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GENE PYRAMIDING THROUGH INTERCROSS POPULATIONS FOR BACTERIAL BLIGHT AND BROWN PLANTHOPPER RESISTANCE IN RICE

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SUMMARY

Conventional breeding approaches often rely on bi-parental crosses, in which analysis occurs only on two alleles, and genetic recombination in such a population is insufficient, limiting genetic diversity. Multi-parent advanced generation inter-cross (MAGIC) populations display large sets of recombinant inbred lines (RILs) that exhibit a genetic mosaic of multiple founder parents. MAGIC populations result in a broader genetic base that has emerged as a powerful tool for genetic analysis and breeding for disease resistance in rice. This study developed the MAGIC population by intermating eight diverse founder parents. Conducting the molecular survey sought to analyze the genes resistant to bacterial blight (BB) and brown planthopper (BPH). The research used primers specific for *Xa*21, *xa*13, *Xa4,* and *Bph*1 in the polymerase chain reaction (PCR). The survey identified combinations of three genes: (*Xa*4+*xa*13+*Xa*21) in BR52 and (*Xa*4+*xa*13+*Bph*1) in BR43, BR44, BR48, and BR58. Two-gene combination (*xa*13 and *Xa*4) materialized in BR53, BR54, BR60, BR73, BR85, and BR86, while identifying a combination of *xa*13 and *Bph*1 in BR11, BR41, and BR99. *Xa*4 was present in 14, *xa*13 in six, and *Bph*1 in three lines. Screening the population against *Xanthomonas oryzae* validated the presence of BB-resistant genes. The consistent finding is that the RILs with a combination of two or more genes express a high level of resistance compared with the RILs with a single gene. The RILs with *Xa*4 and *xa*13 expressed more resistance than the lines with *xa*13 and *Bph*1. Employing the MAGIC populations approach remains to be effective for gene pyramiding.

Keywords: rice (*O. sativa* L.), MAGIC population, bacterial blight, brown planthopper

Key findings: Developing MAGIC populations succeeded in pyramiding multiple resistance genes for bacterial blight and BPH in rice (*O. sativa* L.). A combination of two and three genes pyramided in RILs helped enhance resistance against diseases, leading to durable resistant lines.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most valuable food crops in the world, providing calories to half of the global population (Saud *et al*., 2022). The investment in plant breeding led to the Green Revolution in 1960 by significantly increasing the production of rice, wheat, and maize. The rising population pressure demands to boost yield by 60%–110% by 2050, compared with the present level. Climate change poses tremendous challenges to achieving the objective and reduces yield or causes yield stagnation in many countries (Ray *et al.,* 2019). Pakistan ranks as the 10th largest producer of rice worldwide, producing around 7.5 million tons in 2020 (Gul *et al.,* 2022). Rice is an essential staple food in Pakistan, with an annual rice consumption of around 3.7 million tons and exporting 3.9 million tons. Insect pests and diseases are the major threats to rice production. Biotic stress rendered annual world losses of more than 40% (Noreen *et al*., 2020). Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the prime threats to world rice, reducing the yield by 30% to 50%. The disease incidence occurs by bacteria invasion through wounds or the vascular system. The disease affects the photosynthetic area of rice plants, drastically reducing the grain yield.

About 40 BB resistance genes have become identifiable using different sources (Pradhan *et al*., 2020). Among them, 28 BB resistance genes are dominant, while 17 are recessive. Closely linked markers have tagged and mapped some of these genes on chromosomes, with their resistance pattern determined.

There is continuous evolution in pathogens, developing new strains that break resistance. Researchers in the BB-resistance breeding program have frequently utilized the *Xa*4, *xa*5, *Xa*7, *xa*13, and *Xa*21. Besides this, the brown planthopper (*Nilaparvata lugens*, BPH) is the foremost threat to rice production. BPH damages the plant directly by sucking it and indirectly as a vector for transmitting viral diseases like the ragged stunt virus and the grassy stunt virus, which inhibits panicle production (Chang *et al.,* 2021). Chemical insecticides are the chief source for controlling BPH; however, continuous use of chemicals increases pollution, disruption of field ecological balance, and high production costs, with insects developing chemical resistance (Wu *et al*., 2020). So far, 17 BPH resistance genes attained rice cloning, of which eight BPH resistance genes (*Bph1*, *2*, *7*, *9*, *10*, *18*, *21*, and *26*) are the multiple alleles of a common locus. Hence, the best way for their management is the development of host plant resistance.

