

# Genome-wide association mapping in rice MAGIC *indica* panel detects QTLs and genes for broad-spectrum resistance breeding against African bacterial blight

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## ABSTRACT

Rice (*Oryza sativa*) bacterial blight (BB) is a direct threat to production in Africa. The extent of damage on the continent is not known. We conducted a genome-wide association study (GWAS) to seek out Quantitative trait loci (QTL) that confer the broad-spectrum resistance (BSR) to rice *Xanthomonas oryzae* pv. *oryzae* in the rice Multiparent advanced generation intercross (MAGIC) population. The diverse genetic composition and high levels of recombination, are potential resources for the identification of QTL for BSR. Dealing with the ever-evolving *Xoo* requires identification of novel R gene and resistance quantitative trait loci (QTL).

We conducted a genome-wide association study (GWAS) using 250 rice MAGIC *indica* panel to evaluate for resistance to three *Xoo* strains from Cote D'Ivoire, namely *XooCIkor*, *XooCIgag* and *XooCIcii2* which are prevalent highly virulent pathotypes.

Using the 3 K RG 4.8mio filtered SNP Dataset, a total of 3 QTL associated with BB resistance on chromosome 9, 6 and 5 were identified.

The results of the GWAS identified novel loci that gives useful insight for more investigation and also validated known genes underlying resistance to virulent African strains of BB. Genetic donors identified in this will help plant breeders in improving and broadening resistance to BB.

**Key words:** Africa, Bacterial blight (BB), broad-spectrum resistance (BSR), multiparent advanced generation intercross (MAGIC), Quantitative trait loci (QTL), *Xanthomonas oryzae* pv. *oryzae*

## INTRODUCTION

Plant diseases in different crops cause an average global yield loss to the tune of approximately 16%, and in some cases reach up to 50% in individual crops (Oerke, 2006; Savary et al., 2019; Gupta et al., 2021). Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most devastating rice

diseases worldwide (Savary et al., 2019). It is also an important disease of rice in Africa resulting in heavy yield losses and a direct threat to future expansion and production. The extent of BB damages on the continent is not known. The warm and humid climate conditions in the tropics have accelerated the outbreak of BB, making it a common and the most serious constraint in rice production (Chen et al., 2018; Chen et al., 2019).

In the past, several interventions have been undertaken to combat BB in rice-growing ecologies but the use of chemicals has had limited success due to pathotypes sensitivity towards the applied chemicals. The ever-mutating pathogenic races are a major drawback in developing a durable control of the disease (George et al., 1997). Breeding for resistance is the most feasible and sustainable solution to rice BB.

The resistance of rice to BB is categorized into two viz., qualitative resistance governed by major resistance (*R*) genes, and quantitative resistance conferred by multiple minor genes/quantitative trait loci (QTLs) (Ramalingam et al., 2003; Deng et al., 2012; Bossa-Castro et al., 2018). The rice-*Xoo* interaction follows the classical gene-for-gene model (Nino-Liu et al., 2006).

To date, 46 disease resistance-related genes *Xa1-Xa46(t)* have been identified in different chromosomal regions (Chukwu et al., 2019; Kim et al., 2019; Kim et al., 2018; Neelam et al., 2020; Chen et al., 2020), of which *Xa1*, *Xa3/Xa26*, *Xa4*, *xa5*, *Xa7*, *Xa10*, *xa13*, *Xa21*, *Xa23*, *xa25*, *Xa27*, and *xa41(t)* have been cloned (Yoshimura et al., 1998; Xiang et al., 2006; Iyer et al., 2008; Hu et al., 2017; Chen et al., 2021; Luo et al., 2021; Tian et al., 2014; Wang et al., 2015; Liu et al., 2011; Gu et al., 2005; Hutin et al., 2015; Song et al., 1995; Chu et al., 2006). However, because these genes tend to confer relatively weak and narrow-spectrum disease resistance, only a few dominant genes that provide broad-spectrum disease resistance, including *Xa4*, *Xa21*, *Xa23*, and *Xa39*, have been widely used in rice breeding programs (Hu et al., 2017; Wang et al., 2015; Song et al., 1995). The most recent discovery was the *Xa43(t)*, identified in multi-parent advanced generation inter-cross (MAGIC) population through Genome-Wide Association Analysis (GWAS) (Kim and Reinke 2019), (Lu Wang et al., 2021).

