even the *R*-genes whose resistance has been overcome by the pathogen in the past can be used as components. Thus, this approach allows a sustainable use of *R*-genes in breeding programs.

Because of the limited number of available resistance QTL alleles, only *R*-genes have been used for practical breeding of multiline varieties in rice [75,79]. Multiline varieties that rely on 15 recurrent parents have been developed or are under development in Japan [75]. Of 15 *R*-genes used in the Japanese breeding programs, 13 were incorporated into these varieties (on average, 6.2 per recurrent parent). Despite breeding efforts since the 1980s, only five multiline varieties that rely on four recurrent parents have been released [80,81].

Commercial cultivation of the multiline variety “Sasanishiki BL” started in 1995 in Miyagi Prefecture in Japan [82]. The initial ratio of three NILs having each one of the *Pik*, *Pik-m* and *Piz* genes was 4:4:3. After the increase in the incidence of a pathogen race virulent to the lines carrying *Pik* and *Pik-m* in the fields of “Sasanishiki BL”, the ratio was changed to 3:4:4, followed by the addition of a line carrying *Piz-t*, at a final ratio of *Pik*:*Pik-m*:*Piz*:*Piz-t* = 1:1:4:4. Although this multiline variety has maintained resistance for more than 9 years, pathogen races virulent to each of the NILs have been observed in the field [80,82]. This fact implies

that “Sasanishiki BL” may not stabilize the race composition of the pathogen, and its small area of cultivation might instead explain its continued low disease incidence [80].

Multiline varieties of “Koshihikari”, the leading variety in Japan, were used in Niigata and Toyama prefectures, with different gene components [79,83]. In Niigata Prefecture, the area for the multiline variety “Koshihikari BL” is larger than that for “Sasanishiki BL”. Four out of 11 NILs were used every year, and the choice of the lines and their ratio were based on monitoring temporal race dynamics of the blast pathogen in the prefecture. A theoretical model to slow changes of the estimated pathogen population determined the proportion of resistant plants as 70% [84]. Accordingly, the proportions of the areas of occurrence of leaf blast and panicle blast in the prefecture decreased after the replacement of “Koshihikari” with “Koshihikari BL”, and this trend has been maintained for more than 12 years since 2005 (http://www.pref.niigata.lg.jp/nosanengei/1215712857692.html). This example shows that a multiline variety effectively controls blast damage under appropriate management.

These two cases show that multiline varieties of rice do not stabilize pathogen populations. Therefore, the number of genes in a multiline variety and determination of the components based on the monitoring of temporal changes of pathogen populations are key factors to ensure the durability of resistance. Developing a single multiline variety requires at least 3 (ideally 8 to 10) NILs and their seed production. Sampling of the pathogen and estimation of its population structure are required every year to choose the lines and their relative proportion for the next year. Hence, seed supply requires considerable cost and labor; software that helps seed management has been developed on the basis of simulation studies of temporal pathogen population dynamics in rice [80]. Another aspect of NILs to be considered is the value of their recurrent parent. Because of the high sensitivity of “Sasanishiki” to cold stress at booting stage and because its taste has lost favor among consumers, the cropping area of “Sasanishiki BL” has decreased correspondingly. The life of multiline varieties has become shorter and their market share has decreased because of climate change and diversification in consumers’ requirements. These points suggest that the use of multiline varieties is beneficial for leading varieties but not for varieties grown for diverse purposes.

Gene Pyramiding for Sustainable Control of Blast

Gene pyramiding (combining multiple resistance alleles in the one genetic background) is another way to enhance durable resistance in crop plants. If a single genotype confers durable resistance, this approach is more desirable for breeders because breeding procedures and seed management are simpler than those with multiline varieties.

R-gene pyramids improve resistance to diverse pathogen isolates [85–88]. A comprehensive survey of a series of gene pyramids

detected interaction among genes. Among combinations between one of the *Pigm*, *Pi2*, *Pi9*, *Pi40* and *Piz* genes and one of the *Pi1*, *Pi33* and *Pi54* genes, *Pigm/Pi1*, *Pigm/Pi54* and *Pigm/Pi33* provided the best resistance at both seedling and heading stages [87]. These results highlight the importance of screening for favorable gene combinations to maximize resistance.