Increasing durable resistance is achievable by incorporating multiple resistance genes in a single variety. Conventional breeding approaches often rely on bi-parental crosses, which can limit the genetic diversity of quantitative traits. In a bi-parental population, genetic recombination would be less due to the involvement of two alleles at a locus. MAGIC populations are a newly emerging powerful plant-breeding tool for pyramiding multiple resistance genes in crops. Arabidopsis was the first crop to have the MAGIC population developed, with a discussion on the gene-mapping method. Using 19 genotypes in Arabidopsis developed MAGIC populations. Bandillo *et al*. (2013) developed the *Indica* rice MAGIC population using eight parents, then phenotyping for multiple traits like resistance to blast and bacterial blight, submergence and salinity tolerance, and grain quality. Abbasi *et al.* (2022) reported the improvement of sink size and source capacity in rice by developing MAGIC populations involving eight parent cross. MAGIC populations have resulted in various crops, including tomato, faba bean, barley, maize, sorghum, wheat, oat, and cowpea (Novakazi *et al.,* 2020).

The MAGIC strategy became a proposal to increase recombination and introgress multiple alleles in the crop. It increases the intercrossing and shuffling of the genome. MAGIC populations undergo several cycles of recombination, generating a dense network of recombination events across the genome. Increasing recombination rate enhances the resolution of QTL mapping, enabling precise localization of resistance genes and the identification of smaller genetic intervals

associated with disease resistance (Scott *et al*., 2020). The expanded genetic diversity allows for identifying a broader range of resistance alleles and allelic combinations, including those with novel or complementary effects.

MATERIALS AND METHODS

MAGIC population development

Three rice varieties (IR6, Basmati385, JP5) became choices based on their extensive adaptability, good grain quality, and cultivation in Pakistan. Choosing three landraces: Mushkan (Acc367) from Punjab, Beyan (Acc3394) from Chitral, and Sugdasi (Acc3078) from Sindh transpired based on plant type (Iqbal *et al.*, 2001) and two introgression lines derived from *O. rufipogon* and *O. longistminata* (Abbasi *et al*., 2010a, b). Thus, eight parents inter-mating led to the development of MAGIC populations with a few modifications (Abbasi *et al*., 2022). Initially, the originating parents attained pair-wise inter-mating to develop two-way hybrids (AB, CD, EF, and GH). These continued inter-mating to yield two four-way hybrids of ABCD and EFGH. Subsequently, the two four-way hybrids underwent inter-breeding to develop a bulk of the genome: ABCDEFGH. Selfing and selection ensued from generation four to generation nine.

DNA extraction and PCR amplification

DNA extraction came from 55 RILs of the MAGIC population, IR24, IRBB59, IRBB4, and eight parental lines using the CTAB method for PCR amplification. An amplification reaction volume of 25 µl contained 1.0 µM each of the forward and reverse primer, 50 ng template DNA, 100 µM each of dATP, dCTP, dGTP, and dTTP, 2.5 mM $MgCl_2$, 0.2 unit of Taq DNA polymerase, and 1X Taq polymerase buffer. Amplification of DNA continued in a thermal cycle programmed as initial denaturation for 5 min at 94 °C, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and 72 °C for 2 min (extension). One additional cycle of 10 min at 72 °C happened for the final extension. Electrophoresis began to resolve the amplified product using 2.5% agarose gel, stained with ethidium bromide (10 µl/ml), observed under UV light and scored for the presence and absence of amplified DNA fragments.

Isolation and multiplication of *Xoo*

Collecting rice leaf samples with BB disease symptoms came from different fields of the District Mansehra. A pathogenicity test for all bacterial isolates occurred to get their pathogenic nature using the injection infiltrations technique (Akhtar *et al*., 2011).

Inoculation of RILs of MAGIC population

The bacterial colonies suspension in culture plates emerged by pouring 5 ml of distilled water. The suspension incurred mixing in a plastic container and stirred for uniformity. Water sprayed on plants created dampness to have a favorable condition for disease development. Inoculation transpired by cutting the leaves 5 cm below the tip after dipping the scissor in suspension. The lesion length (LL) recording occurred 14 days post-inoculation. Based on LL, the RILs were markedly resistant with LL from 0 to 5cm, moderately resistant (5.1 to 10 cm), moderately susceptible (10.1 to 15 cm), and susceptible (>15 cm), following the standard method of the International Rice Research Institute.