The narrow genetic basis of BB resistance in rice cultivars has resulted in a dramatic increase in the frequency of *Xoo* pathotype. The disease resistance conferred by a single *R* gene is usually effective against certain strains of the *Xoo* pathogen but the resistance breakdown due to higher selection pressure on pathogen evolution usually occur. Likewise, the resistance conferred by QTLs is presumably non-race specific and considered more durable (Liu et al., 2016). The

ability of QTLs to confer a broader and more durable resistance has drawn a lot of attention and in the past, more than 70 QTLs for BB resistance has been identified (Li et al., 2006, Han et al., 2014; Djedatin et al., 2016; Dilla-Ermita et al., 2017; Zhang et al., 2017; Bossa-Castro et al., 2018).

This has led to quantitative resistance being a more preferred strategy for a durable resistance and vast number of QTLs have been identified for resistance to BB. However, marker assisted selection (MAS) has not been effectively exploited for BB resistance breeding in rice due to the polygenic nature of the trait with each QTL having small effect (Yang et al., 2021). It is difficult to stack multiple QTLs with small effects in breeding for broad-spectrum resistance.

The prerequisite for successful MAS is the availability of the markers that are closely linked with the target gene, in BB resistance, this is limited by the inaccuracy of QTL mapping therefore hindering the application of MAS. Therefore, the use of more powerful approach for genetic dissection of complex traits are critical to address these issues, including the discovery of large-effects QTLs in wider population.

During the last decade, GWAS have been widely conducted to decipher the genetic basis of complex traits in diverse varieties and to identify the causative loci underlying these traits (Wang et al., 2020).

Breeding and deploying resistant cultivar is considered one of the most economical and eco-friendly strategy for managing BB (Ogawa, 2008; Veer Cruz et al., 2000).

Multiparent advanced generation intercross (MAGIC) population were developed and showed to accelerate identification of QTL (Bandillo et al., 2013; Cavanagh et al., 2008; Raghavan et al., 2017). Each rice MAGIC population was generated from multiple founder lines, selected for their different agronomic traits and genetic background. The founders were crossed in a design that ensured an equal representation of each founder in the population.

Benefits of MAGIC populations to QTL mapping are increased recombination, transgressive segregation events revealing novel phenotypes and capacity for fine mapping, which expedite the discovery of new resistance sources against pathogens (Bandillo

et al., 2013; Raghavan et al., 2017).

The expansion and intensification of rice production, coupled with the absence of appropriate phytosanitary controls, have contributed to the increased incidence of rice Bacterial Leaf Streak and Bacterial Blight and the emergence of more virulent pathogen strains (Verdier et al., 2012; Wonni et al., 2014). BB has been an important threat in Africa since 1980, causing up to 50% yield loss, while BLS is a more recently observed disease, causing up to 20% yield loss (Kang et al., 2008; Reddy et al., 1979; Wonni et al., 2015)

The use of resistant varieties is considered the most effective and sustainable way to control these diseases (Tang et al., 2000; Verdier et al., 2012), but sources of resistance for BLS and BB in Africa are lacking (Wonni et al., 2015). Control of BB and BLS is particularly important in sub-Saharan Africa where there are no sources for Broad-spectrum Resistance (BSR) in the currently propagated varieties.

Therefore, the exploitation of genetic resources and discovery of genes conferring resistance to representative *Xoo* strains from African is needed. (Lu Wang et al., 2021) was inoculated with three *Xoo* representative strains of virulent pathotypes from Cote D'Ivoire to evaluate their resistance against BB.

We chose a MAGIC population developed from eight indica founders in this study. Indica is widely grown in the rice ecologies of the tropics and subtropics, including African countries (Garris et al., 2005). Our target was to identify loci (QTL/genes) associated with BSR resistance to BB of rice. We used virulent strains of *Xoo* from West Africa to identify sources of resistance which are limited in the cultivated germplasm in this region (Verdier et al., 2012). BSR is a more sustainable approach for the enhancement of local elite varieties from the regional breeding program. MAGIC populations are very useful new prebreeding materials for breeding durable *Xoo* resistant varieties.

