



## LARGE EFFECT QTLs INVOLVED IN RESISTANCE TO RICE YELLOW MOTTLE VIRUS (RYMV) DISEASE

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### ABSTRACT

*Rice yellow mottle virus* (RYMV) is one of most damaging pathogen of rice in Africa. In order to reduce the impact of this disease, several control methods were proposed by researchers, but selection for resistance is considered to be the most effective management strategy. In the present study, a set of 190 F<sub>2</sub> populations from the cross between an RYMV susceptible genotype IR64 and resistant genotype BM24 were used for QTL analysis. The objective of the study was to identify QTLs associated to RYMV resistance through some agro-morphological traits under RYMV effect. A total of two major QTLs were identified for RYMV resistance on chromosome 12 and associated with day to first symptom (*qDsym*) and disease score (*qDiScore*). These two QTLs explained 14.7% and 16.7% of total phenotypic variation for *qDiScore* and *qDsym*, respectively and showed positive additive effect of 0.363 and 2.129 respectively for *qDiScore* and *qDsym*, indicating that favorable alleles are from resistant parent BM24. In addition two most significant QTLs, (*qFertPan1* and *qFertPan2*) were identified for number of fertile panicle (FertPan) under RYMV effect on chromosome 4 and 12. Such as QTLs for RYMV resistance, these QTLs (*qFertPan1* and *qFertPan2*) showed positive additive effect. QTLs identified in this study are useful for marker-assisted selection and are also suitable for candidate gene identification.

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### INTRODUCTION

Rice (*Oryza sativa* L.), an annual cereal crop, is one of the most important food crop in the world. It is both a common staple food for much of the world's population including that of sub-Saharan Africa. Rice is also the most studied plants due to the comparative advantages of its small genome (Orjuela et al., 2013). It represents an important place in human nutrition. Rice seed contains about 75 to 80% starch, 12% water and 7% protein (Oko et al., 2012). Its seeds are also source of minerals like calcium, magnesium, phosphorus, iron, copper, zinc, manganese, niacin, thiamine and riboflavin (Yousaf, 1992; Oko et al., 2012).

In 2014, rice was grown in more than 117 countries around the world covering a total area of about 162.72 million (M)

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hectares (ha) with a global production of about 741.48 million tons and an average yield of about 4,539 kg.ha<sup>-1</sup> (FAOSTAT, 2017). The major producers were China and India with 206.5 and 157.2 million tons respectively, followed by Indonesia with 70.8 million tons, Bangladesh with 52.2 million tons and Viet Nam with 44.9 million tons (FAOSTAT, 2017). African continent is ranked in the third place with 4.2% of global production after Asian and American continents with their global production of respectively 90 and 5.1%. However, Africa has the lowest average yield (2,590.7 kg.ha<sup>-1</sup>) compared to the average yield of others continents (4,556.9 kg.ha<sup>-1</sup>). This lowest production in the continent can be explained by the effect of several abiotic and biotic constraints in rice.

Among the various biotic stresses that reduce rice yield, *Rice yellow mottle virus* (RYMV) disease is a major cause of losses in sub-Saharan Africa (Ghesquière et al., 1997; Ochola and Tusiime, 2011; Issaka et al., 2012) and can cause yield losses of 100% in highly susceptible varieties (Fomba, 1988).

It is endemic to Africa and was firstly reported in Kenya (Bakker, 1974). The causal agent of RYMV is a *Sobemovirus* that is transmitted by chrysomelid beetles (Bakker, 1971). It has a genomic organization with a single-stranded RNA encoding four open reading frames (Ventelon-Deboutet *et al.*, 2008). Plants infected by RYMV are characterized by leaves mottling and yellowing, plant stunting, partial emergence of panicles and sterility. The diversification of this virus is well described and has revealed different viral strains with gradual differentiation from East Africa, where it originated, to West Africa (Abubakaret *et al.*, 2003; Traoré *et al.*, 2005 and Pinel-Galzi *et al.*, 2015).