Data analysis

Amplified fragments in RILs by PCR attained comparing with those of check varieties/lines for the presence and absence of genes. The results of lesion length gained expression as the mean, with the standard error calculated using Statistix 8.1.

RESULTS

The development of multi-parent advanced generation inter-cross (MAGIC) populations resulted from inter-mating eight diverse founder parents, including wide-cross derivatives, widely cultivated basmati and non-

basmati varieties, *japonica* or *indica* background, and landraces. Selecting the founder's parents depended on grain quality or resistance to bacterial blight. Development of the MAGIC population genotypes totaled 400. Fifty-five recombinant inbred lines reached selection based on plant type, grain quality, yield, and yield-attributing traits for exploring the biotic stress resistance status of the population. The molecular survey continued to examine the RILs and founder parental lines for the bacterial blight (*Xa*4, *xa*13, *Xa*21) and brown planthopper resistance genes (*Bph1*). The presence of these genes proved valid by screening RILs against *Xoo.*

The use of dominant sequence tagged site (STS) marker Npb181 with primer sequences F: 5' ATCGATCGATCTTCACGAGG 3' and R: 5'TGCTATAAAAGGCATTCGGG3' helped detect the presence of *Xa*4 in MAGIC population. Ma *et al.* (1999) developed the STS marker for *Xa*4. The marker generated two distinct sizes of bands in resistant and susceptible recombinant inbred lines. The banding pattern of all the genotypes was either just like IRBB4 (having *Xa*4 gene) or like IR24 (without *Xa*4) gene (Figure 1A). The band corresponding to IR24 is 120 bp in size, while the band corresponding to IRBB4 is 150 bp. During this polymorphic survey, out of 66 rice genotypes, 29 lines, including IRBB4, occurred with the *Xa*4 gene, while 37 genotypes were absent of *Xa*4. Of the eight founder parents, Basmati385, Mushkan, and IL1 (introgression line) derived from *O. rufipogon* exhibited the *Xa*4 gene, indicating that these parental lines could be the source of the *Xa*4 gene, which had transmission to different RILs in MAGIC populations. Fourteen RILs carry the *Xa*4 gene. Six lines showed a combination of *Xa*4 with