Broadening the spectrum of resistance by pyramiding *R*-genes may prompt the counter-evolution of the pathogen; for example, the resistance of a variety with four *R*-genes was overcome one year after its release [89]. The emergence of super-races that overcome the resistance of *R*-gene pyramids might increase over time when a single variety carrying an *R*-gene pyramid is cultivated in a large area. An epidemiological survey and simulation study on pathogen race dynamics suggest that replacement by varieties with different *R*-genes leads to drastic changes in the pathogen population structure that increase the risk of disease outbreak [90]. Hence, *R*-gene pyramids, each resulting in a strong selection pressure against its pathogen, may not improve the durability of resistance. Further study of the effect of *R*-gene pyramids on pathogen population dynamics in the field is necessary to develop the guidelines for their use.

Combining multiple resistance QTL alleles is considered to additively enhance race-nonspecific resistance. However, breeders and researchers know that disease resistance sometimes interacts with genetic backgrounds and/or environmental factors [91–94]. The data on resistance to blast over two decades support this idea in the context of race-specificity and temperature-dependent resistance, and indicate the existence of genetic loci that modulate the resistance or its mode of action [26,54,95,96]. To understand how resistance QTL alleles interact with such factors, it is important to determine the appropriate number and combinations of resistance genes. However, knowledge of the impact of QTL pyramiding on the robustness of plant defense in rice is limited [26,97].

In the genetic background of the susceptible “Aichiasahi”, the average reduction of lesion area by *pi21* in eight field trials was 87% compared with the recurrent parent, whereas that by the minor QTL alleles was 39% by *Pi34*, 45% by *qBR4-2* and 22% by *qBR12-1* [26]. Although the effects of these minor QTL alleles were sometimes undetectable, their combinations dramatically reduced lesion area both in field tests and in glasshouse inoculation tests. The line with four resistance alleles had a lesion area of ≤1%, which was similar to that in the donor and was only 6% of that in the line carrying *pi21* only, suggesting that the QTL pyramid conferred robust resistance. Similar results were obtained for a series of lines with one to four resistance QTL alleles, despite the presence of background noise (effect of unidentified QTLs) from donors [97]. A more important observation is that a QTL pyramid improves the stability of resistance; the coefficient of variation of lesion area across field tests in the line carrying four resistance QTL alleles was smaller than those in lines with only one or two [26]. That study demonstrated the importance of minor QTL alleles

for improving the stability of resistance, even if the effect of each of them is sensitive to the environment.

Histological study and expression analysis supported the idea that the hypersensitive reaction was not induced in the four-QTL pyramid line, unlike in *R*-gene–mediated resistance, but the defense response was greater than in no-QTL or *pi21*-only plants [26]. Therefore, the use of QTL pyramids may maintain an optimal balance between the effective control of the pathogen and selection pressure against it, and thus it may confer durable resistance to blast in rice.

One of the concerns in the use of QTL pyramids is linkage drag. Most resistance QTL alleles have not been used in commercial varieties by conventional breeding programs, possibly because of linkage drag, as in the case of *pi21* [27]. The use of breeding lines with remaining undesirable traits decreases the efficiency of breeding proportionally to the number of resistance QTL alleles, as seen in conventional breeding. Therefore, the resistance alleles should be precisely mapped and the breeding program should start from linkage drag elimination. The fitness cost of resistance is another issue that should be evaluated in the future. The costs or penalties associated with the activation of defense responses in the absence of a pathogen attack may decrease yield [64,98,99]. Unlike the barley *Mlo* mutant [64,65], NILs for *pi21* only appear to have no penalty on yield [28,68]. However, the penalty on plants that carry multiple resistance alleles has not been well clarified, although at least their growth does not appear to be affected (Fukuoka, unpublished data). However, the pleiotropic effects of resistance QTLs may be small and detectable only in large-scale field tests. Further evaluation in multiple environments is required to answer this question.