Genetic studies and resistance to RYMV have been conducted using the cultivated Asian species (*Oryza sativa*, and *Oryza japonica*) and African species (*Oryza glaberrima*). Thus, several resistant accessions have been described in *O. sativa* and *O. glaberrima*, and partial resistance accessions have been observed in *O. japonica* (Thottappilly and Rossel, 1993; Albaret *et al.*, 1998 and Linares, 2002). Three genes conferring resistance to RYMV have been identified: the first, *RYMV1* that encodes an E1F(iso)4G factor has been described by Albar *et al.* (2006); the second (*RYMV2*) is a recessive gene and it was described using *O. glaberrima* accessions by Thiémélé *et al.* (2010) and Orjuela *et al.* (2013) and recently the third resistance gene *RYMV3* was mapped by Pidonet *et al.* (2017) using *O. glaberrima* accessions. Partial resistance was found in varieties Azucena and BM24, and is expressed only at the early stages of infection and was characterized by delayed and lower virus accumulation in leaves and delayed virus invasion in bundle sheath tissues (Ioannidou *et al.*, 2003 and Kam, 2011). The tolerance in Azucena was apparent at the later stages of infection and characterized by low symptom expression despite high virus accumulation (Ioannidou *et al.*, 2000). In addition several major QTLs for RYMV resistance were also identified in Azucena by early studies (Ghesquière *et al.*, 1997 and Boissnard *et al.*, 2007).

Using genetic resistance is efficient to reduce RYMV effect in rice. The QTL identified on chromosome 12, in Azucena, is close to the *indica/japonica* zone of differentiation and is bracketed in an interval of 2.23 Mb containing the marker RM101 (Boissnard *et al.*, 2007). This interval is relatively large and makes the tagging and the fine mapping of the QTL12 implicated in the partial resistance of Azucena difficult. This difficulty hampers the identification and the cloning of the gene(s) involved(s).

However, the high diversity of RYMV and its large geographical distribution may affect the durability of the resistance. In addition, new QTLs are to be detected in crosses from different genetic backgrounds. It would be necessary to use SNP markers to identify QTLs that are involved in the resistance of BM24. Finding of QTLs that are close to SNPs could be used for marker assisted selection (MAS). The objective of the present study was to identify genomic regions associated with RYMV resistance and some agronomical traits under RYMV effect using F<sub>2</sub> population from IR64 X BM24.

## MATERIALS AND METHODS

### Plant materials

F<sub>2</sub> mapping population comprising of 190 lines developed from the cross IR64 X BM24 was used to identify and map

RYMV resistance QTLs. IR64 (*O. s. indica*) is a high-yielding cultivar developed at the International Rice Research Institute (IRRI) and BM24 a traditional *O. sativa* cultivar from Burkina Faso and showing partial resistant to RYMV (Kam, 2011).

### Phenotypic evaluation of mapping population

The 190 F<sub>2</sub> individual plants and the two parents (IR64 and BM24) were grown under control condition at INERA research station of Kamboinssé, Burkina Faso. The experimentation was raised in 8 rows with 24 plants per row and a spacing of 20 cm between rows and between plants. Two weeks after sowing, plantlets were inoculated with an RYMV isolate (KM9) from Burkina Faso. Inoculum was prepared by grinding IR64 infected leaves in 0.05M phosphate buffer (1g/250ml) and adding 600-mesh carborundum abrasive to facilitate mechanical inoculation of the leaves. Plants were mechanically inoculated at 14 days after sowing (DAS) by rubbing the inoculum on leaves. Disease incidence was done based on day to first symptoms appearance (Dsym) and disease score (DiScore). Thus, plants were observed every day from first day after inoculation (DAI) to determine the day to first symptom appearance (Dsym). Disease score (DiScore) were obtained for each F<sub>2</sub> plant by visual scoring two, four and six weeks after inoculation, using 0-9 scale according to the method for RYMV resistance assessment (IRRI, 1996). However, the plant height (PltH), the number of tiller (Tillers), the number of fertile panicle (FertPan), the panicle weight (PanW) and the single plant yield (SinglPY) were recorded at harvest (maturity).