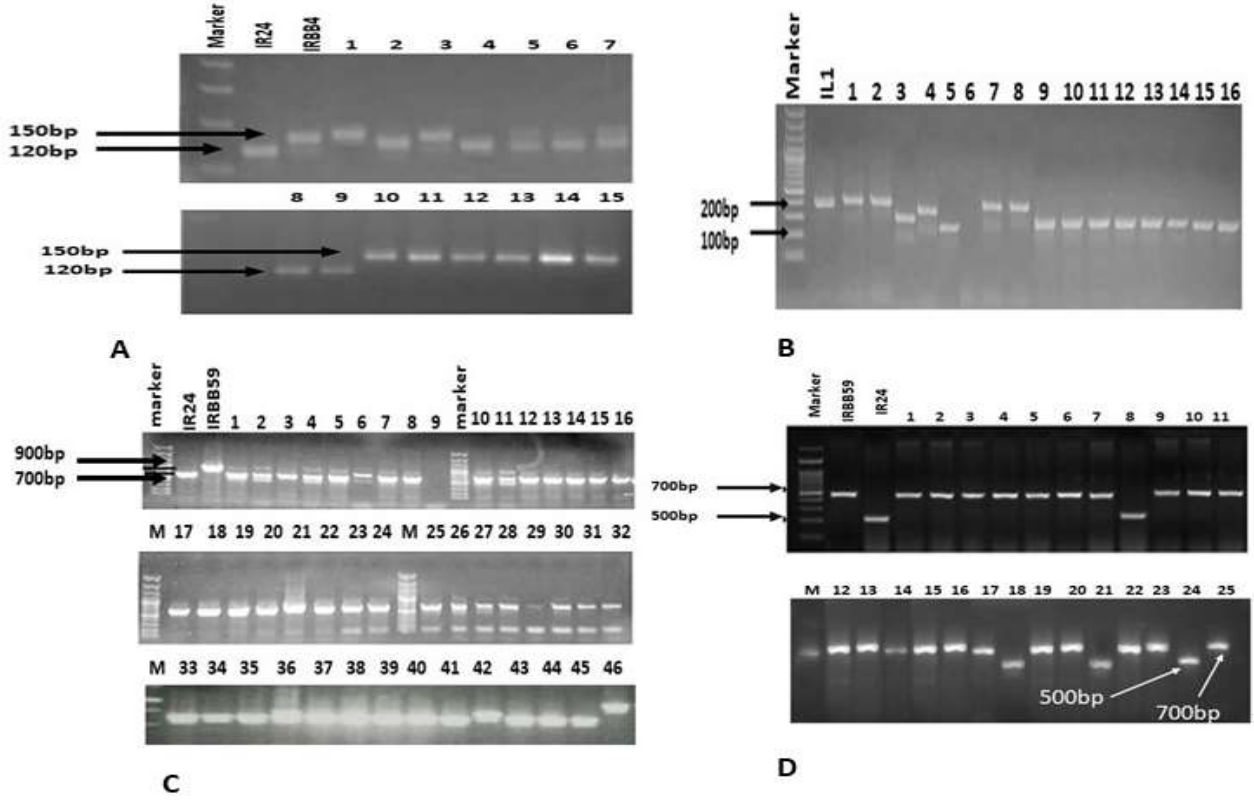

Figure 1 (A-D). Molecular determination of BB and BPH resistance genes in MAGIC population. (A) amplified fragment of 150bp in RILs correspond to the presence of *Xa*4; (B) 200bp fragment detected in RILs representing the presence of *Bph*1; (C) amplification of 900bp fragment in RIls correspond to the presence of *Xa*21; (D) determination of *xa*13 in RILs showing amplification of 700bp fragment.

*xa*13, and four lines exhibited a combination of *Xa*4 with *Bph1* and *xa*13. However, BR52 carries *Xa*4 in combination with *xa*13 and *Xa*21. Three lines' expression (BR43, BR44, and BR48) was heterozygous for *Xa*4.

BPH is one of the principal threats to rice crops in Asian countries by transmitting viral disease or sucking the rice leaves. Resistance varieties are the most effective and environment-friendly approach for reducing yield losses due to this pest. The presented investigation was able to detect the *Bph*1 gene using a closely linked marker RM1103 with primer sequences F: 5'CAGCTGCTGCTACTA CACCG3' and R: 5'CTACTCCACGTCCAT GCATG3'. Park *et al.* (2008) previously reported the marker RM1103. The primers amplified 100 bp and 200 bp fragments in different RILs, indicating the presence and absence of *Bph*1-resistant or susceptible lines. The RILs with amplified fragments of 200 bp seemed to have the *Bph*1 gene, and those lines with amplified fragments of 100 bp lacked this gene (Figure 1B). Out of 55 RILs, and eight founder parents, 11 genotypes (BR11, BR41, BR43, BR44, BR48, BR58, BR99, BR145, BR159, BR168, and IL1) exhibited the *Bph*1 gene, and the rest lacked this gene (Figure 1B). Out of 64 genotypes, a combination of *BPh1* with *BB* genes of *Xa*4 and *xa*13 was notable in BR58, BR48, BR44, and BR43, while a combination of *Bph*1 with a recessive BB gene (*xa*13) was evident in BR99, BR11, and BR41. From the eight parents used in population development, an introgression line (IL1) showed the presence of *Bph*1. IL1 might be the source of *Bph*1 recombining with *Xa*4 and *xa*13 in the population.

The presence of *Bph*1 in IL1 indicates that it might have come from *O.rufipogon*. The research initially developed an introgression line (IL2) by incorporating *Xa*21 from *O. longistaminata* (Abbasi *et al*., 2010 a) and used it as one of the parents in developing the MAGIC population. The IRBB59 and IL2 served as resistance control and IR24 as susceptible check. Previously designing the STS primer pair used (Chunwongse *et al*., 1993) from closely linked marker (pTA248) with sequences F: 5'AGACGCGGAAGGGTGGTTCCCGGA3' and 5'AGACCGGTAATCGAAAGATGAAA3' detected the *Xa*21. During this molecular survey, the banding pattern was either similar to IRBB59 and IL2 with the *Xa*21 gene or similar to IR24 lacking this gene. The size of the fragment amplified in IRBB59 was 9000 bp; however, the band size corresponding to IR24 was 700 bp (Figure 1C). During this determination of the gene, out of 66 genotypes, BR52, IL2, and IRBB59 exhibited a 9000 bp fragment with *Xa*21, while the rest of the lines or varieties showed the absence of this gene.