CONCLUSIONS

Progress in understanding the genetic control of race-nonspecific resistance in Japanese upland rice has led to a breakthrough in rice breeding, and marker-assisted pyramiding of relevant genes guarantees enhancement of the trait. Varieties carrying *pi21* will provide further evidence of the durability of resistance in large cultivation areas. We recommend introducing other resistance QTL alleles into *pi21*-only varieties for robust disease control in disease-prone areas. Removal of undesirable agricultural traits that are tightly linked with the resistance QTL alleles needs to be considered. The cost of enhanced defense response in QTL pyramids has not yet been evaluated and should be optimized according to the risk of disease.

AUTHOR CONTRIBUTIONS

SF and KO wrote the manuscript, and reviewed, corrected, and proofed the final version.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Skamnioti P, Gurr SJ. Against the grain: safeguarding rice from rice blast disease. Trends Biotechnol. 2009;27(3):141-50. doi: 10.1016/j.tibtech.2008.12.002
2. McCouch SR, Nelson RJ, Tohme J, Zeigler RS. Mapping of blast resistance genes in rice. In: Zeigler RS, Leong SA, Teng PS, editors. Rice blast disease. Wallingford (UK): C.A.B. International; 1994. p. 626.
3. Ezuka A. Field resistance of rice varieties to rice blast disease. Rev Plant Prot Res. 1972;5:1-21.
4. Parlevliet JE. Components of resistance that reduce the rate of epidemic development. Annu Rev Phytopathol. 1979;17:203-22. doi: 10.1146/annurev.py.17.090179.001223
5. Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN, Ray S. Rice blast management through host-plant resistance: Retrospect and prospects. Agric Res. 2012;1(1):37-52. doi: 10.1007/s40003-011-0003-5
6. Bonman JM. Durable resistance to rice blast disease—environmental-influences. Euphytica. 1992;63(1-2):115-23. doi: 10.1007/Bf00023917
7. Watanabe S. Breeding by using foreign rice vrieties. In: Yamasaki Y, Kozaka T, editors. Rice blast disease and breeding for resistance to blast. Tokyo (Japan): Hakuyusha; 1980. p. 34-46. Japanese.
8. Morimoto T. Breeding by Chinese upland rice Sensho. In: Yamasaki Y, Kozaka T, editors. Rice blast disease and breeding for resistance to blast. Tokyo (Japan): Hakuyusha; 1980. p. 25-34. Japanese.
9. Higashi T, Saito S. Linkage groups of field resistance genes of upland rice variety “Sensho” to leaf blast caused by *Pyricularia oryzae* Cav. Jpn J Breed. 1985;35:438-48. Japanese with English summary.
10. Maruyama K, Kikuchi F, Yokoo M. Gene analysis of field resistance to rice blast (*Pyricularia oryzae*) in Rikuto Norin Mochi 4 and its use for breeding. Bull Natl Inst Agric Sci Ser D. 1983;35:1-31. Japanese with English summary.
11. Sasaki T. The rice genome project in Japan. Proc Natl Acad Sci U S A. 1998;95(5):2027-8. doi: 10.1073/pnas.95.5.2027
12. Yano M, Sasaki T. Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol. 1997;35(1-2):145-53. doi: 10.1023/A:1005764209331
13. Fukuoka S, Ebana K, Yamamoto T, Yano M. Integration of genomics into rice breeding. Rice. 2010;3(2-3):131-7. doi: 10.1007/s12284-010-9044-9
14. IRGSP. The map-based sequence of the rice genome. Nature. 2005;436(7052):793-800. doi: 10.1038/nature03895
15. Fukuoka S, Okuno K. QTL analysis and mapping of *pi21*, a recessive gene for field resistance to rice blast in Japanese upland rice. Theor Appl Genet. 2001;103:185-90. doi: 10.1007/s001220100611

