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ORIGINAL ARTICLE

Race specific and non-specific resistance to *Magnaporthe oryzae* and QTL mapping in wild introgression lines using the standard differential system

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Abstract

Blast disease caused by *Magnaporthe oryzae* is one of the major challenges to rice crop production worldwide. Wild and related species are potential source of novel alleles or genes for crop improvement especially for biotic stress resistance. This study was designed to characterize blast resistance in a set of wild introgression lines developed in the background of Indian mega rice variety Swarna. The introgression lines (INLs) under this study showed spectra of resistance across the different blast isolates inoculated. Monogenic differential varieties with known specific *Pi* genes and the susceptible checks were used in phenotyping. This study confirmed the usefulness of the differential system consisting of monogenic varieties and differential blast isolates for the systematic characterization of resistance in novel germplasm and to understand the genetic architecture of blast resistance. Twelve race specific quantitative trait loci (QTL) for partial resistance to disease were detected in these wild introgression lines from *O.nivara* using composite interval mapping. Of these, 10 were showing resistance to only one isolate each, while QTL detected in chromosome 3 showed resistance against isolates JPF514 and PHL16. While most of the QTLs mapped to previously reported defense related genes whereas two QTLs *qBL2.2* and *qBL5.1* between RM106—RM5460 and RM5140—RM289 with PVE% of 12.28 and 12.48 respectively are novel with no known blast resistance related genes reported within the locus. This study helps to provide developing the basis for future investigations on race specific or broad-spectrum resistance in rice and related crop species.

Keywords Blast resistance · CSSLs · Differential system · QTL mapping · Wild species

Introduction

Blast disease caused by the fungus *Magnaporthe oryzae* is one of the devastating diseases of rice and considered as one among the 10 fungal plant pathogens of highest scientific and economic importance (Dean et al. 2012). The pathogen infects all the aerials parts of rice plant during the different crop growth and reproduction stages causing devastating damages and yield loss. The disease consistently remained as the most destructive disease to rice crop, for the past several decades (Sharma et al. 2012) that can reduce 70–80% yield potential. The pathogen infection is affecting the production about to feed 60 million people per year and causing a loss of economic value about 70 billion dollars globally (Zarbafi and Ham 2019). *Magnaporthe oryzae,* previously known as *M. grisea*, and also described as *Pyricularia oryzae* (the assexual stage) is a fungal filamentous ascomycete pathogen with seven chromosomes. The 40 Mb genome size with several repetitive segments and

Key message: The chromosome segments containing QTLs *qBL2.2* and *qBL5.1* are candidate genomic regions for identification of novel genes for improvement of blast resistance in rice.

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retro-transposons helps, the blast fungus genome to often alter the virulence mechanisms and overcoming host resistance (Dean et al. 2005). The heterothallic reproduction of this species instigates continuous evolution of races with differential phenotypic virulence (Tharreau et al. 2009) and constantly challenging survival and stable production of the important staple cereal crops supporting human race. The phylogenetic species of *Magnaporthe* complex infect about 50 grass and sedge species including major cereal crops like rice, barley, maize, oats, rye and finger millet (Couch and Kohn 2002) signifying its impact on world agriculture and food production. It was also predicted that the yield losses due to blast will further increase owing to evolution of more virulent pathogenic races in the varying climate change scenario (Milus et al. 2009).

There is a large number of studies conducted in characterization of germplasm, identification of QTLs/ genes and further utilization of them in crop breeding programmes and a number of improved blast resistant varieties are developed and released for cultivation (Koide et al. 2009; Miah et al. 2013; Arunakumari et al. 2016; Rekha et al. 2018). However, the blast resistance studies are mostly depended on the screening using isolates available at hot spot regions or a few virulent isolates of local or regional importance, as in case of most of the biotic stresses (Chen et al. 2006a, b; Skamnioti and Gurr 2009). The effectiveness of different QTLs or genes against various isolates across the genotypic backgrounds is not well studied.

The mega varieties are popular genotypes which are cultivated continuously for many years or decades, over large areas across several countries providing hundreds of millions of consumers with high-quality rice. The mega varieties like IR64, Swarna and Samba Mahsuri, with clear advantage of stable higher yield and stress tolerance across ecosystems, making them near impossible for replacement (Mackill and Khush 2018; Mackill 2018). However, it was also recognized that the popular rice varieties of each country harbor a narrow genetic base due to common origin or parentage and their genome is mostly adaptable to region specific conditions and stresses. Being a globally cultivated and highly exported crop like rice; the lack of broad-spectrum resistance might result in breakdown of resistance to any particular virulent race and further cause pandemic situations. Therefore, the characterization of *Pi* genes /single resistance gene resistance reaction across the isolates from the major cultivated regions and the identification novel broad spectrum resistance loci are highly essential.

Host plant resistance has been extensively used by rice breeders and pathologists as an efficient, economical and environment friendly approach to combat this disease (Balakrishnan et al. 2014). Resistance breeding using both major genes conferring protection against specific races and minor genes convening partial resistance against multiple races are also accomplished. Rice, being the staple crop of large part of the world, originated and grown widely in the Asian continent is represented by a large reservoir of diverse germplasm as cultivars, landraces, wild and related species. The popularization of few cultivars based on consumer preference in most of the rice growing countries drastically narrowed the existing allelic diversity in the genetic base of the cultivated germplasm. Wild species are known to be genetically diverse and source of important novel donor genes for genetic improvement. Much of the existing germplasm are unexplored and are highly important to improve the rice crop to meet the growing demands. Characterization of these untapped genetic resources and transferring these unique alleles to cultivars helps in broadening the genetic base and aids in managing the crop losses from evolving pathogens.

To develop an effective resistance and slowing down the disease breakdown by virulent races, the spectra of resistance mediated by resistance (R) genes (Deng et al. 2009; Wu et al. 2015) is to be determined using a differential system. Previously, standard differential monogenic lines with single different resistance gene were developed by Yamada et al. (1976) and Kiyosawa (1984). NILs in CO39 background with 4 genes *Pi1*, *Piz-5*, *Pi3*, and *Pita* were developed by Mackill and Bonman in 1992. However, to constitute a complete differential system, an additional set in CO39 genetic background with the 20 donor varieties targeting 14 resistance genes *Pib*, *Pik-s*, *Pik*, *Pik-h*, *Pik-m*, *Pik-p*, *Pi1*, *Pi7*, *Pish*, *Pita*, *Pita-2*, *Piz-5*, *Piz-t*, and *Pi5(t)* were developed by Tsunematsu et al. 2000. They also developed monogenic lines of rice blast resistance in the background of susceptible rice variety LTH and US2 by backcross breeding with varieties of known genes of resistance to incorporate 17 and 16 resistance genes, respectively. Koide et al. 2011 explained the usefulness of a set of monogenic standard differential varieties and the differential isolates in determining the appropriate breeding strategies for achieving durable and effective control of blast disease and mapped a resistance gene, *Pi19(t)*.

In the present study, to detect the spectra of resistance available and to facilitate resistance gene characterization in the wild introgression lines, international blast differential system consisting of 26 monogenic lines that target 19 known resistance genes were used along with already characterized 24 international blast isolates. The isolates which are available at JIRCAS, Japan were collected from geographically distinct rice growing regions of Asia and Africa viz., Bangladesh, Benin, Cambodia, China, Indonesia, Japan, Kenya, Nigeria, Philippines and Vietnam. The 90 wild introgression lines under this study, included a subset of 70 marker defined CSSLs from Swarna /*O. nivara* cross and were screened against the blast isolates at the seedling stage. The goal was to detect genetic variation of wild introgression lines against various isolates and to determine if they

contained any novel resistance genes/QTL effective against diverse blast isolates.

Material and methods

Two populations involving 396 wild introgression lines of Swarna /*Oryza nivara* IRGC81832 (BC_2F_{10}) (90 wild introgression Lines (INLs)) (Kaladhar et al. 2008; Balakrishnan et al. 2020) and MTU1010 / *Oryza rufipogon*-IC 309814 (BC_4F_4) (306 lines) (Rao et al. 2018a, b) with chromosome segment substitution lines subsets developed at ICAR-Indian Institute of Rice Research (IIRR), Hyderabad, India; along with parents, were transferred to Japan International Research Center for Agricultural Sciences (JIRCAS), Japan after following all the regulatory protocols such as registration and permission from National Biodiversity Authority (NBA), New Delhi for international germplasm transfer; deposition of same set of materials at National repository NBPGR, India; material transfer agreement between ICAR-IIRR and JIRCAS; export and import permissions with Phyto-sanitary certificate from India and Japan.

The population Swarna /*Oryza nivara* IRGC81832 (BC_2F_{10}) was subjected to blast screening in the months of February-July, 2019 at Tropical Agriculture Research Front (TARF), JIRCAS, Ishigaki, Okinawa, Japan. Ishigaki Island is located at latitude of 24°0.28′N longitude of 124°15'E in the Pacific Ocean with an elevation of 14 m above mean sea level (MSL). Plant material was raised in controlled conditions in trays along with parental checks, differential varieties and susceptible checks like LTH (*Lijiangxin tuan heigu*) and US2 (Universally Susceptible line with no alleles for any known *Pi* genes) in contained glass house conditions at TARF-JIRCAS, Ishigaki. A split-plot Randomized Complete Block Design (Altman and Krzywinski 2015) with 2 replications, with isolates as main plot and INLs as subplot was followed for the experiment and ANOVA and heritability estimates were measured. The mean score of the replications were considered for QTL mapping. Blast spore isolation, plant material preparation, spore cultivation, inoculation and evaluation of blast disease and survey of the germplasm and monogenic lines were carried out following protocols for the sampling of diseased specimens and evaluation of blast disease in rice (Hayashi et al. 2009).

The standard differential blast isolates and differential monogenic lines available at TARF, JIRCAS (Table 1) were used for precise evaluation of the pathogenicity of blast isolates collected from tropical and temperate regions of the world (Fukuta et al. 2004). Set of 24 virulent isolates from 11 countries across Asia and Africa were used to test the resistance in controlled glass house condition. Twenty-four isolates JPF507, JPF500, JPF509, JPF574, JPF494 (Japan), PHL4, PHL8, PHL14, PHL16 and PHL11 (Philippines), LAO12, LAO3 (Lao PDR), KNY135 (Kenya), CHN125 (China), IDN280 (Indonesia), NIG1 (Nigeria), VTN64, VTN119 (Vietnam), BAN440, BAN491 (Bangladesh), CAM116, CAM106 (Cambodia) BEN60, BEN54 (Benin) were used in this study.

Blast spore isolation and incubation of diseased sample

Diseased samples; sections collected from infested plants with symptoms were dried and stored in filter paper. Infected leaf samples, leaf or panicle samples in 3–5 cm, were incubated for 1 day in moist filter paper in a petri plate for single spore isolation.

Single spore isolation

Conidial masses from incubated leaf samples were picked using a very fine tip Pasteur pipette and was spread on a demarcated part of petri plate with solidified 3% water agar medium containing streptomycin (10 mg/250 ml agar). Using an advanced microscopic system with Olympus BX43 and specialized microscopic needle attached and regulated by the microscope, single spores were isolated and transferred to other demarcated segments of the medium and these plates were incubated for 24 h at room temperature. After confirming spore germination without any contamination, selected single spores were allowed to incubate for another 3 days. Isolated and incubated rice agar media with few fungal mycelium blocks were transferred to sterile filter papers placed over rice agar medium and then further dried with silica gel in desiccators for 1 week. Dried filter papers with isolates are made into small pieces of 3–5 mm discs and stored in small envelopes with the proper labelling and further placed in plastic covers containing silica gel desiccant. The covers are stored in cold storage -20° to -70° degree Celsius for long term storage for spore cultivation.

Medium preparation

Oatmeal agar for inoculum production was prepared using powdered oatmeal (20 g), sucrose (8 g), agar (6 g), with 400 ml D_2H_2O and autoclaving following the specific protocol. 1 mg streptomycin was added to the hot solution and the solution was transferred to petri plates inside a laminar air-flow chamber and was allowed to solidify.

Blast isolates cultivation in oatmeal medium

Stored culture of blast isolates in colonized paper disks were placed in an oatmeal agar plate, and was allowed to grow for about two weeks. After two weeks of blast isolate cultivation

Table 1 Details of international blast differential system consisting of monogenic differential varieties and susceptible controls and the International standard differential blast isolates

1a. Differential variety for blast resistance and susceptible controls used in this study

Differential variety					**Reference**
Group[a]	**New name**[b]	**Old name**	**Resistance gene**	**Chromosome located**	
U	ML*Pish-B*	IRBLsh-B	*Pish*	1	Tsunematsu et al. 2000
	ML*Pib-B*	IRBLb-B	*Pib*	2	Tsunematsu et al. 2000
	ML*Pit-K59*	IRBLt-K59	*Pit*	6	Tsunematsu et al. 2000
	——	Lijiangxintuanheigu(LTH)	None	——	Tsunematsu et al. 2000
	ML*Pia-A*	IRBLa-A	*Pia*	11	Tsunematsu et al. 2000
i	ML*Pii-F5*	IRBLi-F5	*Piks*	9	Tsunematsu et al. 2000
	ML*Pi3-CP4*	IRBL3-CP4	*Pi3*	9	Tsunematsu et al. 2000
	LthNIL*Pi5-M*	IRBL5-M[LT]	*Pi5*(t)	9	Telebanco-Yanoria et al. 2010
k	ML*Pik*^{s}*-F5*	IRBLks-F5	*Pik-s*	11	Tsunematsu et al. 2000
	LthNIL*Pi1-CL*	IRBL1-CL[LT]	*Pi1*	11	Telebanco-Yanoria et al. 2010
	LthNIL*Pik-Ka*	IRBLk-Ka[LT]	*Pik*	11	Telebanco-Yanoria et al. 2010
	LthNIL*Pik*^{h}*-K3*	IRBLkh-K3[LT]	*Pik-h*	11	Telebanco-Yanoria et al. 2010
	ML*Pik*^{m}*-Ts*	IRBLkm-Ts	*Pik-m*	11	Tsunematsu et al. 2000
	ML*Pik*^{p}*-K60*	IRBLkp-K60	*Pik-p*	11	Tsunematsu et al. 2000
	ML*Pi7-M*	IRBL7-M	*Pi7*(t)	11	Tsunematsu et al. 2000
z	ML*Piz-Fu*	IRBLz-Fu	*Piz*	6	Tsunematsu et al. 2000
	ML*Piz*^{5}*-CA*	IRBLz5-CA	*Piz-5* (=*Pi2*(t))	6	Tsunematsu et al. 2000
	ML*Piz*^{t}*-T*	IRBLzt-T	*Piz-t*	6	Tsunematsu et al. 2000
	ML*Pi9-W*	IRBL9-W	*Pi9*(t)	6	Tsunematsu et al. 2000
ta	LthNIL*Pita-K1*	IRBLta-K1[LT]	*Pita*	12	Telebanco-Yanoria et al. 2010
	ML*Pita-CP1*	IRBLta-CP1	*Pita*	12	Tsunematsu et al. 2000
	LthNIL*Pita*^{2}*-Pi*	IRBLta2-Pi[LT]	*Pita-2*	12	Telebanco-Yanoria et al. 2010
	ML*Pita*^{2}*-Re*	IRBLta2-Re	*Pita-2*	12	Tsunematsu et al. 2000
	ML*Pi12-M*	IRBL12-M	*Pi12*(t)	12	Tsunematsu et al. 2000
	ML*Pi19-A*	IRBL19-A	*Pi19*(t)	12	Tsunematsu et al. 2000
	ML*Pi20-IE24*	IRBL20-IR24	*Pi20*(t)	12	Tsunematsu et al. 2000
——	——	US-2	None	——	Fukuta et al 2022a, b

Lijiangxintuanheigu (LTH) and US-2 are susceptible contols with genetic background of Japaonica and Indica Groups' rice, respesctively

ML Monogenic lines with geentic background of a susceptible Japonica Group cultivar LTH

LTHNIL Near isogeneic lines with genetic background of LTH

1b. International standard differential blast isolates used in this study

[a] Group for differential varieries for designation of blast races

[b] New name for differential variety were proposed by Fukuta et al. (2022a, b)

Standard differential blast isolates

Entry No	**Isolate name**	**Original country**	**Reference**
BAN440	BD642P	Bangladesh	Khan et al. (2017)
BAN491	BD1092	Bangladesh	Khan et al. (2017)
BEN54	OUED 10.4.5	Benin	Odjo et al. (2014)
BEN43	Bn93	Benin	Odjo et al. (2014)
CAM106	Ca49-1(a)	Cambodia	Fukuta et al. (2014)
CAM116	Ca54(d)	Cambodia	Fukuta et al. (2014)
CHN125	A30-1	China	Fukuta et al. (2022a, b)
IDN280	ID196	Indonesia	Kadeawi et al. (2021)
JPF494	Mu-95	Japan	Hayashi (2005)
JPF500	Kyu92-22	Japan	Hayashi (2005)
JPF506	Kyu9439013	Japan	Hayashi (2005)
JPF507	TH69-8	Japan	Hayashi (2005)

Table 1 (continued)

JPF509	Ina93-3	Japan	Hayashi (2005)
JPF513	Ao92-06–2	Japan	Hayashi (2005)
JPF514	IW81-04	Japan	Hayashi (2005)
JPF517	P-2b	Japan	Hayashi (2005)
KNY135	15ke69	Kenya	Fukuta et al. (2019)
LAO3	H08-40–1	Lao PDR	Xangsayasane et al. (2020)
LAO12	H08-259–1	Lao PDR	Xangsayasane et al. (2020)
NIG1	NI1	Niger	Odjo et al. (2014)
PHL2	B90002	Philippines	Telebanco-Yanoria et al. (2008)
PHL4	43	Philippines	Telebanco-Yanoria et al. (2008)
PHL8	M39-1–3-8–1	Philippines	Telebanco-Yanoria et al. (2008)
PHL10	Ca41	Philippines	Telebanco-Yanoria et al. (2008)
PHL11	Ca89	Philippines	Telebanco-Yanoria et al. (2008)
PHL14	V850196	Philippines	Telebanco-Yanoria et al. (2008)
PHL15	PO6-6	Philippines	Telebanco-Yanoria et al. (2008)
PHL16	C923-49	Philippines	Telebanco-Yanoria et al. (2008)
PHL18	V850256	Philippines	Telebanco-Yanoria et al. (2008)
VTN64	TV414	Vietnam	Nguyen et al. (2020)
VTN119	262–1	Vietnam	Nguyen et al. (2020)

Hayashi, N. Rice blast fungus, MAFF Microorganism Genetic Resources Manual No. 18. National Institute of Agrobiological Sciences, Tsukuba, Inaraki, December 2005

the fungal mycelial growth was removed from non-contaminated Petri plates with a brush for further sporulation. The scraped plates were kept open in a tray, covered with wrapping film with holes, and was left under a fluorescent light for 3–4 days to induce sporulation.