For *xa*13, the previously designed STS primer used in this experiment came from the closely linked marker RG136 (Zhang *et al*., 1996), using sequences F: 5'GCAGACTCCAGTTTGACTTC3' and R: 5'TCCCAGAAAGCTACTACAGC3'. The amplified product generated by this primer did not show any polymorphism. Hence, the product's digestion with *Hinf1* yielded two fragments of 500 and 700 bp. The size of the band amplified in IRBB59 was 700 bp, whereas the band size corresponding to IR24 was 500 bp (Figure 1D). During the investigation, out of 55 RILs, IR24, IRBB59, and eight founder parental lines, 22 samples (34%) exhibited the presence of *xa*13, and the rest lacked this gene. The fragment size corresponding to the fragment of IRBB59 became the line with the *xa*13 gene, and the line exhibiting fragment size similar to IR24 indicated lacking this gene. Of the eight parental lines, the Sugdasi showed the presence of *xa*13. Sugdasi is the source of *xa*13 that might have diffused to the RILs of the MAGIC population (Table 1).

All the selected RILs sustained inoculation with bacterial blight isolates. Recording the lesion length (LL) ensued 14 days post-inoculation. The LL ranged from 1.8 to 19.3 cm. A founder parent (JP5), which is a widely cultivated variety of Khyber Pakhtunkhwa with a lesion length of 19.3 cm, exhibited a susceptible reaction 14 days after injection, and lesions covered the entire inoculated leaf, leading to plant death 24 days after the inocuation. The parental introgression line (IL2) showed a high level of resistance to all the isolates of bacterial blight, with a mean lesion length of 1.9 cm. The RILs carry one to three resistance genes in various combinations.

Table 1. Presence (+) and absence (-) of BB and BPH resistant genes in RILs of rice.

No.	Name of lines	*Xa4*	*Xa13*	*Xa21*	*Bph1*	No	Name of lines	*Xa4*	*Xa13*	*Xa21*	*Bph1*
1	BR-1	+	-	-	-	34	BR-86	+	+	-	-
2	BR-2	+	-	-	-	35	BR-91	-	-	-	-
3	BR-3	-	+	-	-	36	BR-94	-	-	-	-
4	BR-10	-	+	-	-	37	BR-96	+	-	-	-
5	BR-11	-	+	-	+	38	BR-99	-	+	-	+
6	BR-12	-	+	-	-	39	BR-104	-	-	-	-
7	BR-13	+	-	-	-	40	BR-105	-	+	-	-
8	BR-16	+	-	-	-	41	BR-117	-	-	-	-
9	BR-19	+	-	-	-	42	BR-126	-	-	-	-
10	BR-20	+	-	-	-	43	BR-136	-	-	-	-
11	BR-21	+	-	-	-	44	BR-143	-	-	-	-
12	BR-39	-	+	-	-	45	BR-145	-	-	-	+
13	BR-40	-	+	-	-	46	BR-149	-	-	-	-
14	BR-41	-	+	-	+	47	BR-150	-	-	-	-
15	BR-43	+/-	+	-	+	48	BR-151	-	-	-	-
16	BR-44	+/-	+	-	+	49	BR-154	-	-	-	-
17	BR-48	+/-	+	-	+	50	BR-157	-	-	-	-
18	BR-52	+	+	+	-	51	BR-159	-	-	-	+
19	BR-53	+	+	-	-	52	BR-160	-	-	-	-
20	BR-54	+	+	-	-	53	BR-162	-	-	-	-
21	BR-58	+	+	-	+	54	BR-164	-	-	-	-
22	BR-60	+	+	-	-	55	IR168	-	-	-	+
23	BR-66	-	-	-	-	56	Bas-385	+	-	-	-
24	BR-69	+	-	-	-	57	IR-6	-	-	-	-
25	BR-70	+	-	-	-	58	JP-5	-	-	-	-
26	BR-71	+	-	-	-	59	Mushkan	+	-	-	-
27	BR-72	+	-	-	-	60	Sugdasi	-	+	-	-
28	BR-73	+	+	-	-	61	Beyan	-	-	-	-
29	BR-74	+	-	-	-	62	IL1	+	-	-	+
30	BR-76	-	-	-	-	63	IL2	-	-	+	-
31	BR-77	-	-	-	-	64	IR24	-	-	-	-
32	BR-81	+	-	-	-	65	IRBB4	+	-	-	-
33	BR-85	+	+	-	-	66	IRBB59	-	+	+	-