16. Zenbayashi K, Ashizawa T, Tani T, Koizumi S. Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. Theor Appl Genet. 2002;104(4):547-52. doi: 10.1007/s00122-001-0779-y
17. Zenbayashi-Sawata K, Fukuoka S, Katagiri S, Fujisawa M, Matsumoto T, Ashizawa T, et al. Genetic and physical mapping of the partial resistance gene, *Pi34*, to blast in rice. Phytopathol. 2007;97(5):598-602. doi: 10.1094/PHYTO-97-5-0598
18. Kato T, Endo I, Yano M, Sasaki T, Inoue M, Kudo S. Mapping of quantitative trait loci for field resistance to rice blast in upland rice, 'Sensho'. Breed Res. 2002;3:119-24. Japanese.
19. Miyamoto M, Yano M, Hirasawa H. Mapping of quantitative trait loci conferring blast field resistance in the Japanese upland rice variety Kahei. Breed Sci. 2001;54:257-61.
20. Endo T, Yamaguchi M, Kaji R, Nakagomi K, Kataoka T, Yokogami N, et al. Close linkage of a blast resistance gene, *Pias*(t), with a bacterial leaf blight resistance gene, *Xa1-as*(t), in a rice cultivar 'Asominori'. Breed Sci. 2012;62(4):334-9. doi: 10.1270/jsbbs.62.334
21. Mizobuchi R, Sato H, Fukuoka S, Yamamoto S, Kawasaki-Tanaka A, Fukuta Y. Mapping of a QTL for field resistance to blast (*Pyricularia oryzae* Cavara) in Ingngoppor-tinawon, a rice (*Oryza sativa* L.) landrace from the Philippines. Jarq Jpn Agric Res Q. 2014;48(4):425-31. doi: 10.6090/jarq.48.425
22. Hirabayashi H, Sato H, Nonoue Y, Kuno-Takemoto Y, Takeuchi Y, Kato H, et al. Development of introgression lines derived from *Oryza rufipogon* and *O. glumaepatula* in the genetic background of japonica cultivated rice (*O. sativa* L.) and evaluation of resistance to rice blast. Breed Sci. 2010;60(5):604-12. doi: 10.1270/jsbbs.60.604
23. Terashima T, Fukuoka S, Saka N, Kudo S. Mapping of a blast field resistance gene *Pi39*(t) of elite rice strain Chubu 111. Plant Breed. 2008;127(5):485-9. doi: 10.1111/j.1439-0523.2007.01451.x
24. Sato H, Takeuchi Y, Hirabayashi H, Nemoto H, Hirayama M, Kato H, et al. Mapping QTLs for field resistance to rice blast in the Japanese upland rice variety Norin 12. Breed Sci. 2006;56(4):415-8. doi: 10.1270/jsbbs.56.415
25. Fukuoka S, Mizobuchi R, Saka N, Suprun I, Matsumoto T, Okuno K, et al. A multiple gene complex on rice chromosome 4 is involved in durable resistance to rice blast. Theor Appl Genet. 2012;125(3):551-9. doi: 10.1007/s00122-012-1852-4
26. Fukuoka S, Saka N, Mizukami Y, Koga H, Yamanouchi U, Yoshioka Y, et al. Gene pyramiding enhances durable blast disease resistance in rice. Sci Rep. 2015;5:7773. doi: 10.1038/srep07773
27. Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, et al. Loss of function of a proline-containing protein confers durable disease resistance in rice. Science. 2009;325(5943):998-1001. doi: 10.1126/science.1175550
28. Fukuoka S, Mizobuchi R, Yamamoto S-I, Yano M. Rice blast field resistance gene *pi21* enhances early responsiveness to virulent blast pathogen. Breed Res. 2008;10(Suppl 1):37.