Spore preparation and inoculation

For inoculation, these sporulated Petri plates were gently smeared with a paint brush by pouring 10–20 ml distilled water into the Petri dish. Then the conidial suspension was filtered through 4 layers of cheese cloth or through nylon mesh. The conidial concentration of each isolate was determined using a hemocytometer under microscope. 20 micro liter of conidial suspension is checked under microscope and the spore count was recorded. The concentration was adjusted to 10^5 (1,00,000) conidia per milliliter by either diluting with distilled water or adding more spore inoculum. 20 micro litre of spore suspension was placed on slides of the hemocytometer with a cover slip. Spores in 1 mm square demarcated on slide were counted, considering the volume in one mm square is approximately 10^{-4} ml. The number of cells per ml (cells/ml) was calculated as (number of spores per mm square $\times 10^4$)/ml). A drop of tween 20 (final concentration approximately 0.01%) was added to distilled water (10micro litre tween 20 / 500 ml of water) used for removing the spores and mixed thoroughly to aid in the adhesion of the inoculum to the leaves of the plants. The prepared conidial suspension containing spores was sprayed to seedlings for testing, inside a protected glasshouse. They were transferred to dew chamber for incubation at 25 °C, 80RH for 24 h. These seedlings were further transferred to green house (humidity approximately 60%) at 25–30 °C temperature.

Phenotypic evaluation

Evaluation of inoculated entries was carried out after one week based on 1–5 score card on decision criteria of blast resistance to LTH monogenic lines. The resistance reaction of genotypes was classified into four categories; 0–2 score was considered as resistant (R), 3 as moderately resistant (MR), 3–4 as moderately susceptible (MS), and 4–5 score as susceptible (S). LTH, US2 and Swarna were used as susceptible control varieties. Disease assessment was made based on JIRCAS working report No.63 (Hayashi and Fukuta 2009; Hayashi et al. 2009).

Classification of INLs based on the resistance reaction

The phenotypic data on resistance response for 24 blast isolates were used for classifying the wild introgression lines by cluster analysis and comparing the reaction response to differential varieties. Diversity analysis was performed according to Ward's hierarchical analysis (Ward 1963), to identify the clusters and to visualize the pattern of genetic differentiation within and between groups, DARwin v.6.0.021

software (http://darwin.cirad.fr) (Perrier and Jacquemoud-Collet 2006) with 25,000 bootstraps was used for diversity analysis.

QTL mapping

QTL mapping was carried by the single marker analysis (SMA), interval mapping of QTL (IM-ADD), inclusive composite interval mapping (ICIM-ADD), inclusive composite interval mapping of epistatic QTL (ICIM-EPI) functions implemented in the QTL IciMapping v4.2 (www.isbreeding.net) in a stepwise regression for the adjusted means of each trait (Meng et al. 2015). For the mapping function, Kosambi's function was used (Kosambi 1944) using genotypic data from 140SSRs. QTL was detected when the LOD score is above the threshold value corresponding to significance level of 5% and was determined by 1000 permutations. Similarly, CSL (Chromosome Segment Line) mapping function on detecting additive QTLs in non-idealized CSS lines was carried out with QTL IciMapping v4.2 using stepwise regression-based likelihood ratio test (RSTEP-LRT) method which specifically deals with QTL mapping in collections with multiple introgressions per line (Balakrishnan et al. 2020). QTLs identified in this study were further explored using the QTARO database genome viewer (qtaro.abr.affrc.go.jp/ http://qtaro.rd.naro.go.jp/cgi-bin/gbrowse) for reported or predicted functional genes present within or adjacent to the QTL in the specific genomic regions.

Results

To characterize blast resistance quantitative trait loci (QTL), wild introgression lines including chromosome segment substitution lines (CSSLs) developed from cross between the cultivar from *Oryza sativa* and wild accessions from *Oryza nivara* were used. The wild introgression lines, from Swarna /*Oryza nivara* IRGC81832 (BC_2F_{10}) (90 Lines) were phenotyped at the seedling stage with twenty-four blast isolates collected from geographically distinct rice growing regions in two replications. Among the 90 lines tested; one line (NK87), failed to germinate in all experiments. The remaining 89 lines were screened against 24 isolates. Phenotyping or evaluation of blast resistance on 8^{th} day of inoculation showed wide variation in the resistance pattern across the genotypes as well as isolates.

Phenotyping for blast resistance reaction

Most of the isolates induced a resistance response of zero score at least in one of the CSSLs, except BEN54, JPF494, PHL4 and PHL14. Isolates VTN119 and JPF507 showed zero score only in one replication. Two isolates showed a maximum score of less than 5 viz., VTN119 (4.3) and JPF507 (4.5). Highest average disease reaction among the genotypes was expressed while screening with VTN64 (3.84) followed by JPF494 (3.22) and lowest by JPF509(0.76). Most of the isolates induced a maximum range of variation (5.00) of disease scores among the genotypes from highly susceptible to highly resistant except VTN119, JPF507, BEN54, PHL4, PHL14 and JPF494 (Supplementary Table 1). JPF494 was found as the most virulent isolate which produced highest susceptible reaction among majority of the lines while maximum resistance reaction was observed in case of JPF509. Average score of less than 2 was found in JPF509, JPF500, JPF514, IDN280, JPF507 and more than 3 was found in VTN64, PHL14 and JPF494. All isolates caused highly susceptible reaction (score5) at least in one of the INL tested except VTN119 and JPF507.

Analysis of variance revealed that both INLs ($F = 19.85$, $P < 0.01$) and isolates ($F = 13.76$, $P < 0.01$) differed significantly in production blast disease symptoms. A significant interaction ($F = 2.85$, $P < 0.01$) between INLs and isolate was also detected, thus the interaction effects were separated by analyzing the direct effects of isolates and introgression lines (Supplementary Table 2a). Heritability of the reaction of the INLs to the isolates were estimated and found NK28 with lowest value of 36% and NK59 with highest value of 94%. All the resistant ant and susceptible checks showed a higher heritability of 75% and above. The heritability of the virulence of the isolates between the strains were assessed and BEN54 and JPF494 showed a lowest heritability of 45% while JPF500 showed highest heritability of 89% (Supplementary Table 2b).

Average resistance score of INLs ranged from 1.63(NK56) to 3.63(NK15) and 18 lines showed average score of less than 2.0 while 10 INLs showed average score 3.0 or above (Supplementary Table 2c). Maximum susceptibility reaction was observed in NK15 and maximum resistance was observed NK40, NK27, and NK32 while NK28 consistently expressed a resistance reaction score (< 3) to all the isolates. The genotypes NK72, NK49, NK61, NK69, NK50, NK63, NK32, NK68, NK4, NK9, NK82, NK65, NK84, NK56, NK40, NK27, NK62, NK45, NK67, NK42, NK86 and NK28 presented resistant to moderately resistance reaction (< 4.00) for at least 20 isolates with lowest range of resistance score across the isolates. Majority of the lines showed a minimum zero score for at least one isolate, except 9 lines NK74, NK5, NK15, NK79, NK25, NK14, NK53, NK71 and NK54. 60 lines were highly susceptible (score 5.00) to at least one isolate; however, 54 of them showed resistance to at least one isolate (0–2 score). Only 9 lines showed minimum average score above 0.0 while 6 of them NK25, NK54, NK71, NK53, NK14, and NK79 were having minimum score of 1.0 and above. Even though 1.0–1.9 is

also considered under resistance category but they showed initial symptoms demonstrating no genomic loci available to contribute absolute resistance with 0 score to any of the isolates under study. NK46, NK54, NK11, NK81, NK23, NK59, NK51, NK20, NK41, NK77, NK64, NK44 and NK15 had 4–5 score to more than 10 isolates showing they are highly susceptible.

The checks LTH and US2 showed highly susceptible reaction (> 4) to most of the isolates. High susceptibility scores in LTH, US2, Swarna, and *indica* cultivars checks confirmed that inoculation was successful and that disease pressure was enough for genetic analysis of the phenotypic data obtained. Three popular Indian cultivars Swarna, MTU1010 and Samba mahsuri were also screened against the virulent isolates. The recurrent parent Swarna, showed susceptible reaction to most of the isolates except IDN280, BEN60, JPF500, JPF509 and JPF514. Samba mahsuri showed susceptible reaction to all the isolates except BEN60, PHL8, JPF509, IDN280 and JPF514. MTU1010 exhibited varying resistance reaction from highly resistant to moderate resistance reaction across the isolates except 8 isolates with score above 3 to LAO12, PHL14, PHL4, JPF494, BAN491, CAM106, and BAN440 and NIG1. Interestingly, Swarna and MTU1010 showed consistently high resistant reaction to 3 of the Japanese isolates, JPF500, JPF509 and JPF514, and in a lesser extent to the isolates BEN60 and IDN280 while Samba mahsuri showed only a moderately resistant reaction to these isolates.

Classification of genotypes based on reaction pattern to differential monogenic lines

The 89 INLs, recurrent parent Swarna, two susceptible checks LTH and US2, two *indica* checks Samba mahsuri and MTU1010 along with 26 monogenic differential varieties (DV) were clustered using ward's hierarchical clustering method and found that rice accessions were classified into eight cluster groups; Ia, Ib, IIa, IIb, IIIa-1, IIIa-2, IIIb-1 and IIIb-2 (Fig. 1). Introgression lines were categorized only into five clusters; Ia, IIIa-1, IIIa-2, IIIb-1, and IIIb-2 based on their resistance reaction to 24 isolates while differential varieties mostly clustered in the major cluster I and II along with sub cluster IIIa.

Histograms were drawn based on the reactions' data of INLs in each cluster group against 24 blast isolates (Fig. 2a). The means of infection scores per INL varied among cluster groups, and cluster Ia, IIIa-1, IIIa-2, IIIb-1, and IIIb-2, were 3.1, 2.1, 2.2, 2.5, and 2.4, respectively. The score of Swarna was 3.8 indicating the order of resistant among groups as follows; susceptible: Swarna < Ia < IIIb-1 < IIIb-2 < IIIa-1 < IIIa-2: resistant. The relationships between infection scores and accumulations of QTLs for resistance in INLs among cluster groups are indicated in Fig. 2b. Chromosome segments containing QTLs for blast resistance of INLs varied among

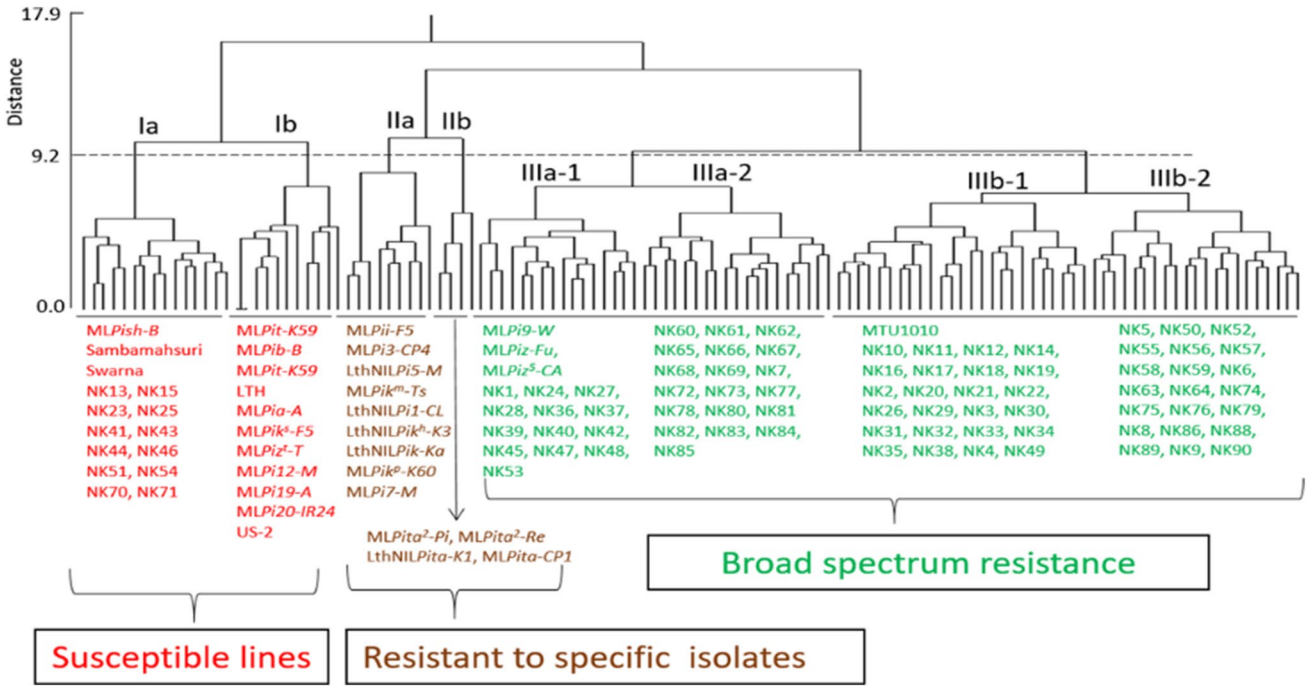
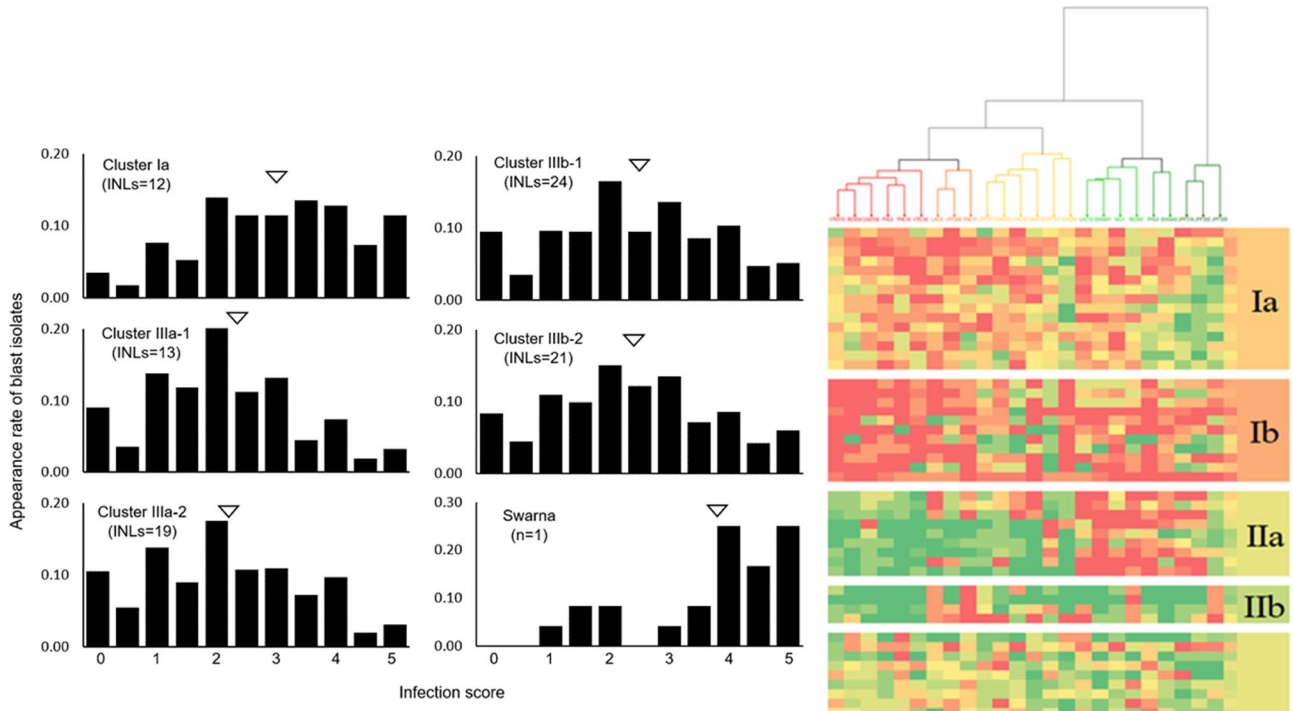
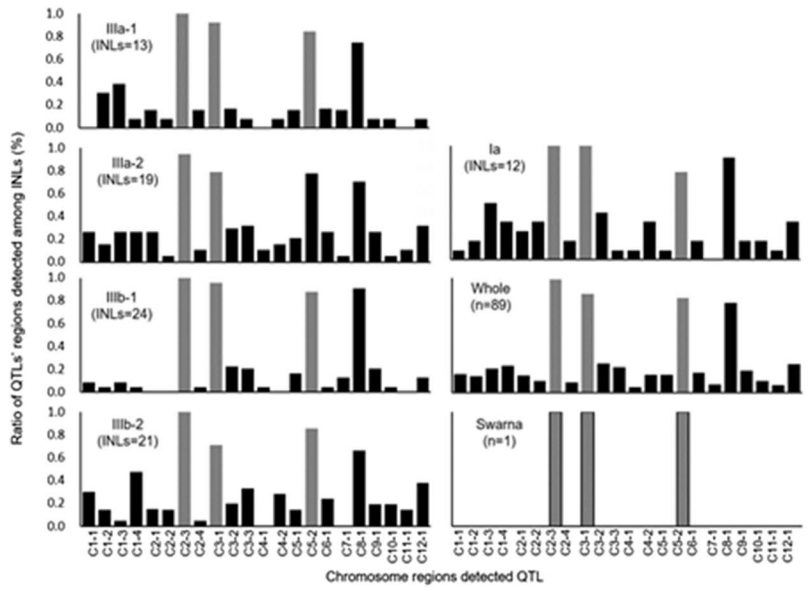