## **MATERIALS AND METHODS**

### **Experimental procedures**

#### **Plant material**

The MAGIC *indica* population used in this study was developed at IRRI, Manila, Philippines (Bandillo et al.,

2013). The population development was initiated by crossing 8 elite indica founders; Fedearroz 50, SANHUANGZHAN-2, IR64633-87-2-2-3-3 (PSBRc82), IR4630-22-2-5-1-3, IR45427-2B-2-2B-1-1, Samba Mahsuri +Sub1, IR77298-14-1-2-10 and IR77186-122-2-2-3 (PSBRc 158) in a half-diallel mating design. A set of 28 F1s were selected to develop 210 4-way crosses by avoiding duplicate crosses. The 4-way crosses were intermated two times and further selfed continuously until the S11 stage. A set of 1361 MAGIC lines were finally derived from the selfed population (Raghavan et al., 2017). A sub-set panel of 250 were randomly selected from the sequenced accessions of the 3 K RGP used in the present experiment

The check variety WITA-9 is widely grown across Africa, developed from Africa Rice Centre. The variety is resistant to BB. Seeds were soaked in sterilizing agent before sowing to prevent seed borne-disease, and germinated in seedling petri dishes in germination chamber. The temperature in the growth chamber was maintained at 25 °C and seeds exposed to 12 hours of light and darkness. The one-week-old seedlings were transplanted to the experimental screen house at the Africa Rice Centre, Mbe, Cote D'Ivoire. All the accessions were grown in two replicates in 10-inch plastic pots. The plants were raised on sterilized mixture of sand and loam soil mixed in the ratio 1:3. Fertilizer for the experiment was applied following (Zhai et al., 2020). The seedlings were flooded watered twice per week and the experiment was maintained for three months including inoculation and data collection.

#### **BB phenotypic evaluation**

The BB resistance of rice plants was evaluated at the maximum tillering stage. Plants were inoculated using the scissor-cutting leaf method and examined 3 weeks later when the lesion length (LL) was stable (Zhang et al., 2015; Kauffman et al., 1973). The average LL of two replicates was used to construct the phenotype matrix for subsequent analyses. On the basis of the LL, rice accessions were classified as follows (Zhang et al., 2017): resistant (R): LL < 5 cm; moderately resistant (MR): 5 cm ≤ LL < 10 cm; moderately susceptible (MS): 10 cm ≤ LL < 15 cm; or susceptible (S): LL ≥ 15 cm.

### GWAS analysis and identification of candidate gene

GWAS analysis, QTL delimitation and identification of candidate gene were performed using the method as described in the study by Zhao et al. 2018. The brute data before filtering had 2721185 SNPs and after filtering the data at MAF  $\leq$  5% and Heterozygosity  $\geq$  0.5, the SNP number was trimmed to 123090 SNPs. All 123090 SNPs and GAPIT version 2 were used for GWAS analysis (Tang et al., 2016).

GWAS was conducted using two models FarmCPU and mixed linear model with kinship matrix, and principal component (PC) was set to 2 in GAPIT. Manhattan and QQ plots were produced using R package qqman (Turner, 2014). A QTL was declared if a region has two or more significant SNPs within a 200 kb interval. The candidate genes were searched from 200 kb upstream and downstream of the most significant SNP in each QTL.

## RESULTS

### MAGIC *indica* panel reveals sources of resistance to *Xoo* strains

The MAGIC *indica* panel was screened with three *Xoo* African strains from Cote D'Ivoire. The responses to *Xoo*CIcii2, *Xoo*CIkor and *Xoo*CIgag, measured as lesion length (LL), showed a large range of phenotypic variation (Table 1). Generally, most of the MAGIC *indica* panel inoculated with the three *Xoo* strains from Cote D'Ivoire exhibited moderate resistance with the highest having a 44.31% resistance (*Xoo*CIcii2), followed by 40.78% (*Xoo*CIkor) and 30.20% (*Xoo*CIgag). The population showed the highest levels of resistance to strain *Xoo*CIkor which accounted for 21.57% of the total population of 55 accessions. Both *Xoo*CIgag and *Xoo*CIcii2 had the average resistance of 20.39%. *Xoo*CIgag was the most virulent of all the

three strains with 39 accessions highly susceptible (15.29%) followed by *Xoo*CIkor affecting 20 accessions (7.84%) and then *Xoo*CIcii2 affecting 17 accessions at 6.67%. The 250 genotypes in the diverse panel showed a distinct population structure (Fig. 1C) which may cause false positive results if not corrected (Zhu et al., 2008). The subpopulations are clustered into three groups of *Xoo*CIcii2, *Xoo*CIkor and *Xoo*CIgag including 110, 100 and 40 genotypes respectively.

### Evaluation of resistance to three *Xoo* strains

The distribution of lesion length (LL) in the 250 accessions inoculated with *Xoo* strains (CIgag, CIcii2 and CIkor) showed large phenotypic variation (Fig. 1A). Among the inoculated cultivars, fifteen accessions were highly resistant to all three strains with LL < 5cm, and 5 were highly susceptible to all three strains with LL  $\geq$  15cm (Fig. 1). Another cultivar WITA9 from Africa Rice that was used as a check conferred high resistance to all the three strains, implying that it could be carrying resistance genes for use in breeding.