### DNA extraction and genotyping

Young fresh leaves of 14 days old from F<sub>2</sub> individual plantlets and parental lines were collected just before viral inoculation for DNA extraction. Then, Genomic DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) method (Saghai-Maroo *et al.* 1994). Quality and quantity of DNA was checked by running aliquots of DNA samples on a 0.8% agarose gel. DNA samples were then normalized and shipped to LGC Genomics in UK (<http://www.lgcgenomics.com>) and genotyped using the Kompetitive Allele Specific PCR (KASP) genotyping platform. Firstly, the two parental lines (IR64 and BM24) were genotyped to find polymorphism SNPs between them using 1111 SNPs markers. Out of 420 segregating SNPs 191 were selected based on their distribution on the chromosomes to genotype the whole F<sub>2</sub> population.

### Statistical analysis

For each trait, data were compiled and means were performed for the two parental lines and F<sub>2</sub> population using XLSTAT-Pro version 7.5. Breeding Management System (BMS) package ("The IBP Breeding Management System Version 3.0.9 (December 2015) The Integrated Breeding Platform. <https://www.integratedbreeding.net/breeding-management-system/>) was used for QTL analysis following by Composite Interval Mapping (CIM) method. A permutation number of 1000 was applied for each trait. The thresholds LOD value was set automatically at 3.107 was selected as minimum to detect the presence of QTL. The percentages of phenotypic variance explained (%Expl. Var) and additive effects were also estimated.

## RESULTS

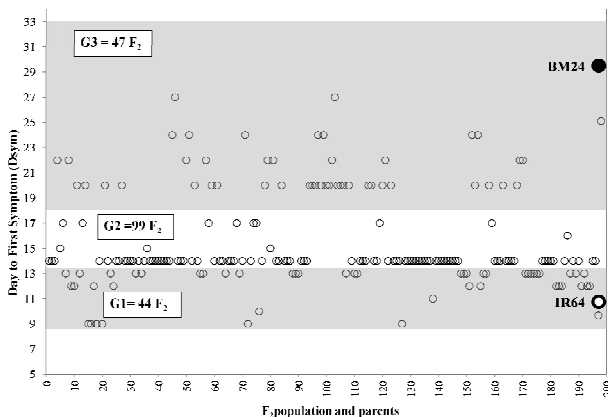
### Performance of F<sub>2</sub> population and the two parental lines

The phenotypic distributions of agronomical traits and RYMV resistance of the two parents and F<sub>2</sub> population are shown in Table 1. The average severity score induced by RYMV (DiScore) for IR64 and BM24 are 7 and 1, respectively. BM24 is observed to be resistant and IR64 susceptible to RYMV. The disease scores of F<sub>2</sub> population were ranged from as low as 3 to as high as 9 with an average of 4.89. Based on disease severity 20 F<sub>2</sub> genotypes of the 190 studied were tolerant with a disease score of 3, and the remaining 170 were susceptible with a disease score ranging from 5 to 9. Generally virus symptom expression varied with time and varieties. The first symptoms were observed at 9 and 35 DAI for IR64 and BM24 respectively, whereas the days to first symptom (Dsym) were ranged from 9 to 27 DAI for the F<sub>2</sub> population. Out of 190 F<sub>2</sub> population, 44 genotypes showed day to first symptom (Dsym) below 14 DAI comparable to susceptible parent IR64, whereas 47 genotypes were recorded with first symptom appearance at 20 days and above considered as tolerant like the parent BM24 (Figure 1). All agronomical traits (FertPan, PltH, Tillers, PanW and SinglPY) estimate under RYMV effect showed high values for resistant parent BM24 than IR64. Almost traits as number of fertile panicles (FertPan), panicle weight (PanW) and single plant yields (SinglPY) showed zero values for IR64 (Table 1) meaning that IR64 plants were completely sterile.