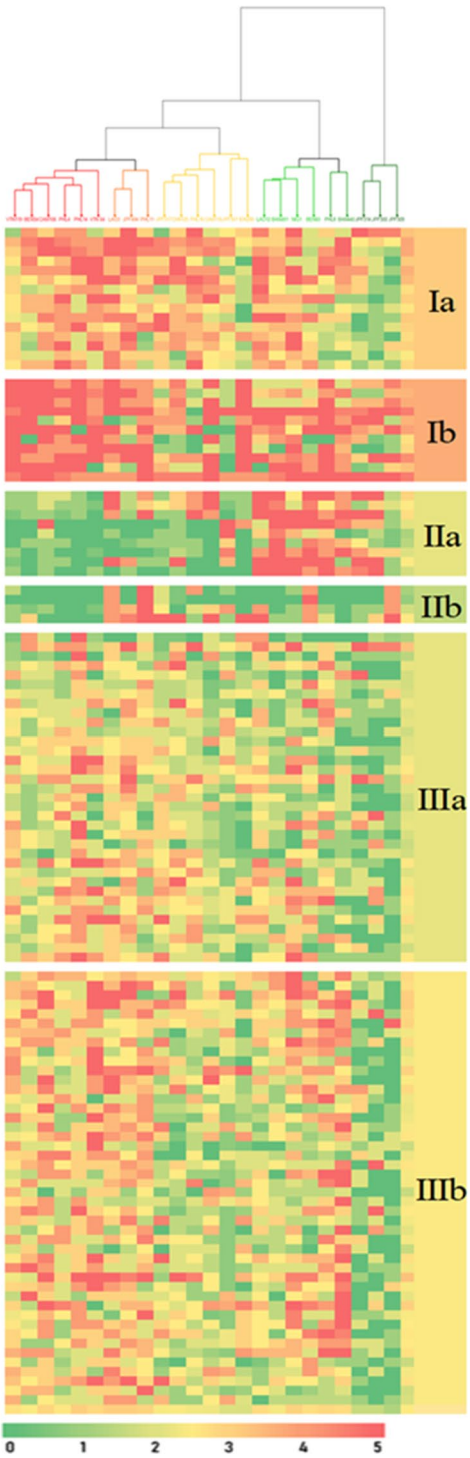
Fig. 1 Classification of introgression lines and monogenic differential varieties based on the reaction patterns to International standard differential blast isolates



a Reactions of standard differential blast isolates to introgression lines for chromosome segments from O. nivara with an Indica Group rice cultivar, Swarna



b Variation of chromosome regions detected QTLs among INLs in each cluster group



c The graphical representation of resistance response of wild introgression lines to different blast isolates

Fig. 2 Genetic variation and classification of disease reaction patterns of in wild introgression lines. **a**. Reactions of standard differential blast isolates to introgression lines for chromosome segments from *O. nivara* with an Indica Group rice cultivar, Swarna. **b**. Variation of chromosome regions detected QTLs among INLs in each cluster group. **c**. The graphical representation of resistance response of wild introgression lines to different blast isolates

cluster groups per INL and the values were 7.2, 5.6, 6.6, 5.0, and 5.8 in the clusters Ia, IIIa-1, IIIa-2, IIIb-1, and IIIb-2 respectively. Black and gray bars indicated the positive or negative effects for resistance with alleles of introgression and Swarna, respectively indicating the order for numbers of chromosome regions locating the QTLs was as follows; Swarna (3.0) < IIIb-1 < IIIa-1 < IIIb-2 < IIIb-2 < Ia. Variation in the ratio of chromosome regions with detected QTLs among INLs especially in chromosome 3, 4 and 7 showed significant difference in each cluster group which might have contributed to the diference in resistance reaction pattern. Significant association between blast resistance reaction and the QTLs accumulated in INLs (Fig. 2b) were observed among the clusters IIIa and IIIb where majority of resistant lines were clustered, however no association between number of QTLs or resistance pattern was observed in other clusters. The resistance response reaction induced by the various isolates is presented as heat map (Fig. 2c).

Eight clusters were characterized on the basis of the infection score range of the respective group for blast resistance. Cluster I was grouped into sub clusters Ia and Ib, where the infection ranges of the lines clustered where towards the susceptible reaction. The cluster Ia had the recurrent parent Swarna, *indica* cultivar Samba mahsuri, along with 12 susceptible INLs (Table 2). One differential variety ML*Pish-B* having gene *Pish* from chr.1 showing similar resistance reaction pattern was also grouped in this cluster. Infection score range of the cluster was 2.4–3.8 and that of the wild introgression lines in the cluster was 2.4–3.6. Cluster Ib had susceptible checks LTH and US2, and differential varieties ML*Pit-K59,* ML*Pib-B* ML*Pia-A,* ML*Pik*^{s}*-F5,* ML*Piz*^{t}*-T,* ML*Pi12-M,* ML*Pi19-A and* ML*Pi20-IR24* harbouring blast resistant genes like *Pit, Pib, Pit, Pia, Pik-s, Piz-t, Pi12*(t), *Pi19*(t) and *Pi20*(t) with a mean infection score ranging from 2.7 to 4.6. No INLs were clustered in this group.

The cluster group II had 2 subgroups containing only differential varieties with resistant reaction to multiple isolates. IIa had 9 DVs viz., MLPii-*F5, MLPi3-CP4,* LthNIL*Pi5-M,* ML*Pik*^{m}*-Ts,* LthNIL*Pi1-CL,* LthNIL*Pik*^{h}*-K3,* LthNIL*Pik-Ka,* ML*Pik*^{p}*-K60* and MLPi*7-M*. The differential monogenic lines of IIa represented the blast resistance genes *Pii, Pi3, Pi5*(t), *Pik-m, Pi1, Pik-h, Pik, Pik-p and Pi7*(t) showing an infection range of 1.2–2.9. IIb had 4 DVs; MLPita^{2}*-Pi,* ML*Pita*^{2}*-Re,* LthNIL*Pita-K1* and ML*Pita-CP1*.DVs clustered in IIb harbored blast genes *Pita-2, and Pita* with lowest infection range of 0.9 to2.6 showing the broad-spectrum resistance of this cluster.

Majority of the INLs grouped into the III cluster having a resistant to moderately resistant reaction ranging from 1.3 to 3.3 score. III-a comprised 32 wild introgression lines and 3 differential varieties ML*Pi9-W*, ML*Piz-Fu* andML*Piz*^{5}*-CA* with *Pi9*(t), *Pish and Piz*-5 genes respectively. The mean resistance reaction of the genotypes in the cluster ranged from 1.3–3.3, while that of introgression lines ranged from 1.7 to 2.9. This cluster is further divided into two clusters IIIa-1 and IIIa-2 with 13 and 19 introgression lines each with average infection score of 2.1 and 2.2 respectively.

Interestingly the last sub cluster III-b comprised of 45 wild introgression lines with MTU1010 showing resistance reaction with an infection score ranging from 1.6–3.33. This is a distinctive cluster where none of the monogenic DVs were grouped and the resistance reaction pattern may be contributed by any novel gene in this genotype and tentatively named as *Pi19(t).* Comparison of resistance pattern of reaction of standard differential blast isolates with differential varieties and *indica* cultivars also showed that MTU10110 is having a novel mechanism contributing for blast resistance other than the genes studied using DVs (Supplementary Table 3). This sub cluster was further grouped into two clusters containing 24 and 21 introgression lines each with a score of 2.5 and 2.4 respectively. Further exploration using bi-parental population derived from MTU1010 and INLs in this study will be employed to confirm the results.

The isolates based on their country of origin are represented in the box plot graphs (Fig. 3) indicating the variation in resistance response of the wild introgression lines. There was only one isolate each collected from China, Indonesia and Niger, two each from Laos, Vietnam, Bangladesh, Benin and Cambodia. While 5 isolates from Philippines and 6 from Japan were used in this study, however the resistance reaction was completely independent of the country of origin showing these races are genetically unrelated. This is also confirmed based on the clustering of the isolates independent of their origin. Isolates from Japan viz., JPF500, JPF507 JPF509 and JPF514, showed the disease reaction skewed towards resistance along with BEN54, IDN280 and PHL16 while all the other isolates induced susceptibility reaction in majority of the lines with a normal frequency distribution. Among the isolates only 11 viz., CHN125, LAO12, LAO3, VTN119, JPF517, PHL4, PHL8, BAN440, BEN60, BEN54, and CAM106 showed the box plot median at 2–3 score range showing the induction of moderately resistant reaction pattern. IDN280, JPF500, JPF509, JPF514, BAN491 and CAM116 induced highly resistance reaction in a greater number of introgression lines as represented by the skewness of boxplot and the median, while BEN60, NIG1 and VTN64 induced more highly susceptible response.

VTN64 induced highest number of highly susceptible reaction while JPF509 induced highest number of resistance reaction among CSSLs and the frequency distribution curves also showed similar results (Fig. 3). BAN440 and VTN64 induced more of susceptible reaction of 4–5 score range, while IDN280, JPF507, JPF500, JPF509 and JPF514 showed more of resistant reaction among the lines in 0–1 score. BEN54, JPF494, JPF507, PHL14, PHL16 and

Table 2 Genetic variation of blast disease resistance of wild introgression lines classified based on reaction patterns to standard differential blast isolates and monogenic differential varieties

Cluster group	Ia	Ib	IIa	IIb	IIIa-1	IIIa-2	IIIb-1	IIIb-2
No. of genotypes	**15**	**11**	**9**	**4**	**16**	**19**	**25**	**21**
(Wild introgression lines)	(12)	(0)	(0)	(0)	(13)	(19)	(24)	(21)
Infection score range (Wild introgression lines)	**2.4–3.8** (2.4–3.6)	**2.7–4.6** (-)	**1.2–2.9** (-)	**0.9–2.6** (-)	**1.2–3.2** (1.7–2.9)	**1.6–2.79** (1.6–2.79)	**1.6–3.0** (1.6–3.0)	**1.6–3.2** (1.6–3.2)
Mean Infection score	**3.1**	**3.7**	**2.1**	**1.6**	**2.1**	**2.2**	**2.5**	**2.4**
Checks	Sambamahsuri, Swarna	LTH, US2	-	-	-		MTU1010	
Standard Differential varieties	ML*Pish-B*	ML*Pit-K59* ML*Pib-B* ML*Pit-K59* ML*Pia-A* ML*Pik^s-F5* ML*Piz^t-T* ML*Pi12-M* ML*Pi19-A* ML*Pi20-IR24*	ML*Pii-F5* ML*Pi3-CP4* LthNIL*Pi5-M* ML*Pik^m-Ts* LthNIL*Pi1-CL* LthNIL*Pik^h-K3* LthNIL*Pik-Ka* ML*Pik^p-K60* ML*Pi7-M*	ML*Pita^2-Pi* ML*Pita^2-Re* LthNIL*Pita-K1* ML*Pita-CP1*	ML*Pi9-W*, ML*Piz-Fu*, ML*Piz^5-CA*			
Reported genes	*Pish*	*Pi9* *Pib* *Pi9* *Pia* *Pik-s* *Piz-t* *Pi12*(t) *Pi19*(t) *Pi20*(t)	*Piks* *Pi3*(t) *Pi5*(t) *Pik-m* *Pi1* *Pik-h* *Pik* *Pik-p* *Pi7*(t)	*Pita-2* *Pita*	*Pi9*(t) *Pish* *Piz-5*		*Pi19(t), unknown*	
Chromosome	1	2,6,11,12	9,11	12	1,6		*unknown*	
CSSLs	NK13, NK15,NK23, NK25, NK41, NK43, NK44, NK46, NK51, NK54, NK70, NK71				NK1,NK24,NK27,NK28,NK36,NK37,NK39,NK40,NK42,NK45,NK47,NK48,NK53	NK60,NK61,NK62,NK65,NK66,NK67,NK68,NK69,NK7,NK72,NK73,NK77,NK78,NK80,NK81,NK82,NK83,NK84,NK85	NK10,NK11,NK12,NK14,NK16,NK17,NK18,NK19,NK2,NK20,NK21,NK22,NK26,NK29,NK3,NK30,NK31,NK32,NK33,NK34,NK35,NK38,NK4,NK49	NK5,NK50,NK52,NK55,NK56,NK57,NK58,NK59,NK6,NK63,NK64,NK74,NK75,NK76,NK79,NK8,NK86,NK88,NK89,NK9,NK90

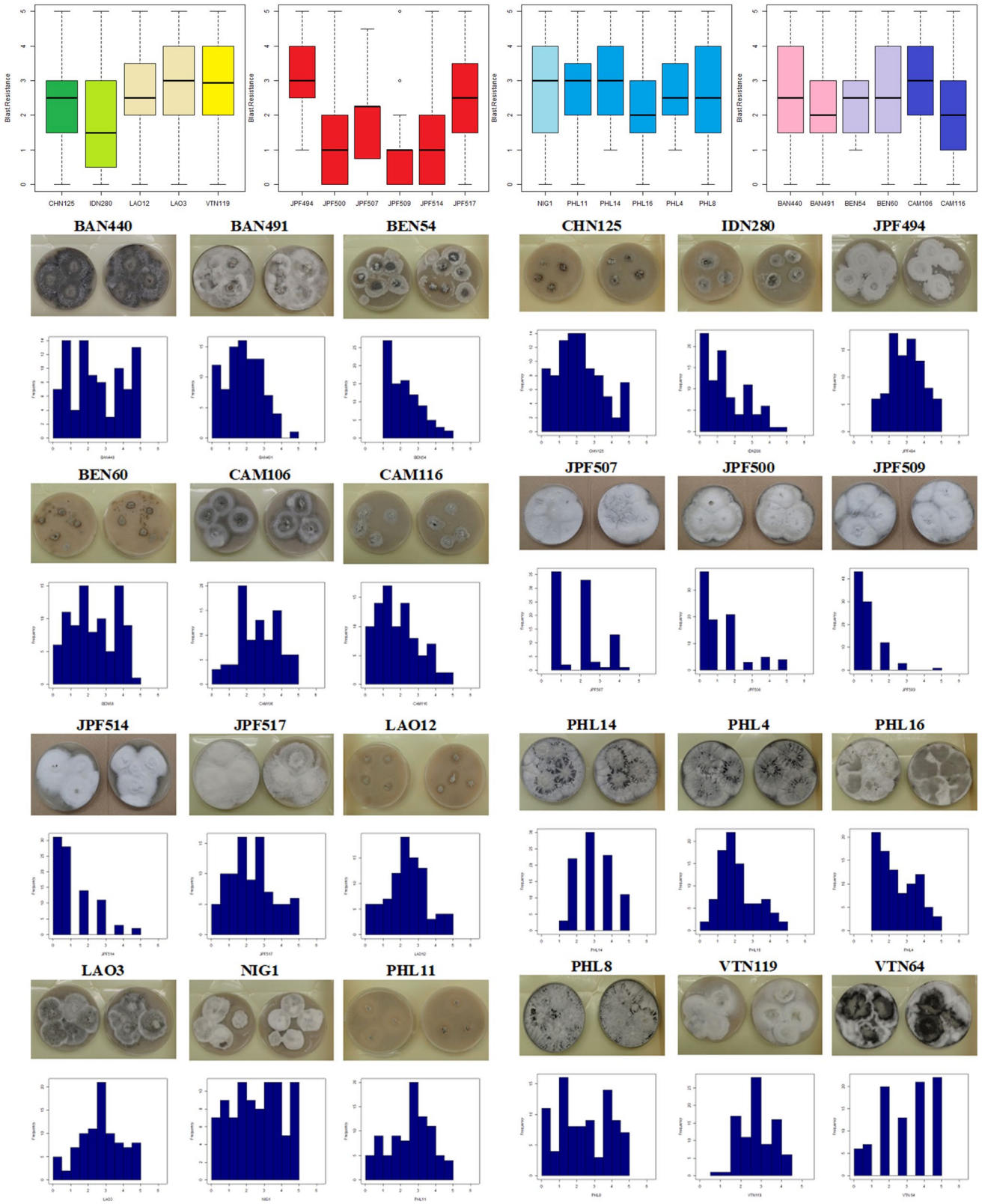


Fig. 3 Box plots and frequency distribution showing resistance reaction of each isolate in the wild introgression lines

Table 3 Additive effect QTLs for blast resistance against different blast isolates detected using QTL IciMapping 4.1

QTL detected	Chromosome	Flanking markers			LOD	PVE (%)	Add	Most linked marker by Single marker analysis			
		Left Marker	Right Marker	Position (cM)				Marker	LOD	PVE (%)	Add
qBL1.1-PHL16	1	RM128	RM431	252	4.19	9.73	-1.07				
qBL2.1-JPF514	2	RM475	RM279	16	3.54	4.9	-1.26				
qBL2.2-PHL11	2	RM106	RM5460	96	2.56	12.28	1.19	RM106	2.6	12.6	1.19
qBL2.3-PHL14	2	RM279	RM6318	41	2.91	14.04	-0.85	RM6318	2.9	14.0	-0.85
qBL3.1-JPF514	3	RM156	RM517	19	2.52	5.09	-1.31				
qBL3.2-JPF514	3	RM517	RM15855	54	2.64	4.99	-1.2				
qBL3.3-PHL16	3	RM517	RM15855	50	4.23	9.71	-1.05				
qBL3.4-VTN119	3	RM570	RM565	206	3.06	15.34	-0.49	RM565	2.9	6.9	-0.43
								RM514	2.9	6.9	-0.43
qBL5.1-JPF500	5	RM5140	RM289	9	6.37	12.48	-1.81	RM5140	2.6	12.5	-0.66
qBL5.2-VTN64	5	RM413	RM122	19	3.01	14.37	1.62	RM122	3.1	14.7	1.63
qBL9.1-JPF514	9	RM215	RM257	19	3.2	5.13	-1.17				
qBL10.1-JPF509	10	RM271	RM474	6	4.71	14.91	-1.2				

VTN119 did not induce highly resistant reaction in any of the INLs showing their virulence in pathogenicity indicating none of the available genes or QTLs in the population is responsible for the absolute resistance against these isolates.