29. van Loon LC, Geraats BPJ, Linthorst HJM. Ethylene as a modulator of disease resistance in plants. Trends Plant Sci. 2006;11(4):184-91. doi: 10.1016/j.tplants.2006.02.005
30. Zhang Y, Zhao JH, Li YL, Yuan ZJ, He HY, Yang HH, et al. Transcriptome analysis highlights defense and signaling pathways mediated by rice *pi21* gene with partial resistance to *Magnaporthe oryzae*. Front Plant Sci. 2016;7:1834. doi: 10.3389/fpls.2016.01834
31. Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, et al. Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. Plant Cell. 2007;19(6):2064-76. doi: 10.1105/tpc.106.046250
32. Maeda S, Hayashi N, Sasaya T, Mori M. Overexpression of *BSR1* confers broad-spectrum resistance against two bacterial diseases and two major fungal diseases in rice. Breed Sci. 2016;66(3):396-406. doi: 10.1270/jsbbs.15157
33. Ashkani S, Rafii MY, Shabanimofrad M, Ghasemzadeh A, Ravanfar SA, Latif MA. Molecular progress on the mapping and cloning of functional genes for blast disease in rice (*Oryza sativa* L.): current status and future considerations. Crit Rev Biotechnol. 2016;36(2):353-67. doi: 10.3109/07388551.2014.961403
34. Koide Y, Kobayashi N, Xu D, Fukuta Y. Resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). Jarq-Jpn Agr Res Q. 2009;43:255-80. doi: 10.6090/jarq.43.255
35. Wang BH, Ebbole DJ, Wang ZH. The arms race between *Magnaporthe oryzae* and rice: Diversity and interaction of *Avr* and *R* genes. J Integr Agr. 2017;16(12):2746-60. doi: 10.1016/S2095-3119(17)61746-5
36. Cesari S, Thilliez G, Ribot C, Chalvon V, Michel C, Jauneau A, et al. The rice resistance protein pair RGA4/RGA5 recognizes the *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 by direct binding. Plant Cell. 2013;25(4):1463-81. doi: 10.1105/tpc.112.107201
37. Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, McAdams SA, et al. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell. 2000;12(11):2033-46. doi: 10.1105/tpc.12.11.2033
38. Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, et al. A B-lectin receptor kinase gene conferring rice blast resistance. Plant J. 2006;46(5):794-804. doi: 10.1111/j.1365-313X.2006.02739.x
39. Zhao HJ, Wang XY, Jia YL, Minkenberg B, Wheatley M, Fan JB, et al. The rice blast resistance gene *Ptr* encodes an atypical protein required for broad-spectrum disease resistance. Nat Commun. 2018;9:2039. doi: 10.1038/s41467-018-04369-4
40. Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T, et al. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer Pikm-specific rice blast resistance. Genetics. 2008;180(4):2267-76.
41. Okuyama Y, Kanzaki H, Abe A, Yoshida K, Tamiru M, Saitoh H, et al. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. Plant J. 2011;66(3):467-79. doi: 10.1111/j.1365-313X.2011.04502.x