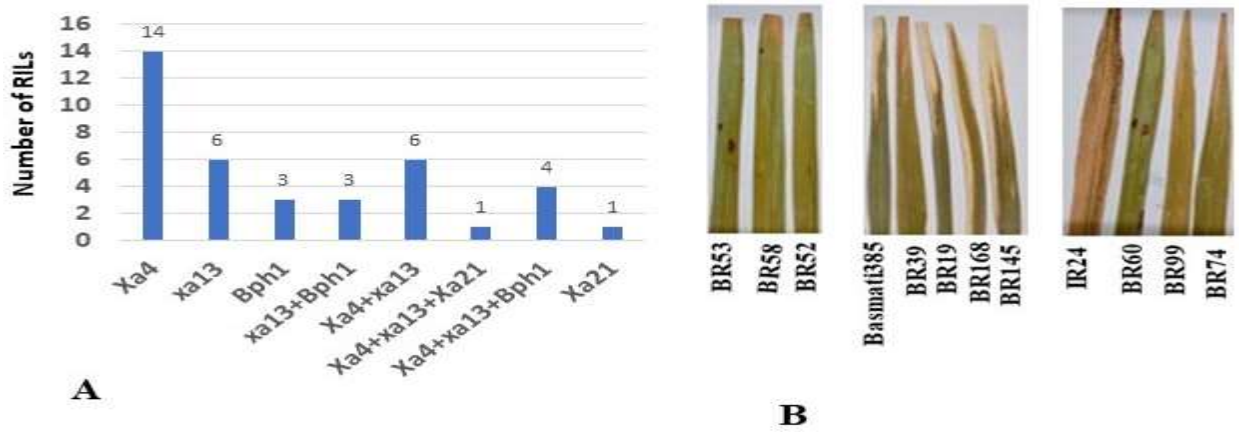

Figure 2 (A-B). Distribution of BB and BPH resistance genes among RILS of MAGIC population and reaction against BB isolates. (A) Combination of resistance genes in RILs. (B) Reaction against *Xoo:* BR52, BR53, BR58, and BR60 express resistant reaction; Basmati385, BR39, BR19, BR74, and BR99 are moderately resistant; BR145 and BR168 are moderately susceptible, and IR24 is susceptible.

Table 2. Resistance reaction of IRLs of MAGIC population of rice against *Xoo* isolates.