QTL mapping

The genome wide genotypic data was used to identify chromosomal regions associated with the blast resistance using 140 SSR markers spanning across 12 chromosomes (Balakrishnan et al. 2020). 12 QTLs were identified for blast resistance to various isolates using the adjusted means of scores of two replications using ICIM method (Table 3). QTLs were detected in the chromosomes 1, 2, 3, 5, 9 and 10 for the isolates JPF500, JPF509, JPF514, PHL11, PHL11, PHL14, PHL16, VTN119 and VTN64 (Fig. 4). The phenotypic variance percentage of detected QTLs ranged from 4.9 to 15.34% while LOD was 2.52 to 6.37. Additive effect was contributed by *O. nivara* in 9 QTLs while the other two were from recurrent parent Swarna. Single marker association showed highly significant marker association representing chr.2, 3, 5 and10 with PVE ranging from 6.92 to 14.75.

QTL for blast resistance was identified in chromosome 1 between flanking markers RM128 to RM431 with a PVE of 9.73% contributing resistance to the isolate PHL16. Three different QTLs identified in chr.2 against JPF514, PHL11 and PHL14 with PVE% 4.9, 12.28 and 14.04 respectively. Four QTLs against 3 isolates namely JPF514, PHL16, VTN119 were mapped in chromosome3 with a PVE% varying from 5.09 to 15.34%, however QTLs against JPF514 and PHL11, which was flanked by RM517 and RM15855 shared a common locus. QTLs were identified against JPF500 and VTN64 with phenotypic variance of 12.48 and 14.37 in chromosome 5 at genotypic locations flanked by markers RM5140-RM289 and RM413-RM122 respectively. Blast resistance QTL were identified against isolate JPF514 between RM215 and RM257 at chromosome 9 with PVE 5.13. Similarly, QTL for JPF509 between RM271-RM474 with PVE% 14.91 at chromosome 10 was mapped. Among these QTLs at least one of the flanking markers of 5 QTLS showed strong association to the blast resistance by single marker association with a major PVE%, confirming the results of composite interval mapping.

22 significant QTLs were identified by interval mapping across 12 chromosomes contributing resistance to 12 isolates, where PVE ranged from 0.69 to 15.76% and LOD from 1.21 to 6.37 (Supplementary Table 4). Among these 9 QTLs showed resistance to more than 1 isolate used in the study and *qBLC11.1* showed resistance to 3 isolates JPF500, PHL8 and CHN125 with a PVE of 2.25 to 4.23%0.10 QTLs for JPF500, 6 QTLS for JPF509, 5QTLs for JPF514, 2 QTLs each for PHL16 and LAO12, 1 QTL each for CHN125, LAO12, PHL11, PHL14, PHL4, PHL8, VTN119 were identified through Interval Mapping (IM). All the QTLs identified through ICIM were detected also through interval mapping.

Epistatic interactions were studied using Inclusive composite interval mapping (ICIM-EPI) method and 108 pairs of significant epistatic QTLs (E-QTLs) were detected for blast resistance for various isolates (Figs. 4, 5). 20 isolates showed epistatic interactions between resistance genes of various chromosomes with JPF500 showing highest number of EQTL pairs of 45 followed by JPF509 with 12 pair of interactions (supplementary Fig. 1). JPF500 showed several minor but significant epistatic interaction above LOD > 2.62.

Four isolates namely CAM116, CHN125, NIG1 and PHL8 showed no epistatic interactions in resistance. 108 epistatic QTLs detected above LOD > 2.5 with 16 major effect EQTLs with PVE% varying from 10.08 to 21.85% and 92 minor effect epistatic interactions with PVE% varying from 0.63 to 8.20%. EQTLs with high PVE % were detected for resistance to isolates like BAN491, BEN54, CAM106, IDN280 and JPF499. E QTLs were detected in all the 12 chromosomes with maximum interactions were linked to chromosome 3 (37 E QTLs) followed by chr.1 (34 E QTLs) and chr.12 (32 E QTLs). Among the 108 E QTLs 56 were having either one or two major QTLs involved in the interactions largely showing negative additive by additive effect. Among the 12 QTLs identified through ICIM also showed various epistatic interactions.

CSL mapping

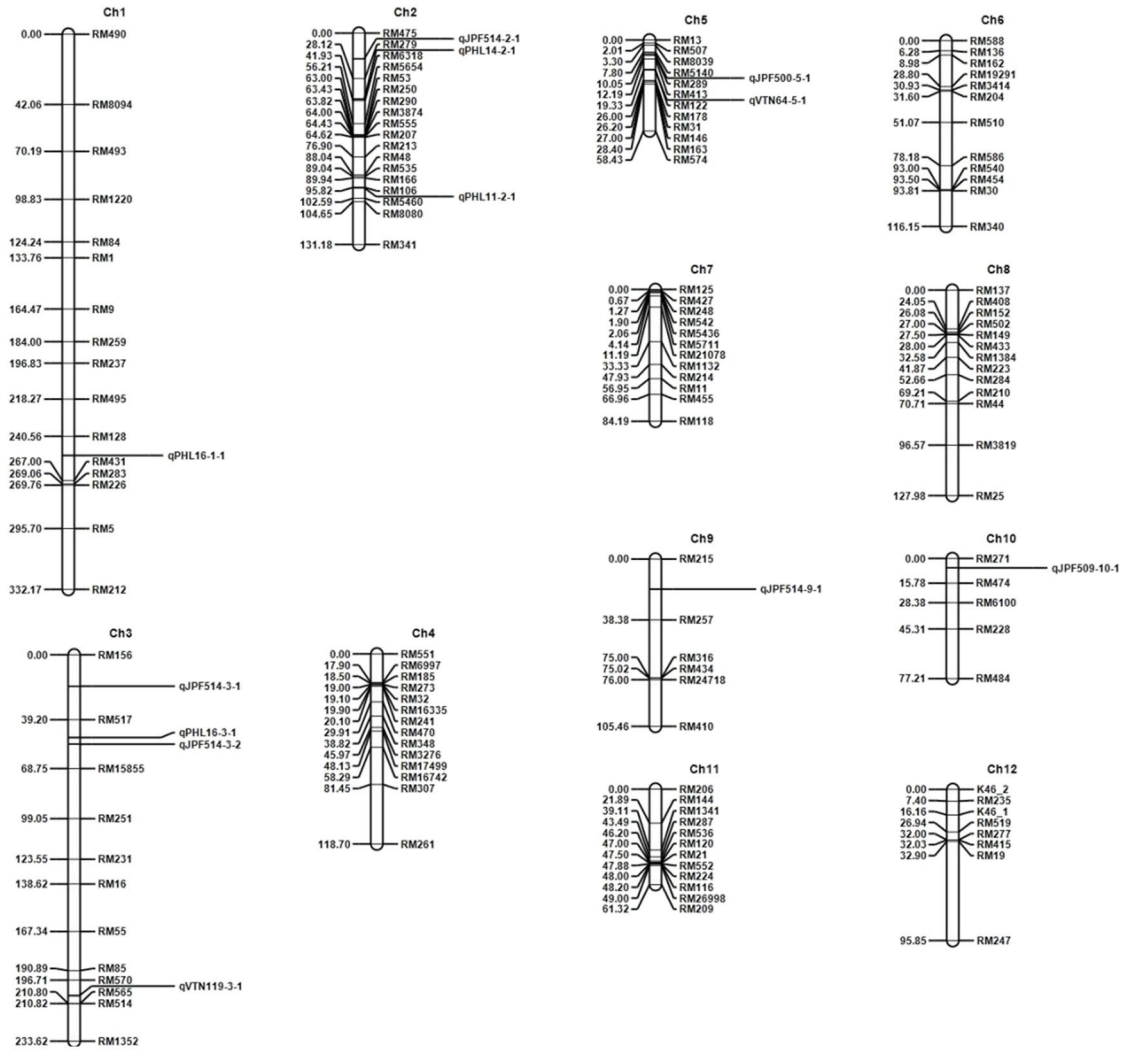
CSL mapping carried out using the 70 CSSL subset (Balakrishnan et al. 2020) with recurrent parent Swarna illustrated the presence of 4 chromosome segments associated to blast disease resistance and contributing to a PVE% ranging from 15.68 to 23.22 towards 4 isolates PHL11, PHL14, PHL16 and VTN64 (Table 4). Chromosome segment at RM128 in chromosome 1 was detected with a QTL region having a PVE of 23.22% and an additive effect of -0.93 from the *O. nivara* allele. Major QTLs for resistance against blast isolates PHL11 and PHL14 was identified in chromosome 2 with a PVE of 15.04 and 15.68% at a chromosome segment represented by RM106 and RM6318 respectively. RM122 represented a chromosome segment at chr.5 which showed a QTL with PVE of 17.07% against the isolate VTN64. Interestingly this QTL showed phenotypic variance contribution from Swarna. These regions were compared with marker defined CSSLs set and found the CSSLs NK25, NK41, NK43, NK53, NK70, NK71 (chr.1); NK51, NK53, NK65, NK13, NK48, NK60, NK67 and NK68 (chr.2); NK24, NK65 and NK67 (chr.5) are containing chromosome segments from *O. nivara* at the QTL regions. Interestingly, the lines with chromosome segment substitution (CSS) from *O. nivara* at chr.1 were grouped under cluster Ia along with susceptible lines, while INLs with chromosome segment substitution from chr.2 and chr.5 were clustered in the resistant group IIIa.

Discussion

Blast disease, caused by *Magnaporthe oryzae* is a major limiting factor in the rice production worldwide because of its global distribution and large-scale reduction to crop yield (Fukuta et al. 2014). Even though there are more than 100 reported genes and 27 of them are cloned and characterized for blast resistance (Koide et al. 2009; Yadav et al. 2019), the rapid genetic evolution of the fungus cause resistance breakdown conferred by major genes within a few years (Fukuta et al. 2019). To manage the blast disease, continuous identification of novel resistant genes, resistance donors and further breeding to introgress these genes to cultivars is essential (Vasudevan et al. 2014). Co-differentiation between rice cultivars and blast races for detection of resistance gene and pathogenicity of blast isolates was carried out using international differential blast isolates and standard differential blast lines (SDBLs). Tsunematsu et al. (2000), Fukuta et al. (2004), Kobayashi et al. (2007), Hayashi and Fukuta (2009) and Telebanco-Yanoria et al. (2010) contributed in development of sets of international differential varieties established from *Pi* resistance genes in the background of Chinese susceptible rice variety LTH as the recurrent parent. These monogenic differential varieties were used to detect the resistance reaction pattern in a set 89 wild introgression lines derived from Swarna/ *O.nivara* and a wide variation in blast resistance was observed, varying from a highly resistant to highly susceptible responses detected while screening with different isolates. The resistance reaction pattern of wild introgression lines was compared with that of differential monogenic varieties and the presence of the *Pi* genes was predicted based on diversity analysis.

The 89 INLs were clustered into two of the three major groups and eight sub groups on the basis of their resistance reaction. Similarly, based on the resistance reaction induced, the 24 isolates were also classified into three major groups and further 5 subgroups. The variability in resistant reaction to diverse isolates showed the existence of polygenic interactions in the resistance mechanism. Broad spectrum resistance to multiple isolates is observed in lines viz., NK32, NK4, NK56 and NK86. This may be either due to the presence of multiple resistance genes and their interactions or due to genomic loci which has a pleiotropic resistance reaction pattern to various isolates. These lines are potential source as a donor in resistance breeding programmes and also to develop mapping populations to detect novel genes with broad spectrum durable resistance. The variation in the virulence and effect of genotypes × isolates interactions were observed endorsing that line and isolate classification as the optimal methodology for this patho-system. Kader et al. (2021) reported a significant isolate × cultivar interaction showing specificity between isolates and cultivars, in a study using winter wheat cultivars against Tan Spot fungus *Pyrenophora tritici-repentis*. Such variable genotypes × isolates specificity indicates a very complex resistance mechanism involving multiple resistance genes with minor or major effect and their interaction with varying inheritance patterns.

Wild species are known resistance source for biotic stress and *O. nivara* an immediate progenitor wild species of rice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218.27 RM495
240.56 RM128
267.00 RM431
269.06 RM283
269.76 RM226
qPHL16-1-1
295.70 RM5
332.17 RM212
Ch2
0.00 RM475
28.12 RM279
41.93 RM6318
56.21 RM5654
63.00 RM53
63.43 RM250
63.82 RM290
64.00 RM3874
64.43 RM555
64.62 RM207
76.90 RM213
88.04 RM48
89.04 RM535
89.94 RM166
95.82 RM106
102.59 RM5460
104.65 RM8080
131.18 RM341
qJPF514-2-1
qPHL14-2-1
qPHL11-2-1
Ch3
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39.20 RM517
68.75 RM15855
99.05 RM251
123.55 RM231
138.62 RM16
167.34 RM55
190.89 RM85
196.71 RM570
210.80 RM565
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233.62 RM1352
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qPHL16-3-1
qJPF514-3-2
qVTN119-3-1
Ch4
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17.90 RM6997
18.50 RM185
19.00 RM273
19.10 RM32
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58.29 RM16742
81.45 RM307
118.70 RM261
Ch5
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10.05 RM289
12.19 RM413
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Ch6
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8.98 RM162
28.80 RM19291
30.93 RM3414
31.60 RM204
51.07 RM510
78.18 RM586
93.00 RM540
93.50 RM454
93.81 RM30
116.15 RM340
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1.90 RM542
2.06 RM5436
4.14 RM5711
11.19 RM21078
33.33 RM1132
47.93 RM214
56.95 RM11
66.96 RM455
84.19 RM118
Ch8
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24.05 RM408
26.08 RM152
27.00 RM502
27.50 RM149
28.00 RM433
32.58 RM1384
41.87 RM223
52.66 RM284
69.21 RM210
70.71 RM44
96.57 RM3819
127.98 RM25
Ch9
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38.38 RM257
75.00 RM316
75.02 RM434
76.00 RM24718
105.46 RM410
qJPF514-9-1
Ch10
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Ch11
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43.49 RM287
46.20 RM536
47.00 RM120
47.50 RM21
47.88 RM552
48.00 RM224
48.20 RM116
49.00 RM26998
61.32 RM209
Ch12
0.00 K46_2
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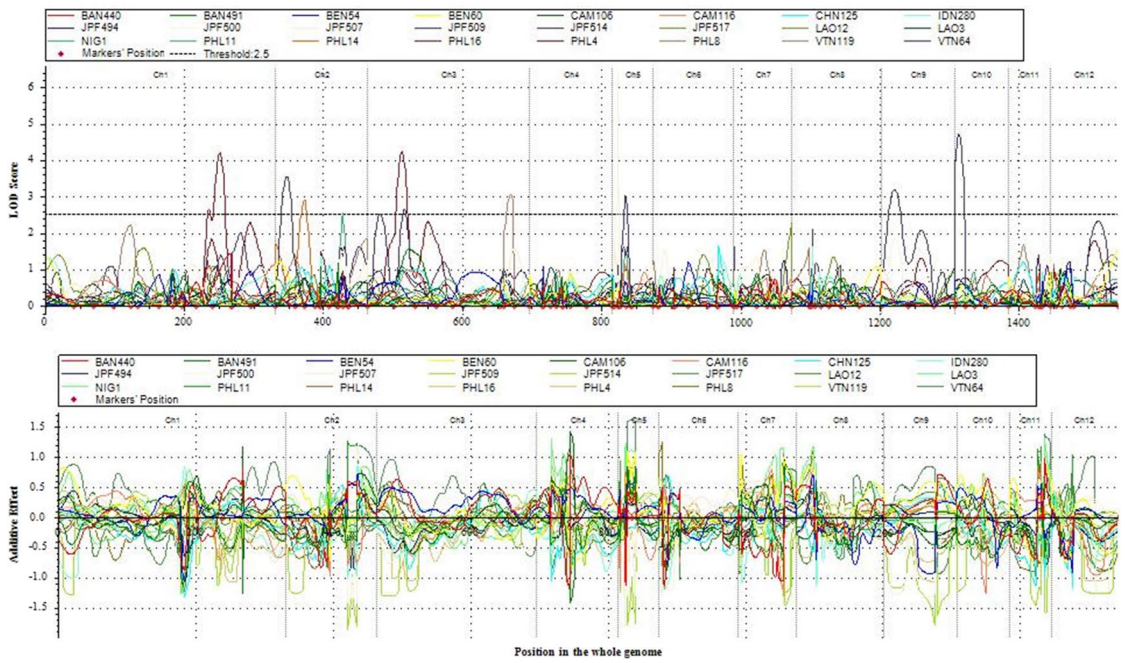
All Traits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NIG1
PHL11
PHL14
PHL16
PHL4
PHL8
VTN119
VTN64
Markers' Position
Threshold:2.5
LOD Score
Additive Effect
Position in the whole genome