42. Lee SK, Song MY, Seo YS, Kim HK, Ko S, Cao PJ, et al. Rice *Pi5*-Mediated Resistance to *Magnaporthe oryzae* Requires the Presence of Two CC-NB-LRR Genes. Genetics. 2009;181(4):1627-38.
43. Williams SJ, Sohn KH, Wan L, Bernoux M, Sarris PF, Segonzac C, et al. Structural basis for assembly and function of a heterodimeric plant immune receptor. Science. 2014;344(6181):299-303. doi: 10.1126/science.1247357
44. Cesari S, Bernoux M, Moncuquet P, Kroj T, Dodds PN. A novel conserved mechanism for plant NLR protein pairs: the “integrated decoy” hypothesis. Front Plant Sci. 2014;5:606. doi: 10.3389/fpls.2014.00606
45. Cesari S, Kanzaki H, Fujiwara T, Bernoux M, Chalvon V, Kawano Y, et al. The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. EMBO J. 2014;33(17):1941-59. doi: 10.15252/embj.201487923
46. Pawson T. Protein modules and signalling networks. Nature. 1995;373(6515):573-80.
47. Ball LJ, Kuhne R, Schneider-Mergener J, Oschkinat H. Recognition of proline-rich motifs by protein-protein-interaction domains. Angew Chem Int Ed Engl. 2005;44(19):2852-69. doi: 10.1002/anie.200400618
48. Huckelhoven R, Eichmann R, Weis C, Hoefle C, Proels RK. Genetic loss of susceptibility: a costly route to disease resistance? Plant Pathol. 2013;62:56-62. doi: 10.1111/ppa.12103
49. Chu ZH, Yuan M, Yao LL, Ge XJ, Yuan B, Xu CG, et al. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Gene Dev. 2006;20(10):1250-5. doi: 10.1101/gad.1416306
50. Li S, Shen L, Hu P, Liu Q, Zhu X, Qian Q, et al. Developing disease-resistant thermosensitive male sterile rice by multiplex gene editing. J Integr Plant Biol. 2019. doi: 10.1111/jipb.12774.
51. Nguyen TTT, Koizumi S, La TN, Zenbayashi KS, Ashizawa T, Yasuda N, et al. *Pi35*(t), a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188. Theor Appl Genet. 2006;113(4):697-704. doi: 10.1007/s00122-006-0337-8
52. Takahashi A, Hayashi N, Miyao A, Hirochika H. Unique features of the rice blast resistance Pish locus revealed by large scale retrotransposon-tagging. BMC Plant Biol. 2010;10:175. doi: 10.1186/1471-2229-10-175
53. Fukuoka S, Yamamoto SI, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, et al. Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. Sci Rep. 2014;4:4550. doi: 10.1038/srep04550
54. Xu X, Hayashi N, Wang CT, Fukuoka S, Kawasaki S, Takatsuji H, et al. Rice blast resistance gene *Pikahei-1*(t), a member of a resistance gene cluster on chromosome 4, encodes a nucleotide-binding site and leucine-rich repeat protein. Mol Breed. 2014;34(2):691-700. doi: 10.1007/s11032-014-0067-6
55. Xu X, Chen H, Fujimura T, Kawasaki S. Fine mapping of a strong QTL of field resistance against rice blast, *Pikahei-1*(t), from upland rice Kahei, utilizing a novel resistance evaluation system in the greenhouse. Theor Appl Genet. 2008;117(6):997-1008. doi: 10.1007/s00122-008-0839-7

56. Fujii K, Hayano-Saito Y, Saito K, Sugiura N, Hayashi N, Tsuji T, et al. Identification of a RFLP marker tightly linked to the panicle blast resistance gene, *Pb1*, in rice. Breed Sci. 2000;50(3):183-8. doi. 10.1270/jsbbs.50.183
57. Fujii K, Hayano-Saito Y. Genetics of durable resistance to rice panicle blast derived from an indica rice variety Modan. Jpn J Plant Sci. 2007;1:69-76.
58. Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, et al. 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. 2010;64(3):498-510. doi: 10.1111/j.1365-313X.2010.04348.x
59. Liu XQ, Inoue H, Hayashi N, Jiang CJ, Takatsuji H. CC-NBS-LRR-Type R Proteins for Rice Blast Commonly Interact with Specific WRKY Transcription Factors. Plant Mol Biol Rep. 2016;34(2):533-7. doi: 10.1007/s11105-015-0932-4
60. Inoue M, Morimoto T, Tanabe K, Shumiya A, Fujii K. Breeding of field resistance of rice varieties superior to their parents to blast. Res Bull Aichi Agric Res Cent. 1983;15:63-9.
61. Saka N, Fukuoka S, Terashima T, Kudo S, Shirota M, Ando I, et al. Breeding of a new rice variety “Chubu 125” with high field resistance for blast and excellent eating quality. Res Bull Aichi Agric Res Ctr. 2010;42:171-83.
62. Takehara K, Murata K, Yamaguchi T, Yamaguchi K, Chaya G, Kido S, et al. Thermo-responsive allele of sucrose synthase 3 (*Sus3*) provides high-temperature tolerance during the ripening stage in rice (*Oryza sativa* L.). Breed Sci. 2018;68(3):336-42. doi: 10.1270/jsbbs.18007
63. Kojima Y, Iyama Y, Yamaguchi T, Murata K, Kidani Y, Muraoka Y, et al. Development of a new rice cultivar “FUFUFU”. Breed Res. 2018;20(Suppl 2):97.
64. Brown JK. Yield penalties of disease resistance in crops. Curr Opin Plant Biol. 2002;5(4):339-44. doi: 10.1016/S1369-5266(02)00270-4
65. Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, et al. The barley *Mlo* gene: a novel control element of plant pathogen resistance. Cell. 1997;88(5):695-705.
66. Saka N, Tomita K, Nagano K, Kataoka T, Ando I, Nagayoshi F, et al. Effect of rice blast resistance gene *pi21* on yield under different environments. Breed Res. 2012;14(3):77-82. Japanese.
67. Johnson R. A critical analysis of durable resistance. Annu Rev Phytopathol. 1984;22:309-30. doi: 10.1146/annurev.py.22.090184.001521
68. Pilet-Nayel ML, Moury B, Caffier V, Montarry J, Kerlan MC, Fournet S, et al. Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Front Plant Sci. 2017;8:1838. doi: 10.3389/fpls.2017.01838
69. Jensen NF. Intra-varietal diversification in oat breeding. Agron J. 1952;44(1):30-4. doi: 10.2134/agronj1952.00021962004400010009x
70. Browning JA, Frey KJ. Multiline cultivars as a means of disease control. Annu Rev Phytopathol. 1969;7:355-82. doi: 10.1146/annurev.py.07.090169.002035
71. Wolfe MS. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. Annu Rev Phytopathol. 1985;23:251-73. doi: 10.1146/annurev.py.23.090185.001343