No.	Lines	Mean	Resistance reaction	No	Mean	Lines	Resistance reaction
1	BR-1	6.76 ± 0.39	MR	34	BR-86	4.33 ± 0.33	R
2	BR-2	6.83 ± 0.60	MR	35	BR-91	16.83 ± 0.44	S
3	BR3	8.2 ± 0.37	MR	36	BR-94	15.83 ± 0.44	S
4	BR10	8.5 ± 0.29	MR	37	BR-96	6.66 ± 0.66	MR
5	BR-11	8.66 ± 0.88	MR	38	BR99	8.05 ± 0.34	MR
6	BR12	8.5 ± 0.25	MR	39	BR-104	14.83 ± 0.44	MS
7	BR-13	6.66 ± 0.33	MR	40	BR-105	8.66 ± 0.33	MR
8	BR-16	7.96 ± 0.57	MR	41	BR-117	14.33 ± 0.88	MS
9	BR-19	6.66 ± 0.33	MR	42	BR126	16.4 ± 0.34	S
10	BR-20	6.86 ± 0.46	MR	43	BR136	17.7 ± 0.64	S
11	BR-21	7.33 ± 0.33	MR	44	BR143	14.1 ± 0.64	MS
12	BR-39	7.93 ± 0.63	MR	45	BR145	12.7 ± 0.43	MS
13	BR-40	8.8 ± 0.41	MR	46	BR149	15.9 ± 0.05	S
14	BR-41	8.5 ± 0.76	MS	47	BR150	15.2 ± 0.11	S
15	BR-43	4.56 ± 0.47	R	48	BR151	13.3 ± 0.37	MS
16	BR-44	4.8 ± 0.15	R	49	BR154	12.7 ± 0.37	MS
17	BR-48	4.43 ± 0.29	R	50	BR157	16.5 ± 0.53	S
18	BR-52	1.8 ± 0.35	R	51	BR159	13.03 ± 0.08	MS
19	BR-53	3.83 ± 0.60	R	52	BR160	15.5 ± 0.50	S
20	BR-54	4.5 ± 0.28	R	53	BR162	13.1 ± 0.58	MS
21	BR-58	4.6 ± 0.37	R	54	BR164	13.3 ± 0.33	MS
22	BR-60	4.33 ± 0.33	R	55	BR168	12.5 ± 0.28	MS
23	BR-66	16.56 ± 0.99	S	56	Bas-385	8.33 ± 0.33	MR
24	BR-69	6.83 ± 0.44	MR	57	IR-6	12.33 ± 0.33	MS
25	BR-70	8.43 ± 0.29	MR	58	JP-5	19.3 ± 0.56	S
26	BR-71	8.33 ± 0.66	MR	59	Mushkan	6.33 ± 0.33	MR
27	BR-72	7.66 ± 0.33	MR	60	Sugdasi	8.66 ± 0.33	MR
28	BR-73	4.33 ± 0.33	R	61	Beyan	13.33 ± 0.40	MS
29	BR-74	7.66 ± 0.33	MR	62	IL1	4.8 ± 0.15	R
30	BR76	12.9 ± 0.05	MS	63	IL2	2.3 ± 0.15	R
31	BR77	16.2 ± 1.01	S	64	IR24	19.2 ± 0.9	S
32	BR-81	7.83 ± 0.44	MR	65	IRBB4	4.9 ± 0.70	R
33	BR-85	4.66 ± 0.33	R	66	IRBB59	1.9 ± 0.6	R

The lines carrying the *Xa*4 gene have lesion lengths from 6.66 to 8.43 cm and exhibited moderate resistance. However, the lines without the *Xa*4 gene showed moderate susceptibility to susceptible reactions. The RILs carrying *xa*13 expressed the lesion length from 7.93 to 8.66 cm, with a resistance reaction of moderate. The lines having triple BB resistance genes (*Xa*21, *Xa*4, *xa*13), as well as two genes (*Xa*4 and *xa*13), exhibited lesion lengths from 1.8 to 4.9 cm, showing resistant reaction to *Xoo*, revealing the effectiveness of resistance gene pyramiding (Figure 2). Out of 55 RILs, 11 lines showed resistance reaction, 22 were moderately resistant, 12 were moderately susceptible, and 10 exhibited susceptible reaction. The lesion length of IL2 carrying *Xa*21 (2.3cm) was slightly higher than IRBB59, with a lesion length of 1.9 cm (Table 2).

DISCUSSION

We developed the rice multi-parent advanced generation inter-cross (MAGIC) populations using eight diverse parents, and the approach remains very efficient in pyramiding multiple BB and BPH resistance genes. MAGIC is a newly emerging breeding approach leading to a reshuffling of the genome and identifying gene-trait associations with high resolution (Arrones *et al*., 2020; Putri *et al*., 2023). It

could be due to the inter-mating of multiple founders with diverse genetic backgrounds, which increases genetic and phenotypic diversity (Scott *et al*., 2020). In the MAGIC population, the several rounds of inter-mating, followed by selfing, increase the number of desirable recombinants and, thus, improve the population accurately.

Genetic transformation technologies enhanced conventional breeding since 1980, but these mainly focused on monogenic traits, while most agronomic traits are quantitative and polygenically controlled. Breeders used backcrossing as the primary method for enhancing the resistance level against diseases. Molecular marker-assisted selection (MAS), combined with backcross breeding, has already been reported to increase breeding efficiency (Yugander *et al.,* 2018). However, due to the relatively large amount of work needed in the MAS process, the conventional backcross approach has been widely used, yet it may not accurately transfer multiple genes into a cultivar based on phenotypic screening (Crossa *et al*., 2017). In a conventional bi-parental population, crossing consists only of two parents, and these suffer from a shortage of genetic diversity due to a narrow genetic base and have limited opportunity for genetic recombination.