◂**Fig. 4** Linkage map showing QTLs located on the 12 chromosomes with additive effect by ICIM QTL mapping

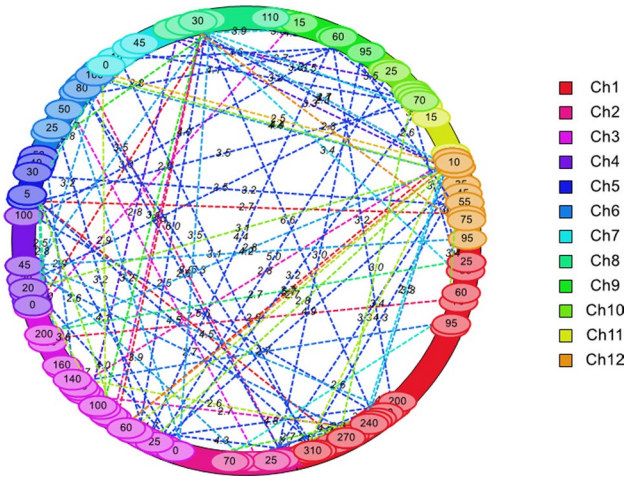

Fig. 5 Cyclic diagram of epistatic QTLs (EQTL) for Blast resistance located across the 12 chromosomes by ICIM-EPI QTL mapping

was contributed much to resistance breeding by acting as a donor for well-known resistance genes (Haritha et al. 2018). Eizenga et al. (2013) identified QTLs for blast (*qBLAST8-1*, *qBLAST12*) using *O. nivara* accessions RGC100898 and IRGC104705 as donors using AB-QTL strategy. Similarly, Devi et al. (2015) identified potential donors for neck blast resistance in a cross between PR114 and *O. nivara* (acc. 105,410). Several blast resistance genes like *Pita*, *Pi33*, *Pir4* and *Pi56* were detected from *O. rufipogon*, another wild species in AA genome family (Huang et al. 2008; Berruyer et al. 2003; Utami et al. 2008; Pawar et al. 2019). Qu et al. (2006) cloned *Pi9* a broad-spectrum blast *R* gene in rice from the wild rice species *O. minuta* (Amante-bordeos et al. 1992) using a map-based cloning strategy. *Pi40* from an EE genome wild *Oryza* species, *O. australiensis* (Acc. 100,882) was fine mapped conferring broad spectrum of resistance to Korean and Philippine blast isolates (Jeung, et al. 2007). Devi et al. (2020) identified a major QTL from the wild species *O. glumaepatula* designated as *Pi68(t)* for resistance to leaf as well as neck blast.

The mapping populations has a greater role in the precision of QTL mapping, advanced and secondary mapping populations like BILs, INLs and CSSLs are of high importance in the dissection of genetic architecture of complex traits like blast resistance compared to primary mapping populations with large interference between the introgressions and the genetic background (Doi et al. 1997; Ebitani et al. 2005). CSSLs are valuable tools for harnessing the genetic diversity from the wild and distant-related species. These are resources for genetic map construction, mapping QTLs, genes or gene interactions and their functional analysis for crop improvement (Eshed and Zamir 1995; Balakrishnan et al. 2018). These populations have much less recurrent parent background effect and are directly useful for molecular marker assisted selections and breeding and further release as improved varieties. In the last few decades, the utility of CSSLs in identification of novel genomic regions influencing a wide range of traits including disease resistance has been well demonstrated in rice (Ali et al. 2010). Zhang et al. 2012 detected 11 QTLs for blast resistance using 114 of the single-segment substitution lines (SSSLs) against 16 rice blast isolates. Fifteen of the SSSLs showed different resistance response from the recipient parent HJX74 indicating the SSSLs are powerful tools to detect genetic architecture of complex traits. The CSSLs with chromosome segments contributing high phenotypic variance towards the blast resistance are potential candidates for further crossing to develop advanced fine mapping populations or secondary single segment subset substitution lines to dissect the novel genes responsible for resistance.

More than 500 QTLs were reported since 1994 (Wang et al. 1994) across the globe and in several genetic backgrounds against blast disease due to advances in molecular breeding techniques and comparatively easy phenotyping which resulted in identification of genomic regions with a spectrum of resistance (Li et al. 2019). Majorly QTL mapping studies against any biotic stress were carried out by screening using only single or few isolates. So, the QTLs which are observed against one isolate are generally considered as universal resistance gene ignoring the major role of interaction between genetic background and specific isolates. Breeding using non race specific genes with partial quantitative resistance controlled by multiple QTLs are gaining more importance against *Magnaporthe* sp. Such resistance helps in reduction of selection pressure to pathogen population and avoids its further multiplication resulting in

Table 4 QTLs identified through CSL analysis for blast resistance based on resistance reaction to 24 blast isolates detected using QTL IciMapping4.1

Isolate	Marker Name	LOD	PVE (%)	Add	M(QQ)	M(qq)
PHL16	RM128	4.07	23.22	-0.93	2.29	4.16
PHL11	RM106*	2.51	15.04	1.22	2.95	0.50
PHL14	RM6318*	2.63	15.68	-0.75	3.16	4.66
VTN64	RM122*	2.88	17.07	1.65	3.31	0.00

* SSR markers showing trait association in both ICIM and SMA

durable broad-spectrum resistance (Niks et al. 2015; Ning et al. 2020). Several studies demonstrated the presence of candidate genes in QTL regions like biotic stress expressed sequence tags, defense genes, and resistance gene analogs (RGA) (Wang et al. 2001; Ramalingam et al. 2003; Wen et al. 2003; Wisser et al. 2005).

Partial resistance is considered more durable in case of ever evolving pathogen like *Magnaporthe oryzae* compared to absolute resistance by monogenic lines. As complete or absolute resistance genes cause breakdown of resistance comparatively faster by triggering pathogen evolution especially due to heterothallic nature. Multiple genes pyramiding is more durable than monogenic complete resistance, however pyramiding involving both complete resistance and partial resistance genes are expected to contribute much durable defense. Several genes like *Pi21*, *Pb*, *Pi3*, *Pi63*, *bsr-d1*, *Bsr-k* and *Pid3-I1* (Fukuoka et al. 2009; Hayashi et al. 2010; Fukuoka et al. 2014; Xu et al. 2014; Li et al. 2017; Zhou et al. 2018; Inukai et al. 2019) conferring partial and durable resistance have been identified and available for crop improvement against blast disease (Zhang et al. 2016; Pilet-Nayel et al. 2017). Genetic mapping for disease resistance gained more importance as QTLs imparts partial resistance which is more durable and broader spectrum (Bonman et al. 1989; Wang et al. 1994); later it was reported that QTLs can be isolate specific (Lopez-Gerena 2006; Roumen 1992; Talukder et al. 2004) as in our study. Wang et al. (1994) reported that QTLs conferring partial resistance may have race-non-specific effects. However, Talukder et al. (2004), detected both race specific and race nonspecific QTLs against 3 blast isolates CD100, CM28 and PH19 in a segregating mapping population derived from Bala / Azucena, implying existence of QTL × isolate interaction. Host species specificity or isolate specificity can be explained in terms of the classical gene-for-gene concept and are reported between *Oryza* and *Eleusine* isolates and these genes might be having alternative species-specific functions (Skamnioti and Gurr 2009). A metagenomic analysis to assess the specificity of infection pattern across the isolates of *Magnaporthe oryzae* and the related *graminaeceous* species might elucidate the details of genetics of pathogen-plant interactions and to understand any cross infections.

In this study virulent races gave varying resistance responses and resulted in identification of major QTLs with high phenotypic variance and a total of 12 QTLs identified against 8 isolates. Among these 2 QTLs against isolate PHL16 and 4 QTLs against JPF514 were detected and also observed in interval mapping. 5 QTLs showed a major effect with more than 10 PVE% and were confirmed by the significant single marker association of the flanking markers. Among the QTLs detected, only one QTL region at chr.3 flanking markers RM517 and RM15855 showed resistant against two isolates. Similarly, chromosome segments at chr.1, 2 and 5 represented by RM128, RM106, RM6318 and RM122 also showed the presence of QTLs harboring genes for blast resistance by CSL mapping. SSR markers RM5140 in chr.5 and RM565 in chr.3 showed strong association with blast resistance by consistently appearing as associated by ICIM, IM and SMA.

Accumulation of QTLs in chromosome segments of INLs were studied and found that the scores for infection were comparatively less in III cluster group where majority of the INLs were clustered. Cluster Ia contained susceptible genotypes and INLs in clusters IIIa-1, IIIa-2, IIIb-1, and IIIb-2 were majorly resistant. INLs in Cluster IIIa was showing more resistant response than that of cluster IIIb, therefore cluster III was divided into two subgroups in clusters IIIa and IIIb, and there were a few exceptions in resistant and susceptible reactions observed in these clusters and were further subdivided into two sub clusters each.

The presence of number of QTLs identified in the study showed significant association with resistance reaction especially in case of INLs present in the clusters IIIa and IIIb where most of the lines were either resistant or moderately resistant to majority of the isolates. The results indicated a tendency that the resistance among INLs was increased according to accumulations of QTLs, except for cluster group Ia (Supplementary Table 5 and 6). This might be due to the presence of undetected minor QTLs or due to the interaction of the QTLs among each other and varying genome percentage from donor and recurrent parents in the background. The combinations or interactions of QTLs also play an important role for positive and negative resistance reactions pattern which is evident from this study. Seven chromosomal regions viz., *qBLC1.1, qBLC1.4, qBLC2.1, qBLC3.3, qBLC6.1, qBLC11.1,* and *qBLC12.1* showed the effects of resistance according to increasing number of introgressed segments and some of these segments were involved in epistatic interactions which altered their phenotypic effect. The genotypic clusters IIIa-1 (mean score: 2.1) and IIIa-2 (2.2), and IIIb-1 (2.5) and IIIb-2 (2.4) showed that these chromosomal regions corresponded with the additive effects of resistant according to the accumulations. The chromosomal region *qBLC5.2* was detected in all cluster groups with high ratio, and it was expected the one of the most effective QTLs for the resistance among INLs. QTL on *qBLC1.4* might play the role for negative effect with the other QTL(s).

Although several QTLs for blast tolerance have been identified in rice, the comparison of additive QTLs for different isolates has rarely been conducted, and the epistatic interaction between QTLs is not studied. In this study, A total of 16 digenic interactions were detected for blast resistance which showed a cumulative phenotypic effects of 11–21% against isolates VTN119, BAN491, BEN54, CAM106, IDN280, JPF494, JPF507, LAO12, LAO3,

PHL14 and major QTLs *qBLC1.1, qBLC1.3, qBLC1.4, qBLC2.1, qBLC3.2, qBLC3.3, qBLC9.1* and *qBLC10.1* were involved in these major effect epistatic interactions also (Supplementary Table 7). Interestingly, only one significant main effect QTLs is involved in EQTLs with non-significant loci in other chromosomes. Among these 16, eight of the EQTLs were independent of the main effect QTL and majorly negative digenic interactions were observed with negative additive by additive effect. An epistatic interaction between chromosome 3 (RM16-RM55) and chromosome 5 (RM163-RM574) explained a high phenotypic variance of 21.85% for resistance to isolate JPF507. Additive by additive effects of epistatic QTLs were lower than additive effect of any of their corresponding major effect QTL and it may be due to presence of segregation distortion for QTL combinations, interference from other loci and low phenotypic variance raised from epistatic interactions (Mackay 2001). Epistatic interactions play a major role in expression of phenotype of complex quantitative traits and it depends upon magnitude and direction of the QTLs involved as pair (Wang et al. 2018). To avoid underestimation of total genetic effects of a trait the epistatic interaction must be considered to plan breeding programmes to pyramid multiple QTLs. Participating QTLs of the E QTLs with large cumulative or complimentary effect are useful in marker assisted pyramiding for resistance improvement of cultivars (Wang et al. 2012; Balakrishnan et al. 2020).

The blast resistance QTL on chromosome 1 with the flanking markers RM128 and RM431 was found associated with yield and biomass related traits PH, BM and TDM in previous mapping studies in the same population (Swamy et al. 2014; Surapaneni et al. 2017; Haritha et al. 2018; Balakrishnan et al. 2020) and also in other mapping studies using different population (Qiao et al. 2016). Chen et al. 2005 reported mapping of *Pi37(t)*, gene closely linked to RM128 conferring resistance to rice blast against isolates collected from the South China region. The 8.15 Mb region covering QTL ***qBL1.1*** is an important genomic region containing several QTLs / genes linked to plant height and yield like *sd-1*(Li et al. 2003) and *yld1.1*(Cho et al. 2007) and several agronomic and domestication related traits. The linked marker RM431 which is closely linked to the semi dwarf *SD1* gene (Spielmeyer et al. 2002) was also previously reported as associated to blast resistance indicating a reverse relationship of plant height to blast resistance (Wang et al. 2015). This region was identified to be harbouring resistance gene clusters responsible for plant defensive mechanisms like *Pi35(t)* (Nguyen et al. 2006), *Pi37*(Lin et al. 2007), OsWRKY13 (Qiu et al. 2008), OsGH3.1 (Domingo et al. 2009), GH3-2 (Fu et al. 2011) and RACK1A (Nakashima et al. 2008). Nguyen et al. 2006 identified *Pi35(t)* with a major effect contributing to non-race specific durable resistance by reducing the extent of pathogen multiplication at this locus. In the same region, blast resistance gene *Pi37* encoding an NBSLRR protein was identified by Lin et al. 2007. Qiu et al. 2008 detected OsWRKY13 imparting resistance for both bacterial blight and blast resistance in this gene cluster.

Pathogen infection induces DNA-binding activity of OsWRKY13, which activates of the salicylic acid dependent pathway and a suppress jasmonic acid dependent pathways, which intervene the resistance mechanism regulating the expression of a subset of genes acting both upstream and downstream of these pathways either directly or indirectly. Another gene in this region with pleiotropic effect OsGH3.1 controlling auxin content, cell growth and resistance to fungal pathogens plays a major role in activating a significant number of defense-related genes (Domingo et al. 2009). *GH3-2*, encodes indole-3-acetic acid (IAA)-amido synthetase, imparting a broad-spectrum resistance to both bacterial blight and blast by suppressing pathogen-induced IAA accumulation (Fu et al. 2011). This QTL region is also closely placed near the receptor for Activated C-Kinase 1A (RACK1A) which plays a role in the production of reactive oxygen species (ROS) by providing innate immunity against rice blast infection by interacting with multiple proteins in the Rac1 immune complex (Nakashima et al. 2008).

qBL2.1 detected in this study is collocated with qDLA2, mapped by Wu et al. 2005 for percentage diseased leaf area against isolate C923-39 from Philippines. The same region is very closely linked to R gene loci *Pib* and contained genes for BLB resistance like OsHPL2 coding for *Hydroperoxide lyase* 2 (Gomi et al. 2009) and OsWRKY71 (Jiang et al. 2017) which are reportedly playing a defensive mechanism against fungal and bacterial pathogens. The third QTL region flanking between RM106 and RM5460 was already known to have QTLs giving resistance against sheath blight (Pinson et al. 2005) and insect resistance (Fujita et al. 2002) but no QTL or gene for blast resistance was reported. Interestingly Silicon uptake related gene lsi1 (Low silicon rice 1) was previously detected in this region imparting pest resistance (Ma et al. 2008). RM279 flanking ***qBL2.1*** and RM106 flanking ***qBL2.2*** was previously reported as associated with blast and sheath blight resistance (Anupam et al. 2017; Pinson et al. 2008; Li et al. 1995). A major QTL detected between RM279-RM6318 was having genes for blast resistance qCA-2 (Rao et al. 2005) and RAR1 (Thao et al. 2007). Rao et al. 2005 indicated the necessity for partial resistance mapping using advanced recombinant inbred line (RIL) subpopulations where the interference of major resistance genes is not significant and reported the gene qCA-2 which imparts partial resistance to neck blast. Thao et al. 2007 reported formation protein complexes by RAR1 along with Rac1, HSP90, and HSP70 in rice cells for important components of *R* gene–mediated disease resistance and suggest that these proteins play important roles in

innate immunity in rice against both *Magnaporthe oryzae* and *Xanthomonas oryzae*. ***qBL3.1*** which was found closely located to a pathogen-inducible rice OsAOS2 gene encoding *Alleneoxide synthase* in jasmonic acid pathway controlling resistance to *Magnaporthe oryzae* (Mei et al. 2006).