72. Zhu YY, Chen HR, Fan JH, Wang YY, Li Y, Chen JB, et al. Genetic diversity and disease control in rice. Nature. 2000;406(6797):718-22. doi: 10.1038/35021046
73. Mundt CC. Use of multiline cultivars and cultivar mixtures for disease management. Annu Rev Phytopathol. 2002;40:381-410. doi: 10.1146/annurev.phyto.40.011402.113723
74. Chin KM, Wolfe MS. The spread of *Erysiphe graminis* f. sp Hordei in mixtures of barley varieties. Plant Pathol. 1984;33(1):89-100. doi: 10.1111/j.1365-3059.1984.tb00592.x
75. Koizumi S. Rice blast control with multilines in Japan. In: Mew TW, Borromeo E, Hardy B, editors. Exploiting Biodiversity for Sustainable Pest Management. Los Baños (Philippines): IRRI; 2001. p. 143-57.
76. Garrett KA, Mundt CC. Epidemiology in mixed host populations. Phytopathology. 1999;89(11):984-90. doi: 10.1094/Phyto.1999.89.11.984
77. Lannou C, Devallavieillepope C, Goyeau H. Induced resistance in host mixtures and its effect on disease-control in computer-simulated epidemics. Plant Pathol. 1995;44(3):478-89. doi: 10.1111/j.1365-3059.1995.tb01670.x
78. Ashizawa T, Zenbayashi K, Koizumi S. Severity of panicle blast on Sasanishiki near-isogenic lines after inoculation with avirulent isolates of rice blast fungus and disease suppression by pre-inoculation. Jpn J Phytopathol. 2002;68:305-8. doi: 10.3186/jjphytopath.68.305
79. Ishizaki K, Hoshi T, Abe S, Sasaki Y, Kobayashi K, Kasaneyama H, et al. Breeding of blast resistant isogenic lines in rice variety “Koshihikari” and evaluation of their characters. Breed Sci. 2005;55:371-7. doi: 10.1270/jsbbs.55.371
80. Ashizawa T. Current status and problem in rice blast control with multilines. Plant Prot. 2003;57(12):537-40. Japanese.
81. Maeda H. The current status and prospects for disease and insect resistance in rice breeding. Plant Prot. 2015;69:60-4. Japanese.
82. Sasahara M, Koizumi S. Rice blast control with Sasanishiki multilines in Miyagi Prefecture. In: Kawasaki S, editor. Rice Blast: Interaction with rice and control. Dordrecht (The Netherlands): Kluwer Academic Publishers; 2004. p. 201-7.
83. Kojima Y, Ebitani T, Tamamoto Y, Nagamine T. Development and utilization of isogenic lines Koshihikari Toyama BL. In: Kawasaki S, editor. Rice Blast: Interaction with rice and control. Dordrecht (The Netherlands): Kluwer Academic Publishers; 2004. p. 209-14.
84. Marshall DR, Pryor AJ. Multiline varieties and disease control .1. Dirty crop approach with each component carrying a unique single resistance gene. Theor Appl Genet. 1978;51(4):177-84. doi: 10.1007/Bf00273143
85. Khan GH, Shikari AB, Vaishnavi R, Najeeb S, Padder BA, Bhat ZA, et al. Marker-assisted introgression of three dominant blast resistance genes into an aromatic rice cultivar Mushk Budji. Sci. Rep. 2018;8:4091. doi: 10.1038/s41598-018-22246-4
86. Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet. 2000;100(7):1121-8. doi: 10.1007/s001220051395