In the latest investigation, a diverse set of founder parents, including introgression lines derived from wild relatives, cultivated *indica and japonica* varieties*,* and landraces from different origins, were used as founder parents. These founder lines can resist bacterial blight and brown planthopper and possess desirable agronomic and grain quality traits. Earlier reports indicated introducing wide-cross derivatives as a founder parent increased the genetic variability in the population, which is a vital point for QTL identification (Gramazio *et al*., 2020).

A high level of resistance exhibited in RILs of MAGIC populations against virulent races of *Xoo* is comparable to the donor parents. These results are similar to earlier reports (Suh *et al.,* 2013). The consistent finding is that the RILs that carry a combination of three genes exhibited higher resistance reactions against *Xoo* than the RILs with two or single-gene combinations. In the presented study, it is clear that the lines with two-gene combinations (*Xa*4 and *xa*13) exhibited higher resistance reactions with shorter lesion lengths, while RILs having *xa*13 and *Bph1* were relatively less effective. Lines with *Xa*21 combined with *Xa*4 and xa13 promise to advocate the usefulness of *Xa*21 in getting higher levels of resistance in the MAGIC population.

It could be due to quantitative complementation among the resistant genes that result in enhanced resistance levels in RILs with minimum lesion length. Sanchez *et al*. (2000) observed a similar reaction where the line carrying multiple resistance genes exhibited a higher resistance level (shorter lesion length) than the line with a single resistance gene. The pyramided lines carrying more than one resistance gene are presumably more durable and adaptable against the dynamic nature of pathogens. *Xa*4 and *Xa*21 are dominant resistant genes. Resistance gene *xa*13 is recessive and could result in mutation in the promoter region of susceptibility gene Os-8N3 homolog of the nodulin MtN3. Having a dominant susceptibility allele in rice, when infected by *Xoo,* the noduline gene expression is upregulated. It indicates that for growing *Xanthomonas oryzae* on rice, the upregulation of this gene is necessary. The rice with the *xa*13 gene may stop the upregulation of this gene, which hinders the growth of bacteria on rice, leading to resistance expression*.*

A study reported a combination of four BB resistance genes (*Xa*4, *xa*5, *xa*13, and *Xa*21) confers broad-spectrum resistance against *Xoo* in different parts of the world (Neelam *et al.,* 2020). Earlier studies conducted in various locations revealed that such a combination of genes did not break down resistance, making it a promising approach for controlling disease. A combination of three genes (*xa*13, *Xa*21, and *xa*5) has shown complete resistance against all pathotypes (Sakthivel *et al*., 2017). *Xa*4 and *Xa*21 dominant resistance genes confer resistance, independently or cumulatively, exhibiting their role in two distinct resistance pathways. Earlier studies have successfully generated a combination of three genes in

several rice varieties, including Ranidahan (*Xa*4, *xa*5, *xa*13, and *Xa*21) (Pradhan *et al.,* 2022).

Brown planthopper is also one of the threats to rice by spreading the viral disease in rice or sucking the leaves and causing a significant reduction in yield. We initially incorporated the *Bph1* gene from *O. rufipogon* into Basmati385, using the introgression line as one of the founder parents in the MAGIC population. The International Rice Research Institute (IRRI) transfers the BPH resistance gene from *O. australiensis* into *O. sativa* (IR65682-136-4-3-2) and localized on the chromosome of *O. sativa* by genomic *in situ* hybridization (Abbasi *et al.*, 2010). Earlier reports indicated that around 70 BPH resistance genes have been derivatives from wild rice. This study was able to combine *Bph*1 to BB resistance genes in RILs. Similar results for BPH and bacterial blight-resistant gene pyramiding came from He *et al*. (2019). The MAGIC approach is the most successful breeding method for multiple gene pyramiding.

CONCLUSIONS

The MAGIC approach in plant breeding is the most effective for multiple gene pyramiding. It increases recombination compared with the bi-parental approach**.** The RILs that carry a combination of three genes exhibit higher resistance reactions against *Xoo* than the RILs having two or single-gene combinations. It could be due to quantitative complementation among the resistance genes that results in enhanced resistance levels in RILs with minimum lesion length. The selection of diverse founder lines may enhance the genetic variability of the MAGIC population.

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