qBL3.2 and ***qBL3.3*** between RM517- RM15855 contained a hot spot region for multiple resistance genes like OsAOS2 (Mei et al. 2006), OsBRR1 (Peng et al. 2009), OsMAPK5 (Xiong and Yang 2003), OsFAD7 (Yara et al. 2007), OsWRKY31 (Zhang et al 2008), phyB (Xie et al. 2011), OsAOC (Riemann et al. 2013) and OsSWAP70A (Yamaguchi et al. 2012). Peng et al. 2009 detected OsBRR1 putative leucine-rich repeat receptor kinase, which mediates resistance to rice blast. Another gene located at this region, OsMAPK5, for mitogen-activated protein kinase, plays an important role in controlling stress response mechanisms for resistance to fungal and bacterial pathogens and tolerance to cold, drought and salinity (Xiong and Yang 2003). Similarly, OsWRKY31, located in this QTL region is involved in many regulatory roles in response to biotic and abiotic stresses which is induced by the rice blast fungus *Magnaporthe oryzae*. This WRKY transcription factor regulates constitutive expression of the pathways of many defense-related genes, as well as early auxin-response genes (Zhang et al. 2008). PhyB, a phytochrome regulating the *jasmonate* (JA) or salicylic acid (SA) signaling pathways required for inducing pathogenesis-related class 1 (PR1) proteins is identified in this region (Xie et al. 2011) and also demonstrated that the transcript levels of genes involved in these defense pathways were regulated by leaf age and functional phytochromes. OsAOC encoding allene oxide cyclase a functional enzyme for *jasmonate* mediated defense to *Magnaporthe oryzae* was reported in this genomic region by Riemann et al. 2013. Yamaguchi et al. 2012 detected OsSWAP70A which cause a Chitin elicitor-induced defense response and mediates reactive oxygen species production. SWAP70 functions as a Rac/Rop guanine nucleotide-exchange factor in rice and regulates immune responses through activation of important signaling factors like Rho GTPase OsRac1.OsFAD7 encoding fatty acid desaturase7 was identified as imparting disease resistance against blast by suppressing of ω-3 Fatty Acid desaturases which contribute to signaling on defense responses (Yara et al. 2007). Interestingly this region chr3:6,165,992…29,813,274 was also harbouring several QTLs and genes for grain size related traits like GS3, qGW3, qGY3, qGL3, qLWR3, GL3.1 and genes like pdhk encoding pyruvate dehydrogenase kinase (Mao et al. 2003; Miyata et al. 2007; Wan et al. 2005; Mukherjee et al. 2012). Gene bph19(t) is also located in this region (Chen et al. 2006a, b).

qBL3.4 between RM570 and RM565 contains gene for OsCPK10, a calcium-dependent protein kinase 10, inducing resistance to *Magnaporthe oryzae* by activating expression of SA- and JA-related defense genes and playing a crucial regulator role in plant immune responses (Fu et al. 2013). Another gene in this locus, OsADF is a lectin receptor-like kinase contribute to innate immune responses to *Magnaporthe oryzae* and *Xanthomonas oryzae* and brown planthopper resistance as well as seed germination indicating of progressive adaptive evolution in rice with a common physiological pathway connecting germination and immunity in plants (Cheng et al. 2013). QTLs for blast resistance were not documented in 5.676Mbp region of ***qBL5.1*** and this is a potential candidate region to identify novel genes. However, this region has a stress resistance gene OsHI-XIP encoding rice *xylanase*-inhibiting protein (XIP)-type which induce defense compounds against herbivores and wounding (Xin et al. 2014). Overexpression of this *xylanase* inhibitor gene reported to play an important role in plant defense against fungal pathogens. Several QTLs for grain weight viz., QKw5 for 1000 kernel weight, qTHJ-5 for grain thickness; qSW5 for seed width; qGW5 1,000-grain weight (Li et al. 1997; Onishi et al. 2007; Tian et al. 2006), OsEXPA3 for salinity tolerance (Qiu et al. 2014) and qLTG-5 for germinability at low temperature and cold tolerance (Suh et al. 1999) were also collocated in this region. Two megabases downstream from the qBL5.1 region, there are four genes OsWRKY45 (Tao et al. 2011; Shimono et al. 2007, 2012), WRKY45 (Akagi et al. 2014), OsWRKY53 (Chujo et al. 2014), d1(dwarf1 mutant) (Suharsono et al. 2002) inducing blast and *Xoo* resistance were reported. OsWRKY45 genes are known to regulate resistance mechanisms by mediating salicylic acid and jasmonic acid pathways (Tao et al. 2011), WRKY45 (OsWRKY45) cause BTH-induced disease resistance by mediating SA signalling (Shimono et al. 2012) and *OsWRKY53 is a* chitin oligosaccharide elicitor-induced gene for defense signaling pathways (Chujo et al. 2014), though they induce different resistance expressions which are pathogen specific however the race specific mechanisms are not well studied. Suharsono et al. 2002 found d1 Gα gene in this region using *dwarf1* (*d1*) mutants playing a pivotal role in activation of *R* gene-mediated disease resistance of rice.

qBL5.2 contained an already reported gene responsible for resistance to both *M. oryzae* and *X.oryzae ie.* OsMT2b encoding metallothionein2b (Wong et al. 2004). Metallothioneins are ubiquitous Cys-rich proteins which act as a ROS scavengers regulated by OsRac1 signals defense mechanisms. The rice *xa5* gene for disease resistance to *Xanthomonas oryzae pv. oryzae* has been located in this QTL region which encodes the gamma subunit of transcription factor IIA (TFIIAγ) (McCouch et al. 1991; Jiang et al. 2006; Iyer and McCouch 2004) where only two nucleotide substitutions result in an amino acid change between resistant and susceptible cultivars. Ashkani et al. 2012 found flanking marker RM413 was significantly associated with blast resistance to pathotype 7.2 of *M. oryzae* in rice. ***qBL9.1*** region between markers had one previously reported defense

gene OsSGT1 encoding salicylic acid glucosyltransferase1 and found to contribute to the chemically induced SA O-β-glucoside (SAG) up-regulation which results in SA signaling (Umemura et al. 2009). One important blast resistance gene, LYP4, lysin motif containing protein 4 (Liu et al. 2012) is located 2 Mb upstream of this region. It induces plant innate immunity playing a dual role of detection of microbe-associated molecular patterns (MAMPs) of pathogens through pattern recognition receptors (PRRs) and causing blast and *xoo* resistance by combined ROS generation, defense gene activation, and callose deposition. In the same region grain yield QTLs *yldp9.1* (Marri et al. 2005) and gy9 (Cho et al. 2007) were also reported.

qBL10.1 was harbouring OsNAC111 a blast disease–responsive transcription factor encoding NAC domain-containing protein 111 enhanced blast resistance by regulating the expression of a specific set of PR genes (Yokotani et al. 2014). Another gene OsAT1 in Spl18 from Spotted leaf 18 mutant causing resistance to blast and bacterial blight was also reported in the locus. Transcription of the gene in leaf blade, sheath and young panicles upregulated PR proteins, increased accumulation of phytoalexins, and improved the resistance to blast disease (Mori et al. 2007). Talukder et al. (2004) identified race specific QTL 12.2 for lesion size, number of lesions per leaf at this region. Liu et al. 2004 reported candidate defense gene PR-1 at this locus using recombinant inbred (RI) and advanced backcross (BC) populations derived from a blast-resistant cultivar, Sanhuangzhan 2 (SHZ-2) resulting in a quantitative blast resistance of 15.8% of diseased leaf area (DLA) variation and 18.0% of total phenotypic variation.QTL flanking markers RM156(Li et al. 2007) and RM5140 (Sabouri et al. 2011) were previously reported associated to blast resistance.

Even though only a few of the flanking markers linked to the QTLs identified in our study were recorded previously as having a significant association with blast resistance, most of the identified chromosome segments/ loci were harbouring genes for defense mechanisms against either blast or bacterial leaf blight disease with pathogen specific or non-specific expression. The clustering of most of the resistance genes in certain genomic regions were reported by Ballini et al. (2008) in a genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci. The detailed characterization of both host R and pathogen *Avr* genes will reveal genetic architecture of specific host–pathogen interactions and the evolution and functioning of pleiotropic loci at R gene clusters (Lin et al. 2007). Many of these flanking markers and the linked loci were found to be showing trait association with yield contributing traits like plant height and biomass. Interestingly, association to plant hopper resistance and sheath blight resistance were found with some of the flanking markers in earlier QTL mapping studies showing a cross talk between resistance mechanisms among different biotic stress. There was no QTL identified against 12 of the isolates studied either by ICIM or IM even though the phenotypic trait segregation was observed; this might be mainly because of the network of undetected minor genes and their epistatic interactions. In this study we used some of the well-known previously reported markers linked to *Pi* genes like RM259 (*Pi27t*), RM212(*Pi37*) at chr.1, RM166 (*Pig(t)*) at chr.2, RM136(*Pi2/Pi9*) at chr.6, RM44(*Pi33*) at chr.8, RM206 and RM21(*Pi38*), RM144 an RM224(*Pikh/Piks*) at chr.11 (Zhu et al. 2004; Chen et al. 2004; Fjellstrom et al. 2004; Gowda et al. 2006; Deng et al. 2006; Berruyer et al. 2003) (Supplementary Table 8). However, none of these markers were flanked any of the novel QTLs identified in this study. The presence of reported favourable alleles in the lines did not show any significant association to the resistance pattern. Further characterization using linked molecular markers to additional major genes are required for confirmation of novelty of the detected resistance loci and the study is under progress. It was found that INLs harbouring the *O.nivara* alleles for *Pi38* or *PiKs* were found to show a highly resistant average score within 0–2.

The lines which demonstrated resistance but not containing any of the reported genes might harbour different gene(s) other than the profiled ones (Azameti et al. 2020) or having the novel genes from *O.nivara*. The chromosome segment pattern in the INLs are studied and it was found RM273 in chr.4 and RM137 in chr.8 were found to have bearing *O.nivara* alleles across the clusters in 75% of the INLs independent of resistance response. RM1384 (chr.8), RM535(chr.2), RM235(chr.2) and RM137 (chr.8) were showing majorly *O.nivara* alleles in the highly resistant lines. There was no specific CSS found which can distinguish the set of resistant and susceptible lines and the resistance level and was completely independent of parental genome constitution, number of recombination etc., however the network of combination of various alleles and their interaction might have resulted in variability in resistance response. In the INLs we found NK31, NK70 harbored CSS for ***qBL1.1***, NK15, NK75 and NK55 for ***qBL2.1***, NK31, NK79, NK60, NK67, NK46 harbored *O.nivara* CSS for ***qBL.2.3***, NK11, NK20, NK8, NK47 for ***qBL3.4***, NK67 for ***qBL5.2***, NK14, NK35 for ***qBL9.1***, NK54 andNK39 for ***qBL10.1***.Similarly NK51 and NK46 had heterozygous CSS for ***qBL2.2*** and ***qBL5.1*** respectively and are potential INLs for fine mapping by further selfing. Two CSSLs NK40 (***qBL3.1***, ***qBL10.1***) and NK60 (***qBL3.2***, ***qBL3.3***) which are resistant to moderately resistant against most of the lines, harbored chromosome segments for two QTLs each and another CSSL NK53 also contained QTLs identified from CSL mapping (Supplementary Table 5). The presence of donor genome alleles of the QTLs in the INLs showed a positive correlation with resistance response, while no significant relation was found due to variation in percentage of

recurrent parent alleles/ genome recovery. These lines can be used for further genetic dissection by crossing to develop fine mapping populations or sub-CSSLs and to identify candidate genes. The multiple isolate resistant line NK4(7 K) showed stability and high mean for 12 yield contributing traits in our previous genotype × environment interaction study for yield traits (Balakrishnan et al. 2014). This wild introgression line also a proven donor for seedling vigour (Addanki et al. 2019) and high photosynthetic rate compared to the parent Swarna (Rao et al. 2018a, b). These lines are potential donors in resistance breeding programmes and also to develop mapping populations to detect novel genes with broad spectrum durable resistance. QTL mapping as well as comparison using resistance reaction of differential monogenic lines resulted in the identification of CSSLs with multiple known genomic regions as well as novel resistance loci from *O. nivara*.

Resistance to moderately resistant reaction was observed to all the isolates in the lines NK4 and NK86 with an average disease score of 1.5 and a total of 16 lines showed resistance reaction below an average score of 2. Among the 24 isolates used in the study; JPF494, PHL14 and VTN64 were highly virulent causing maximum disease susceptibility among these wild introgression lines. Interestingly resistance response was not depending on the geographical origin of the isolates indicating their phylogenic diversity. Broad spectrum resistance to multiple isolates is observed in lines viz., NK56(152 K), NK4(7 K), NK86(7-4 K), NK62(163 K), NK68(194 K), NK32(83 K), NK40(104 K), NK61(158 K), NK84(271 K), NK27(54 K), NK69(197 K), NK9(16 K), NK24(50 K), NK67(192 K), NK90(262-1 K) and NK28(59 K). This might be either due to the presence of multiple resistance genes or due to gene clusters in specific associated genomic loci which has a pleiotropic resistance reaction pattern to various isolates. In this study, chromosomal segments harboring gene clusters of defense related genes contributing to quantitative disease resistance were identified across 12 chromosomes. These QTLs contained numerous pathogen specific and nonspecific resistance gene analogues along with several agronomic traits for crop improvement. The genetic dissection and fine mapping of these QTL intervals are essential for further functional analysis.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40858-023-00614-4.

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Author contributions YF designed the experiments, analyzed the data and supervised the project. DB performed the experiments carried out the QTL mapping and wrote the manuscript. SN facilitated the development and sharing the mapping population for the experiment. YF and DB prepared the final manuscript.

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Data availability The data generated and analyzed in this study are included in the manuscript and supplementary files.

Introgression lines (INLs) described in the manuscript is available for non-commercial research purposes from ICAR-IIRR subject to the Material Transfer Agreement conditions. Obtaining any permissions will be the responsibility of the requestor.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Addanki KR, Balakrishnan D, Yadavalli VR, Surapaneni M, Mesapogu S, Beerelli K, Neelamraju S (2019) Swarna× *Oryza nivara* introgression lines: A resource for seedling vigour traits in rice. Plant Genetic Resources 17:12–23

Akagi A, Fukushima S, Okada K, Jiang CJ, Yoshida R, Nakayama A, Shimono M, Sugano S, Yamane H, Takatsuji H (2014) WRKY45-dependent priming of diterpenoid phytoalexin biosynthesis in rice and the role of cytokinin in triggering the reaction. Plant Molecular Biology 86:171–183

Ali ML, Sanchez PL, Si-bin Yu, Lorieu M, Eizenga GC (2010) Chromosome segment substitution lines: A powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*). Rice 3:218–234

Altman N, Krzywinski M (2015) Split plot design. Nature Methods 12:165–166

Amante-Bordeos A, Sitch LA, Nelson R, Dalmacio RD, Oliva NP, Aswidinnoor H, Leung H (1992) Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theoretical and Applied Genetics 84:345–354

Anupam A, Imam J, Quatadah SM, Siddaiah A, DasSP VM, Mandal NP (2017) Genetic diversity analysis of rice germplasm in Tripura state of Northeast India using drought and blast linked markers. Rice Science 24:10–20

Arunakumari K, Durgarani CV, Satturu V, Sarikonda KR, Chittoor PDR, Vutukuri B, Laha GS, Nelli APK, Gattu S, Jamal M, Prasadbabu A, Hajira S, Sundaram RM (2016) Marker-assisted pyramiding of genes conferring resistance against bacterial blight and blast diseases into indian rice variety MTU1010. Rice Science 23:306–316

Ashkani S, Rafii MY, Rusli I, Sariah M, Abdullah SNA, Harun AR, Latif MA (2012) SSRs for marker-assisted selection for blast

resistance in rice (*Oryza sativa* L.). Plant Molecular Biology Reports 30:79–86

Azameti MK, Vishalakshi B, Umakanth B, Marathi Balram M, Srinivas Prasad M, Madhav S (2020) Molecular characterization of popular rice (*Oryza sativa* L.) varieties of India and association analysis for blast resistance. Genetic Resources and Crop Evolution 67:2225–2236

Balakrishnan D, Robin S, Rabindran R, Senthil S, Raveendran M, John Joel A (2014) Marker assisted backcross breeding approach to improve blast resistance in indian rice (*Oryza sativa*) variety ADT43. Euphytica 200:61–77

Balakrishnan D, Surapaneni M, Raju AK, Rao YV, Mesapogu S, Kavitha B, Sarla N (2020) Detection of chromosome segment substitution lines and yield QTLs with additive, epistatic and QTL × environment interaction effects from *Oryza sativa*/ *O. nivara* IRGC81832 cross. Scientific Reports 10:7766

Balakrishnan D, Surapaneni M, Mesapogu S, Neelamraju S (2018) Development and use of chromosome segment substitution lines as a genetic resource for crop improvement. Theoretical and Applied Genetics 132:1–25

Ballini E, Morel J-B, Droc G, Price A, Courtois B, Notteghem J-L, Tharreaul D (2008) A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. Molecular Plant-Microbe Interactions 21:859–868

Berruyer R, Adreit H, Milazzo J, Gaillard S, Berger A, Dioh W, Lebrun MH, Tharreau D (2003) Identification and fine mapping of Pi33, the rice resistance gene corresponding to the *Magnaporthe grisea* avirulence gene ACE1. Theoretical and Applied Genetics 107:1139–1147

Bonman JM, Estrada BA, Bandong JA (1989) Leaf and neck blast resistance in tropical lowland rice cultivars. Plant Disease 73:388–390

Chen QH, Wang YC, Zheng XB (2006a) Genetic diversity of *Magnaporthe grisea* in China as revealed by DNA fingerprint haplotypes and pathotypes. Journal of Phytopathology 154:361–369

Chen XW, Li SG, Xu JC (2004) Identification of two blast resistance genes in a rice variety. Journal of Phytopathology 152:77–85