Based on the LL of the accessions, the *Xoo* strains were divided into three clade groups namely Korhogo strain group (*CIkor*), Gagnoa strain (*CIgag*) and Central group (*CIcii2*) (Fig. 1C).

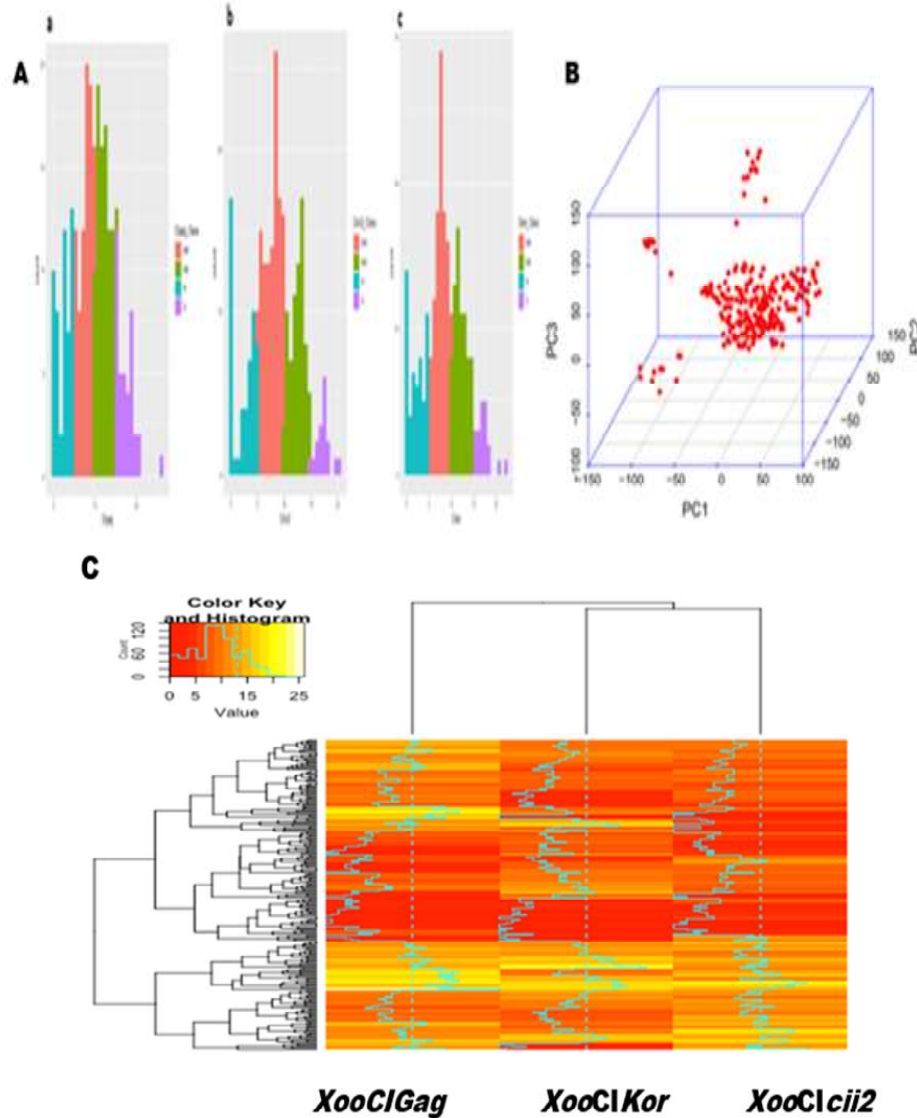
### Hotspot chromosomes associated with BB resistance

The GWAS results indicated chromosomes 5, 6 and 9 as hotspots for BB resistance. Chromosome 5, contained one QTL against strain *Xoo*CIKo overlapping with *LOC\_Os05g01710* (*Xa5*), a recessive gene encoding transcription initiation factor IIA subunit 2, responsible for broad-spectrum resistance (Huang et al., 2016; Iyer-Pascuzzi et al., 2008; Mishra et al., 2013). Chromosome 6 is a hotspot for *Xa7* which encodes an executor R protein that confers broad-spectrum resistance to *Xoo* strains. However, *Xa7* has some unique characteristics, including extremely durable