87. Wu YY, Xiao N, Chen Y, Yu L, Pan CH, Li YH, et al. Comprehensive evaluation of resistance effects of pyramiding lines with different broad-spectrum resistance genes against *Magnaporthe oryzae* in rice (*Oryza sativa* L.). Rice. 2019;12:11. doi: 10.1186/s12284-019-0264-3
88. Ellur RK, Khanna A, Yadav A, Pathania S, Rajashekara H, Singh VK, et al. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Sci. 2016;242:330-41. doi: 10.1016/j.plantsci.2015.08.020
89. Kiyosawa S. Disease resistance 2: Blast disease. In: Matsuo T, editor. Collected data of plant genetic resources Vol. 1. Tokyo (Japan): Kodansha Scientific; 1989. p. 365-8.
90. Ashizawa T, Moriwaki J, Hirayae K. Development of a simulator of the rice blast race dynamics in multiline. Bull NARO Agric Res Cent. 2015;24:15-29. doi: 10.24514/00001587. Japanese with English summary.
91. Yunoki T, Ezuka A, Morinaka T, Sakurai Y. Studies on the varietal resistance to Rice blast. 4. Variation of field resistance due to fungus strains. Bull Chugoku Agric Exp Stn Ser A. 1970;6:21-41.
92. Hashioka Y. Effects of environmental factors on development of causal fungus, infection, disease development, and epidemiology in rice blast disease. In: The rice blast disease: Proceedings of a symposium at the International Rice Research Institute, July 1963. Baltimore (US): Johns Hopkins Press; 1985. p. 153-61.
93. Matsuyama N, Dimond AE. Effect of nitrogenous fertilizer on biochemical processes that could affect lesion size of rice blast. Phytopathology. 1973;63(9):1202-3. doi: 10.1094/Phyto-63-1202
94. Ohata K, Goto K, Kozaka T. Effect of low air temperature on the susceptibility of rice plants to blast disease, with special reference to some chemical components in the plants. Bull Natl Inst Agric Sci. 1966;20:1-65. Japanese with English summary.
95. Inoue H, Nakamura M, Mizubayashi T, Takahashi A, Sugano S, Fukuoka S, et al. Panicle blast 1 (*Pb1*) resistance is dependent on at least four QTLs in the rice genome. Rice. 2017;10:36. doi: 10.1186/s12284-017-0175-0
96. Zenbayashi-Sawata K, Ashizawa T, Koizumi S. *Pi34-AVRPi34*: a new gene-for-gene interaction for partial resistance in rice to blast caused by *Magnaporthe grisea*. J Gen Plant Pathol. 2005;71(6):395-401. doi: 10.1007/s10327-005-0221-4
97. Suwannual T, Chankaew S, Monkham T, Saksirirat W, Sanitchon J. Pyramiding of four blast resistance QTLs into Thai rice cultivar RD6 through marker-assisted selection. Czech J Genet Plant. 2017;53(1):1-8. doi: 10.17221/51/2016-Cjgpb
98. Heil M, Baldwin IT. Fitness costs of induced resistance: emerging experimental support for a slippery concept. Trends Plant Sci. 2002;7(2):61-7. doi: 10.1016/S1360-1385(01)02186-0

99. Brown JKM, Rant JC. Fitness costs and trade-offs of disease resistance and their consequences for breeding arable crops. Plant Pathol. 2013;62:83-95. doi: 10.1111/ppa.12163

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