Chen JW, Wang L, Pang XF, Pan QH (2006b) Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene bph19(t). Molecular Genetics and Genomics 275:321–329

Chen S, Wang L, Que Z, Pan R, Pan Q (2005) Genetic and physical mapping of Pi37 (t), a new gene conferring resistance to rice blast in the famous cultivar St. No. 1. Theoretical and Applied Genetics 111:1563–1570

Cheng X, Wu Y, Guo J, Du B, Chen R, Zhu L, He G (2013) A rice lectin receptor-like kinase that is involved in innate immune responses also contributes to seed germination. Plant Journal 76:687–698

Cho YI, Jiang W, Chin JH, Piao Z, Cho YG, McCouch S, Koh HJ (2007) Identification of QTLs associated with physiological nitrogen use efficiency in rice. Molecules and Cells 23:72–79

Chujo T, Miyamoto K, Ogawa S, Masuda Y, Shimizu T, Kishi-Kaboshi M, Takahashi A, Nishizawa Y, Minami E, Nojiri H, Yamane H, Okada K (2014) Overexpression of phosphomimic mutated OsWRKY53 leads to enhanced blast resistance in rice. PLoS ONE 9:e98737

Couch BC, Kohn LM (2002) A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from M. grisea. Mycologia 94:683–693

Dean R, van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD (2012) The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13:414–430

Dean RA, Talbot NJ, Ebbole DJ, Farman ML, Mitchell TK, Orbach MJ, Thon M, Kulkarni R, Xu JR, Pan H, Read ND, Lee YH, Carbone I, Brown D, Oh YY, Donofrio N, Jeong JS, Soanes DM, Djonovic S, Kolomiets E, Rehmeyer C, Li W, Harding M, Kim S, Lebrun MH, Bohnert H, Coughlan S, Butler J, Calvo S, Li-Jun Ma LJ, Nicol R, Purcell S, Nusbaum C, Galagan JE, Birren BW (2005) The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434:980–986

Deng Y, Zhu X, Shen Y, He Z (2006) Genetic characterization and fine mapping of the blast resistance locus Pigm(t) tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety. Theoretical and Applied Genetics 113:705–713

Deng Y, Zhu X, Xu J, Chen H, He Z (2009) Map-based cloning and breeding application of a broad-spectrum resistance gene *Pigm* to rice blast. In: Wang GL, Valent B. (eds) Advances in genetics, genomics and control of Rice blast disease. Springer, Dordrecht. pp. 161–171. https://doi.org/10.1007/978-1-4020-9500-9_16

Devi SR, Singh K, Umakanth B, Vishalakshi B, Renuka P, Sudhakr KV, Prasad MS, Viraktamath BC, Babu VR, Madhav MS (2015) Development and identification of novel rice blast resistant sources and their characterization using molecular markers. Rice Science 22:300–308

Devi SJSR, Singh K, Umakanth B, Vishalakshi B, Rao KVS, Suneel B, Sharma SK, Kadambari GKM, Prasad MS, Senguttvel P, Syamaladevi DP, Madhav MS (2020) Identification and characterization of a large effect QTL from *Oryza glumaepatula* revealed Pi68(t) as putative candidate gene for rice blast resistance. Rice 13:1–13

Doi K, Iwata N, Yosimura A (1997) The construction of chromosome substitution lines of African rice (*Oryza glaberrima* Steud) in the background of japonica rice (*O. sativa* L). Rice Genetics Newsletter 14:39–41

Domingo C, Andrés F, Tharreau D, Iglesias DJ, Talon M (2009) Constitutive expression of *OsGH3.1* reduces auxin content and enhances defense response and resistance to a fungal pathogen in rice. Molecular Plant-Microbe Interactions 22:201–210

Ebitani T, Takeuchi Y, Nonoue Y, Yamamoto T, Takeuchi K, Yano M (2005) Construction and evaluation of chromosome segment substitution lines carrying overlapping chromosome segments of indica rice Cultivar 'Kasalath' in a genetic background of japonica elite cultivar 'Koshihikari. Breeding Science 55:65–73

Eizenga GC, Prasad B, Jackson AK, Jia MH (2013) Identification of rice sheath blight and blast quantitative trait loci in two different *O. sativa*/ *O. nivara* advanced backcross populations. Molecular Breeding 31:889–907

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Fjellstrom R, McClung A, Gibbons J, Deren C (2004) Development of DNA markers suitable for marker assisted selection of three Pi genes conferring resistance to multiple *Pyricularia grisea* pathotypes. Crop Science 44:1790–1798

Fu J, Liu H, Li Y, Yu H, Li X, Xiao J, Wang S (2011) Manipulating broad-spectrum disease resistance by suppressing pathogen-induced auxin accumulation in rice. Plant Physiology 155:589–602

Fu L, Yu X, An C (2013) Overexpression of constitutively active *OsCPK10* increases Arabidopsis resistance against Pseudomonas syringae pv. tomato and rice resistance against *Magnaporthe grisea*. Plant Physiology and Biochemistry 73:201–210

Fujita D, Yoshimura A, Yasui H (2002) Detection of QTLs associated with antibiosis to green rice leafhopper, Nephotettixcincticeps Uhler, in four Indica rice varieties. Rice Genetics Newsletter 19:90–91

Fukuoka S, Yamamoto SI, Mizobuchi R, Yamanouchi U, Ono K, Kitazawa N, Yasuda N, Fujita Y, Nguyen T TT, Koizumi S, Sugimoto K, Matsumoto T, Yano M (2014) Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. Scientific Reports 4:1±7

Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. Science 325:998±1001

Fukuta Y, Koga I, Ung T, Sathya K, Kawasaki-Tanaka A, Koide Y, Kobayashi N, Obara M, Yagana H, Hayashi N (2014) Pathogenicity of rice blast (*Pyricularia oryzae* Cavara) isolates from Cambodia. Japan Agricultural Research Quarterly 48:155–166

Fukuta Y, Telebanco-Yanoria MJ, Imbe T, Tsunematsu H, Kato H, Ban T, Ebron LA, Hayashi N, Ando I, Khush GS (2004) Monogenic lines as an international standard differential set for blast resistance in rice (*Oryza sativa* L.). Rice Genetics Newsletter 21:70–72

Fukuta Y, Suzuki T, Yanagihara S, Obara M, Tomita A, Ohsawa R, Machungo CW, Hayashi N, Makihara D (2019) Genetic variation of blast (*Pyricularia oryzae* Cavara) resistance in rice (*Oryza sativa* L.) accessions widely used in Kenya. Breeding Science 69:672–679

Fukuta Y, Koide Y, Kobayashi N, Kato H, Saito H, Telebanco-Yanoria MJ, Ebron LA, Mercado-Escueta D, Tsunematsu H, Ando I, Fujita D (2022a) Lines for blast resistance genes with genetic background of Indica Group rice as international differential variety set. Plant Breeding 141:609–620

Fukuta Y, Koide Y, Kobayashi N, Kato H, Saito H, Telebanco-Yanoria MJ, Ebron LA, Mercado-Escueta D, Tsunematsu H, Ando I, Fujita D, Obara M, Tomita A, Hayashi N, Imbe T (2022b) Lines for blast resistance genes with genetic background of Indica Group rice as international differential variety set. Plant Breeding 141:609–620

Gomi K, Satoh Masaru, Ozawa Rika, Shinonaga Yumi, Sanada Sachiyo, Sasaki Katsutomo, Matsumura Masaya, Ohashi Yuko, Kanno Hiroo, Akimitsu Kazuya, Takabayashi Junji (2009) Role of hydroperoxide lyase in white-backed planthopper (*Sogatella furcifera* Horváth)-induced resistance to bacterial blight in rice, *Oryza sativa L.* The Plant Jounal 61:46–57

Gowda M, Barman SR, Chattoo BB (2006) Molecular mapping of a novel blast resistance gene *Pi38* in rice using SSR and AFLP markers. Plant Breeding 125:596–599

Haritha G, Malathi S, Divya B, Swamy BPM, Mangrauthia SK, Sarla N (2018) *Oryza nivara* Sharma et Shastry. In: Mondal T, Henry R (eds) The Wild *Oryza* Genomes. Compendium of Plant Genomes. Springer, Cham

Hayashi N, Inoue H, Kato T, Funao T, Shirota M, Shimizu T, Kanamori H, Yamane H, Hayano-Saito Y, Matsumoto T, Yano M, Takatsuki H (2010) 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 Journal 64:498–510

Hayashi N, Fukuta Y (2009) Proposal for a new international system of differentiating races of blast (*Pyricularia oryzae* Cavara) by using LTH monogenic lines in rice (*Oryza sativa* L.). In: JIRCAS Working Report No. 63. Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan, pp. 11–15

Hayashi N, Kobayashi N, Vera Cruz CM, Fukuta Y (2009) Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. JIRCAS Working Report No. 63. International Research Center for Agricultural Sciences, Tsukuba City, Ibaraki, Japan, 17–33

Hayashi N (2005) Rice blast fungus, MAFF Microorganism Genetic Resources Manual No. 18. National Institute of Agrobiological Sciences, Ibaraki, Japan

Huang CL, Huang SY, Hwang YC, Chiang T, Lin TP (2008) Molecular evolution of the Pi-ta gene resistant to rice blast in wild rice (*Oryza rufipogon*). Genetics 179:1527–1538

Inukai T, Nagashima S, Kato M (2019) Pid3-I1 is a race-specific partial-resistance allele at the Pid3 blast resistance locus in rice. Theoretical and Applied Genetics 132:395±404

Iyer AS, McCouch SR (2004) The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. Molecular Plant-Microbe Interaction 17:1348–1354

Jeung JU, Kim BR, Cho YC, Han SS, Moon HP, Lee YT, Jena KK (2007) A novel gene, Pi40(t), linked to the DNA markers derived from NBS-LRR motifs confers broad spectrum of blast resistance in rice. Theoretical and Applied Genetics 115:1163–1177

Jiang C, Shen QJ, Wang B, He B, Xiao S, Chen L, Yu T, Ke X, Zhong Q, Fu J, Chen Y (2017) Transcriptome analysis of WRKY gene family in *Oryza officinalis* Wall ex Watt and WRKY genes involved in responses to *Xanthomonas oryzae pv. oryzae* stress. PLoS One 12:e0188742

Jiang GH, Xia ZH, Zhou YL, Wan J, Li DY, Chen RS, Zhai WX, Zhu LH (2006) Testifying the rice bacterial blight resistance gene xa5 by genetic complementation and further analyzing xa5 (Xa5) in comparison with its homolog TFIIAgamma1. Molecular Genetics and Genomics 275:354–366

Kadeawi S, Swaruno NA, Hairmansis A, Telebanco-Yanoria MJ, Obara M, Hayashi N, Fukuta Y (2021) Pathogenicity of isolates of the rice blast pathogen (*Pyricularia oryzae*) from Indonesia. Plant Disease 105(3):675–683

Kader KA, Hunger RM, Payton ME (2021) Isolate virulence and cultivar response in the winter wheat: *Pyrenophora tritici-repentis* (tan spot) pathosystem in Oklahoma. Plant Pathology Journal 37(4):339–346

Kaladhar K, Swamy BPM, Babu AP, Reddy CS, Sarla N (2008) Mapping quantitative trait loci for yield traits in BC_2F_2 populationderived from Swarna x *O. nivara* cross. Rice Genetics Newsletter 24:34–36

Khan MAI, Latif MA, Khalequzzaman M, Tomita A, Ali MA, Fukuta Y (2017) Genetic variation in resistance to blast (*Pyricularia oryzae* Cavara) in rice (*Oryza sativa* L.) germplasms of Bangladesh. Breeding Science 67:493–499

Kiyosawa S (1984) Establishment of differential varieties for pathogenicity test of rice blast fungus. Rice Genetics Newsletter 1:95–97

Kobayashi N, Telebanco-Yanoria MJ, Tsunematsu H, Kato H, Imbe T, Fukuta Y (2007) Development of new sets of international standard differential varieties for blast resistance in rice (*Oryza sativa* L.). Japan Agricultural Research Quarterly 41:31–37

Koide Y, Ebron LA, Kato H, Tsunematsu H, Telebanco-Yanoria MJ, Kobayashi N, Yokoo M, Maruyama S, Imbe T, Fukuta Y (2011) A set of near-isogenic lines for blast resistance genes with an Indica-type rainfed lowland elite rice (*Oryza sativa* L.) genetic background. Field Crop Research 123:19–27

Koide Y, Kobayashi N, Xu D, Fukuta Y (2009) Resistance genes and selection DNA markers for blast disease in rice (*Oryza sativa* L.). Japan Agricultural Research Quarterly 43:255–280

Li WT, Chern MS, Yin JJ, Wang J, Chen XW (2019) Recent advances in broad-spectrum resistance to the rice blast disease. Current Opinion in Plant Biology 50:114±120

Li WT, Zhu ZW, Chern M, Yin JJ, Yang C, Ran L, Cheng MP, He M, Wang K, Wang J, Zhou XG, Zhu XB, Chen ZX, Zhao WC, Ma BT, Qin P, Chen WL, Wang YP, Liu JL, Wang WM, Wu XJ, Li P, Wang JR, Zhu LH, Li SG, Chen XW (2017) A natural allele of a transcription factor in rice confers broad- spectrum blast resistance. Cell 170:114±126

Li Z, Pinson S, Marchetti M, Stansel J, Park W (1995) Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (*Rhizoctonia solani*). Theoretical and Applied Genetics 91:382–388

Li YB, Wu CJ, Jiang GH, Wang LQ, He YQ (2007) Dynamic analyses of rice blast resistance for the assessment of genetic and environmental effects. Plant Breeding 126:541–547

Li Z, Pinson SR, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453–465

Li ZK, Yu SB, Lafitte HR, Huang N, Courtois B, Hittalmani S, Vijayakumar CH, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS (2003) QTL x environment interactions in rice. I. Heading date and plant height. Theoretical and Applied Genetics 108:141–153

Lin F, Chen S, Que Z, Wang L, Liu X, Pan Q (2007) The blast resistance gene Pi37 encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. Genetics 177:1871–1880

Liu B, Li JF, Ao Y, Qu J, Li Z, Su J, Zhang Y, Liu J, Feng D, Qi K, He Y, Wang J, Wang HB (2012) Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in Rice innate immunity. The Plant Cell 24:3406–3419

Liu B, Zhang S, Zhu X, Yang Q, Wu S, Mei M, Mauleon R, Leach J, Mew T, Leung H (2004) Candidate defense genes as predictors of quantitative blast resistance in rice. Molecular Plant-Microbe Interaction 17:1146–1152

Lopez-Gerena J (2006) Mapping QTL controlling durable resistance to rice blast in the cultivar Oryzica Llanos 5. Ph.D. thesis, Universidad del Valle, Plant Pathology College of Agriculture, Cali, Colombia and Kansas State University, Manhatten, KS, U.S.A.

Ma JF, Yamaji N, Mitani N, Xu XY, Su YH, McGrath S, Zhao FJ (2008) Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences 105:9931–9935

Mackay TFC (2001) The genetic architecture of quantitative traits. Annual Review of Genetics 35:303–339

Mackill DJ, Khush GS (2018) IR64: a high-quality and high-yielding mega variety. Rice 11:18

Mackill DJ (2018) Special Issue: Iconic Rice Varieties. Rice (N Y) 11:16

Mackill DJ, Bonman JM (1992) Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746–749

Mao BB, Cai WJ, Zhang ZH, Hu ZL, Li P, Zhu LH, Zhu YG (2003) Characterization of QTLs for harvest index and source-sink characters in a DH population of rice (*Oryza sativa* L.). Yi Chuan xue bao= Acta Genetica Sinica 30:1118–1126

Marri PR, Sarla N, Reddy LV, Siddiq EA (2005) Identification and mapping of yield and yield related QTLs from an Indian accession of *Oryza rufipogon*. BMC Genetics 6:33

McCouch SR, Abenes ML, Angeles R, Khush GS, Tanksley SD (1991) Molecular tagging of a recessive gene, xa-5, for resistance to bacterial blight of rice. Rice Genetics Newsletter 8:143–145

Mei C, Qi M, Sheng G, Yang Y (2006) Inducible overexpression of a rice allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression, and host resistance to fungal infection. Molecular Plant-Microbe Interaction 19:1127–1137

Meng L, Li H, Zhang L, Wang J (2015) QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. The Crop Journal 3:269–283

Miah M, Rafii MY, Ismail MR, Puteh AB, Rahim HA, Asfaliza R, Latif MA (2013) Blast resistance in rice: a review of conventional breeding to molecular approaches. Molecular Biology Reports 40:2369–2388

Milus EA, Kristensen K, Hovmøller MS (2009) Evidence for increased aggressiveness in a recent widespread strain of Puccinia striiformis f.sp. tritici causing stripe rust of wheat. Phytopathology 99:89–94

Miyata M, Yamamoto T, Komori T, Nitta N (2007) Marker-assisted selection and evaluation of the QTL for stigma exsertion under japonica rice genetic background. Theoretical and Applied Genetics 114:539–548

Mori M, Tomita C, Sugimoto K, Hasegawa M, Hayashi N, Dubouzet JG, Ochiai H, Sekimoto H, Hirochika H, Kikuchi S (2007) Isolation and molecular characterization of a spotted leaf 18 mutant by modified activation tagging in rice. Plant Molecular Biology 63:847–860

Mukherjee R, Gayen S, Chakraborty A, Bhattacharyya J, Maiti MK, Basu A, Sen SK (2012) Double-stranded RNA-mediated down-regulation of pdhk gene expression to shorten maturation time of a late maturing native indica Rice cultivar. Badshahbhog Crop Science 52:1743–1753