**Table 1.** Responses of MAGIC *indica* panel to *Xanthomonas oryzae* pv. *oryzae* strains used in this study.

Sn.	<i>Xoo</i> CIkor	Class count	%	<i>Xoo</i> CIgag	Class count	%	<i>Xoo</i> CIcii2	Class count	%
1.	MR	104	40.78	MR	77	30.20	MR	113	44.31
2.	MS	76	29.80	MS	87	34.12	MS	73	28.63
3.	R	55	21.57	R	52	20.39	R	52	20.39
4.	S	20	7.84	S	39	15.29	S	17	6.67

\*MR: Moderately Resistant, \*MS: Moderately Susceptible, \*R: Resistant, \*S: Susceptible



**Fig. 1.** Distribution of lesion length (cm) of magic indica panel. (a) Screening of 340 MAGIC indica panel with *Xoo* CIgag (Cote D'Ivoire) (b) *Xoo* CIcii2 and (C) *Xoo* CIkor. **B.** 3D Principal component analysis for the 250 accessions **C.** Hierarchical cluster of accessions and strains based on lesion length (LL) of three *Xoo* strains.

and heat-tolerant resistance (Vera Cruz et al., 2000; Webb et al., 2010). First, the durable resistance of *Xa7* may be partly explained by the distinctive functions of its cognate avirulence gene *avrXa7* in *Xoo*.

**DISCUSSION**

The sustainable and economical management of BB in rice growing ecologies will depend on breeding for

broad spectrum resistance. The MAGIC population used in these studies provide a diverse gene pool for resistance genes to the African *Xoo* strains. The MAGIC population carrying genes of resistance can be used by breeders to improve the African rice germplasm. But to achieve success in the large-scale genetic studies, accurate genotyping is done (Tam et al., 2019). Compared to 44 K, 55 K and 700 K SNP arrays, SNPs identified from the 3 K RG 4.8mio filtered

SNP Dataset (derived from the 3 K RGP) in this study were distributed more densely and covered almost all registered genes in the Nipponbare reference genome (Mansueto et al., 2017; McCouch et al., 2016; Mengetal.2017; Zhaoetal, 2011).

The sequencing data of the 3 K RGP have provided a basis for identifying QTL associated with agronomic traits, abiotic resistance and sheath blight resistance in rice (Shi et al., 2017; Zhaietal., 2018; Zhang et al., 2017b; Zhang et al., 2019). In this study, QTL against BB in a diverse MAGIC rice panel from the 3 K RGP were dissected through GWAS, and the results obtained provide novel information for rice breeding. However, further genetical studies with the MAGIC rice population to more bacterial blight strains are recommended to better understand the different modes of resistance.

### Diverse reactions to *Xoo* strains among different accessions

We evaluated the resistance of 250 rice accessions from the 3 K RGP to BB caused by three *Xoo* strains from the different ecological regions of Cote D'Ivoire. All the three strains are new virulent strains prevalent in the rice-growing regions of cote D'Ivoire (Hutin et al., 2015). In all, only fifteen high resistant cultivars with LL<5cm) for all the three strains were identified (Table 1). These sources of resistant germplasm in different genetic backgrounds can provide valuable material for facilitating breeding for BB resistance.

### QTL application in rice breeding

It is known that chromosome 11 is an important and complex region of the rice genome with respect to BB resistance, containing mapped or finely-mapped BB *R* genes *Xa22(t)*, *Xa30(t)*, *Xa32(t)*, *Xa35(t)*, *Xa36(t)*, *Xa39*, *Xa40*, *xa41(t)*, *Xa43(t)* and *xa44(t)*, and cloned genes *Xa3/Xa26*, *Xa4*, *Xa10*, *Xa21*, *Xa23* (<https://shigen.nig.ac.jp/rice/oryzabase/>).

### CONCLUSION

Our study provides new insights into the geographical distribution and bacterial blight resistance in rice. The MAGIC panel accounted for about 44.31% resistance to African strains of BB suggesting that novel genes can be deployed for rice breeding. The findings reported herein provides useful knowledge for future crop

improvement. Further validation of the effects of these candidate genes and their functional variants is required. We will deploy more genomic tools to verify that these genes confer bacterial blight resistance to rice.

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### Authors contributions

M.O., M.O.S., J.L., G.O., and R.O designed the studies. M.O performed the experiments. J.O. performed bioinformatics analysis. M.O performed the analysis and wrote the manuscript. T.L.O., T.G., P.T., S.B.M., and W.P provided useful advice. All authors read and approved the final manuscript.

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### Availability of Data and Materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

### Ethics Approval and Consent to participate

Not applicable.

### Competing interests

The authors declare that they have no competing interest.

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