Nakashima A, Chen L, Thao NP, Fujiwara M, Wong HL, Kuwano M, Umemura K, Shirasu K, Kawasaki T, Shimamoto K (2008) RACK1 functions in rice innate immunity by interacting with the Rac1 immune complex. The Plant Cell 20:2265–2279

Nguyen TMN, Nguyet HL, Nguyen GN, Nguyen TN, Nguyen TT, Hayashi N, Fukuta Y (2020) Diversity and distribution of rice blast (*Pyriculariaoryzae* Cavara) races in Vietnam. Plant Disease 104:381–387

Nguyen TT, Koizumi S, La TN, Zenbayashi KS, Ashizawa T, Yasuda N, Imazaki I, Miyasaka A (2006) Pi35(t), a new gene conferring partial resistance to leaf blast in the rice cultivar Hokkai 188. Theoretical and Applied Genetics 113:697–704

Niks RE, Qi XQ, Marcel TC (2015) Quantitative resistance to biotrophic filamentous plant pathogens: Concepts, misconceptions, and mechanisms. Annual Reviews of Phytopathology 53:445±470

Ning X, Yunyu W, Aihong L (2020) Strategy for use of Rice blast resistance genes in Rice molecular breeding. Rice Science 27:263–277

Odjo T, Kawasaki-Tanaka A, Noda T, Ahohuendo BC, Sere Y, Kumashiro T, Yanagihara S, Fukuta Y (2014) Pathogenicity analysis of blast (*Pyricularia oryzae* Cavara) isolates from West Africa. Japan Agricultural Research Quarterly 48:403–412

Onishi K, Horiuchi Y, Ishigoh-Oka N, Takagi K, Ichikawa N, Maruoka M, Sano Y (2007) A QTL cluster for plant architecture and its ecological significance in Asian wild Rice. Breeding Science 57:7–16

Pawar DV, Mainkar P, Marathe A, Prajapat RK, Sharma TR, Singh NK (2019) Characterization and molecular modelling of Pi56 ortholog from *Oryza rufipogon*. International Journal of Current Microbiology Applied Sciences 8:790–798

Peng H, Zhang Z, Li Y, Lei C, Zhai Y, Sun X, Sun D, Sun Y, Lu T (2009) A putative leucine-rich repeat receptor kinase, OsBRR1, is involved in rice blast resistance. Planta 230:377–385

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin. Accessed 23 May 2022

Pilet-Nayel ML, Moury B, Caffier V, Montarry J, Kerlan MC, Fournet S, Durel CE, Delourme R (2017) Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Frontiers in Plant Science 8:1838

Pinson SR, Oard JH, Groth D, Miller R, Marchetti MA, Shank AR, Jia MH, Jia Y, Fjellstrom RG, Li Z (2008) Registration of TIL: 455, TIL: 514, and TIL: 642, three rice germplasm lines containing introgressed sheath blight resistance alleles. Journal of Plant Registrations 2:251–254

Pinson SRM, Capdevielle FM, Oard JH (2005) Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. Crop Science 45:503–510

Qiao W, Qi L, Cheng Z, Su L, Li J, Sun Y, Ren J, Zheng X, Yang Q (2016) Development and characterization of chromosome segment substitution lines derived from *Oryza rufipogon* in the genetic background of O. sativa spp. indica cultivar 9311. BMC Genomics 17:89

Qiu D, Xiao J, Xie W, Liu H, Li X, Xiong L, Wang S (2008) Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. Molecular Plant 1:538–551

Qiu S, Ma N, Che S, Wang Y, Peng X, Zhang G, Wang G, Huang J (2014) Repression of OsEXPA3 expression leads to root system growth suppression in Rice. Crop Science 54:2201–2213

Qu S, Liu G, Zhou B, Bellizzi M, Zeng L, Dai L, Han B, Wang GL (2006) The broad-spectrum blast resistance gene Pi9 encodes an NBS-LRR protein and is a member of a multigene family in rice. Genetics 172:1901–1914

Ramalingam J, Vera Cruz CM, Kukreja K, Chittoor JM, Wu JL, Lee SW, Baraoidan M, George ML, Cohen MB, Hulbert SH, Leach JE, Leung H (2003) Candidate defence genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. Molecular Plant-Microbe Interaction 16:14–24

Rekha G, Abhilash Kumar V, Viraktamath BC, Pranathi K, Kousik MBVN, Laxmi Prasanna B, Backiyalakshmi C, Sinha P, Ravindra RK, Bhaskar S, Hajira SK (2018) Improvement of blast resistance of the popular high-yielding, medium slender-grain type, bacterial blight resistant rice variety, improved samba mahsuri by marker-assisted breeding. Journal of Plant Biochemistry and Biotechnology 27:463–472

Rao YV, Raju AK, Malathi S, Sukumar M, Kavitha B, Divya B, Sarla N (2018a) Interspecific hybridization for the development of chromosome segment substitution lines of rice in India. Oryza 55:511–522

Rao YV, Divya B, Raju AK, Mesapogu S, Kiran TV, Subrahmanyam D, Voleti SR, Sarla N (2018b) Characterization of backcross introgression lines derived from *Oryza nivara* accessions for photosynthesis and yield. Physiology and Molecular Biology of Plants 24:1147–1164

Rao ZM, Wu JL, Zhuang JY, Chai RY, Fan YY, Leung H, Zheng KL (2005) Genetic dissections of partial resistances to leaf and neck blast in rice (*Oryza sativa* L.). Acta Genetica Sinica 32:555–565

Riemann M, Haga K, Shimizu T, Okada K, Ando S, Mochizuki S, Nishizawa Y, Yamanouchi U, Nick P, Yano M, Minami E, Takano M, Yamane H, MoritoshiIino (2013) Identification of rice Allene Oxide Cyclase mutants and the function of jasmonate for defence against *Magnaporthe oryzae*. The Plant Journal 74:226–238

Roumen EC (1992) Small differential interactions for partial resistance in rice cultivars to virulent isolates of the blast pathogen. Euphytica 64:143–148

Sabouri H, Sabouri A, Jafarzadeh MR, Mollashahi M (2011) Detection of QTLs controlling field blast resistance in rice (*Oryza sativa* L.). Plant Omics 4:1–5

Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN, Ray S (2012) Rice blast management through host-plant resistance: Retrospect and prospects. Agricultural Research 1:37–52

Shimono M, Koga H, Akagi AYA, Hayashi N, Goto S, Sawada M, Kurihara T, Matsushita A, Sugano S, Jiang CJ, Kaku H (2012) Rice WRKY45 plays important roles in fungal and bacterial disease resistance. Molecular Plant Pathology 13:83–94

Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. The Plant Cell 19:2064–2076

Skamnioti P, Gurr SJ (2009) Against the grain: safeguarding rice from rice blast disease. Trends in Biotechnology 27:141–150

Spielmeyer W, Ellis MH, Chandler PM (2002) Semidwarf (sd-1), 'green revolution' rice, contains a defective gibberellin 20-oxidase gene. Proceedings of the National Academy of Sciences USA 99:9043–9048

Suh JP, Ahn SN, Moon HP, Suh HS (1999) QTL analysis of low temperature germinability in a Korean weedy rice (*Oryza sativa* L.). Rice Genetics Newsletter 16:53–55

Suharsono U, Fujisawa Y, Kawasaki T, Iwasaki Y, Satoh H, Shimamoto K (2002) The heterotrimeric G protein alpha subunit acts upstream of the small GTPase Rac in disease resistance of rice. Proceedings of the National Academy of Sciences 99:13307–13312

Surapaneni M, Balakrishnan D, Mesapogu S, Addanki KR, Yadavalli VR, Tripura Venkada VGN, Neelamraju S (2017) Identification of major effect QTLs for agronomic traits and CSSLs in rice from swarna/*oryza nivara* derived backcross inbred lines. Frontiers in Plant Science 8:1027

Swamy BPM, Kaladhar K, Reddy AG, Viraktamath BC, Sarla N (2014) Mapping and introgression of QTL for yield and related traits in two backcross populations derived from *Oryza sativa* cv. Swarna and two accessions of *O. nivara*. Journal of Genetics 93:643–653

Talukder ZI, Tharreau D, Price AH (2004) Quantitative trait loci analysis suggests that partial resistance to rice blast is mostly determined by race specific interactions. New Phytologist 162:197–209

Tao Z, Kou Y, Liu H, Li X, Xiao J, Wang S (2011) OsWRKY45 alleles play different roles in abscisic acid signalling and salt stress tolerance but similar roles in drought and cold tolerance in rice. Journal of Experimental Botany 62:4863–4874

Telebanco-Yanoria MJ, Imbe T, Kato H, Tsunematsu H, Ebron LA, Vera Cruz CMV, Kobayashi N, Fukuta Y (2008) A set of standard differential blast isolates (*Magnaporthe grisea* (Hebert) Barr.) from the Philippines for rice (*Oryza sativa* L.) resistance. Japan Agricultural Research Quarterly 42:23–34

Telebanco-Yanoria MJ, Koide Y, Fukuta Y, Imbe T, Kato H, Tsunematsu H, Kobayashi N (2010) Development of near-isogenic lines of Japonica-type rice variety Lijiangxintuanheigu as differentials for blast resistance. Breeding Science 60:629–638

Thao NP, Chen L, Nakashima A, Hara S, Umemura K, Takahashi A, Shirasu K, Kawasaki T, Shimamoto K (2007) RA and HSP90 form a complex with Rac/Rop GTPase and function in innate immune responses in rice. The Plant Cell 19:4035–4404

Tharreau D, Fudal I, Andriantsimialona D, Santoso L, Utami DW, Fournier E, Lebrun MH, Nottéghem JL (2009) World population structure and migration of the rice blast fungus, *Magnaporthe oryzae*. In: Wang G-L, Valent B (eds) Advances in Genetics, Genomics and Control of Rice Blast Disease. Springer-Verlag, New York, pp 209–215

Tian F, Li DJ, Fu Q, Zhu ZF, Fu YC, Wang XK, Sun CQ (2006) Construction of introgression lines carrying wild rice (*Oryza rufipogon* Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. Theoretical and Applied Genetics 112:570–580

Tsunematsu H, Yanoria MJT, Ebron LA, Hayashi N, Ando I, Kato H, Imbe T, Khush GS (2000) Development of monogenic lines of rice for blast resistance. Breeding Science 50:229–234

Umemura K, Satou J, Iwata M, Uozumi N, Koga J, Kawano T, Koshiba T, Anzai H, Mitomi M (2009) Contribution of salicylic acid glucosyltransferase, OSSGT1, to chemically induced disease resistance in rice plants. The Plant Journal 57:463–472

Utami DW, Moeljopawiro S, Hanarida I, Tharreau D (2008) Fine mapping of rice blast QTL from *Oryza rufipogon* and IR64 by SNP markers. SABRAO Journal of Breeding and Genetics 40:105–115

Vasudevan K, Casiana M, Vera C, Gruissem W, Navreet KB (2014) Large scale germplasm screening for identification of novel rice blast resistance sources. Frontiers in Plant Science 505:1–9

Wan XY, Wan JM, Weng JF, Jiang L, Bi JC, Wang CM, Zhai HQ (2005) Stability of QTLs for rice grain dimension and endosperm chalkiness characteristics across eight environments. Theoretical and Applied Genetics 110:1334–1346

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete

and partial resistance to blast in a durably resistant rice cultivar. Genetics 136:1421±1434
Wang X, Jia MH, Ghai P, Lee FN, Jia Y (2015) Genome-wide association of rice blast disease resistance and yieldrelated components of rice. Molecular Plant-Microbe Interactions 28:1383–1392
Wang X, Jin L, Zhu H, Wang S, Zhang G, Liu G (2018) QTL epistatic analysis for yield components with single-segment substitution lines in rice. Plant Breeding 137:346–354
Wang Z, Cheng J, Chen Z, Huang J, Bao Y, Wang J, Zhang H (2012) Identification of QTLs with main, epistatic and QTL environment interaction effects for salt tolerance in rice seedlings under different salinity conditions. Theoretical and Applied Genetics 125:807–815
Wang Z, Yamanouchi U, Katayose Y, Sasaki T, Yano M (2001) Expression of the Pib rice-blast-resistance gene family is up-regulated by environmental conditions favouring infection and by chemical signals that trigger secondary plant defences. Plant Molecular Biology 47:653–661
Ward JH (1963) Hierarchical grouping to optimize an objective function. Journal of the American Statistical Association 58:236–244
Wen N, Chu Z, Wang S (2003) Three types of defence-responsive genes are involved in resistance to bacterial blight and fungal blast diseases in rice. Molecular Genetics and Genomics 269:331–339
Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. Genetics 169:2277–2293
Wong HL, Sakamoto T, Kawasaki T, Umemura K, Shimamoto K (2004) Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. Plant Physiology 135:1447–1456
Wu Y, Xiao N, Yu L, Pan C, Li Y, Zhang X, Liu G, Dai Z, Pan X, Li A (2015) Combination patterns of major R genes determine the level of resistance to the *M. oryzae* in rice (*Oryza sativa* L.). PLoS One 10:e0126130
Wu JL, Fan YY, Li DB, Zheng KL, Leung H, Zhuang JY (2005) Genetic control of rice blast resistance in the durably resistant cultivar gumei 2 against multiple isolates. Theoretical and Applied Genetics 111:50–56
Xangsayasane P, Boualaphanh C, Bounphanousay C, Bounphanousay V, Manivong P, Voradeth S, Inthapanya P, Sato T, Obara M, Hayashi N, Fukuta Y (2020) Genetic variation of rice blast (*Pyricularia oryzae*) isolates in Laos. Plant Health Progress 21:248–255
Xie XZ, Xue YJ, Zhou JJ, Zhang B, Chang H, Takano M (2011) Phytochromes regulate SA and JA signaling pathways in rice and are required for developmentally controlled resistance to *Magnaporthe grisea*. Molecular Plant 4:688–696
Xin Z, Wang Q, Yu Z, Hu L, Li J, Xiang C, Wang B, Lou Y (2014) Overexpression of a xylanase inhibitor gene, OsHI-XIP, enhances resistance in rice to herbivores. Plant Molecular Biology Reports 32:465–475
Xiong L, Yang Y (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. The Plant Cell 15:745–759
Xu X, Hayashi N, Wang CT, Fukuoka S, Kawasaki S, Takatsuji H, Jiang CJ (2014) 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. Molecular Breeding 34:691–700
Yadav M, Aravindan S, Ngangkham U, Raghu S, Prabhukarthikeyan S, Keerthana U, Marndi B, Adak T, Munda S, Deshmukh R, Pramesh D, Samantaray S, Rath P (2019) Blast resistance in Indian rice landraces: genetic dissection by gene specific markers. PLoS ONE 14:e0213566
Yamada M, Kiyosawa S, Yamaguchi T, Hirano T, Kobayashi T, Kushibuchi K, Watanabe S (1976) Proposal of a new method for differentiating races of Pyricularia oryzae Cavara in Japan. Annual Phytopathological Society of Japan 42:216–219
Yamaguchi K, Imai K, Akamatsu A, Mihashi M, Hayashi N, Shimamoto K, Kawasaki T (2012) SWAP70 functions as a Rac/Rop guanine nucleotide-exchange factor in rice. The Plant Journal 70:389–397
Yara A, Yaeno T, Hasegawa M, Seto H, Montillet JL, Kusumi K, Seo S, Iba K (2007) Disease resistance against *Magnaporthe grisea* is enhanced in transgenic rice with suppression of omega-3 fatty acid desaturases. Plant and Cell Physiology 48:1263–1274
Yokotani N, Tsuchida-Mayama T, Ichikawa H, Mitsuda N, Ohme-Takagi M, Kaku H, Minami E, Nishizawa Y (2014) OsNAC111, a blast disease–responsive transcription factor in rice, positively regulates the expression of defense-related genes. Molecular Plant-Microbe Interactions 27:1027–1034
Zarbafi SS, Ham JH (2019) An overview of rice QTLs associated with disease resistance to three major rice diseases: blast, sheath blight, and bacterial panicle blight. Agronomy (Basel) 9:177
Zhang J, Peng Y, Guo Z (2008) Constitutive expression of pathogen-inducible OsWRKY31 enhances disease resistance and affects root growth and auxin response in transgenic rice plants. Cell Research 18:508–521
Zhang Y, Yang J, Shan Z, Chen S, Qiao W, Zhu X, Xie Q, Zhu H, Zhang Z, Zeng R, Ding X, Zhang G (2012) Substitution mapping of QTLs for blast resistance with SSSLs in rice (*Oryza sativa* L.). Euphytica 184:141–150
Zhang Y, Zhao J, Li Y, Yuan Z, He H, Yang H, Qu H, Ma C, Qu S (2016) Transcriptome analysis highlights defense and signaling pathways mediated by rice pi21 gene with partial resistance to *Magnaporthe oryzae*. Frontiers in Plant Science 7:1834
Zhou XG, Liao HC, Chern M, Yin JJ, Chen YF, Wang JP, Zhu XB, Chen ZX, Yuan C, Zhao W, Li WT, He M, Ma BT, Wang JC, Qin P, Chen WL, Wang YP, Liu JL, Qian YW, Wang WM, Wu XJ, Li P, Zhu LH, Li SG, Ronald PC, Chen XW (2018) Loss of function of a rice TPR-domain RNA-binding protein confers broad-spectrum disease resistance. Proceedings of National Academy of Sciences 115:3174±3179
Zhu M, Wang L, Pan Q (2004) Identification and characterization of a new blast resistance gene located on rice chromosome 1 through linkage and differential analyses. Phytopathology 94:515–519