**Table 1** Phenotypic performance of the F<sub>2</sub> population and its parents under RYMV pressure

TYPE OF TRAITS	TRAITS	PARENTS		F <sub>2</sub> POP	
		BM24	IR64	F <sub>2</sub> s' Mean	F <sub>2</sub> s' Range
RYMV Component	Dsym	35	9	15.5	9 - 27
	DiScore	1	7	4.9	3 - 9
	PltH	84	25	59.8	0 - 97 cm
Agronomical traits	Tillers	2	2	4.6	0 - 12
	FertPan	2	0	0.22	0 - 6
	PanW	5.4	0	0.17	0 - 4.5 g
	SinglPY	4.2	0	0.10	0 - 3.1 g

RYMV: Rice yellow mottle virus, Dsym: day to first symptom, DiScore: disease score, PltH: plant height, FertPan: Number of fertile panicles, PanW: panicle weight, SinglPY: single plant yields, Tillers: number of tillers,



**Figure 1** distribution of IR64 X BM24 F<sub>2</sub> population for day to first symptom of RYMV

### Identification of QTL involved in RYMV resistance and agro-morphological traits

Polymorphism level varied with parental combinations used. In this study, a parental polymorphism survey using 191 SNPs markers revealed that 180 SNPs (94.24%) were polymorphic

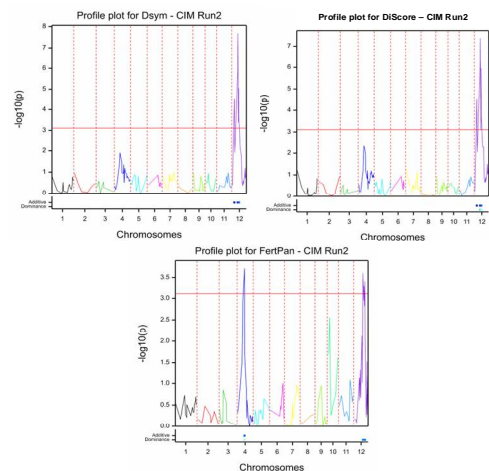
between IR64 and BM24. These 180 polymorphic SNPs markers were used for genotyping 190 F<sub>2</sub> population.

Phenotypic data of RYMV resistance and related traits (day to first symptom and disease score) and agro-morphological traits under RYMV effect were used conjunctly with genotyping data for QTL analysis. A total of four (04) QTLs were detected in this study. Of these, two (02) QTLs were associated to fertile panicle (FertPan) and two to RYMV resistance related traits (Table 2 and Figure 2). Two QTLs located in chromosome 4 on locus id4005526 at position 50.37 and chromosome 12 on locus K\_id12006801 at position 72.54 were detected for panicle fertility (FertPan) under RYMV effect. The LOD values were 3.54 and 3.45 for *qFertPan1* and *qFertPan2*, respectively. The two QTLs contribution to the total phenotypic variance explained (PVE) were 2.11% and 9.75% respectively. The high value allele of the two QTLs were on parent BM24 and the dominant allele on IR64. The two QTLs had a total contribution of 11.86%.

Similarly, two other QTLs were detected on chromosome 12 for days to first symptom appearance and disease score. The two major QTLs, *qDiScore* and *qDsym*, were mapped on chromosome 12 on SNP marker K\_id12003066 at position 48.54. The QTL associated to day to first symptom (*qDsym*) was detected at a LOD score of 7.24 and the one associated to disease score (*qDiScore*) at LOD score of 7.37. The contributions of these QTLs to the total phenotypic variance were of 14.7% and 16.7% for *qDiScore* and *qDsym*, respectively. The high value alleles of these two QTLs are from the parent BM24.

**Table 2** QTLs for RYMV resistance and agronomical traits in IR64 × BM24 F<sub>2</sub> population Composite Interval Mapping (CIM)

TRAITS	QTLs	Locus	LG Position	LOD	% Expl. Var	Add. Eff.	High value allele
FertPan	<i>qFertPan1</i>	id4005526	4	50.4	3.54	9.75	0.386 BM24
	<i>qFertPan2</i>	K_id12006801	12	72.5	3.45	2.11	0.180 BM24
RYMV	<i>qDiScore</i>	K_id12003066	12	48.54	7.37	14.75	0.363 BM24
	<i>qDsym</i>	K_id12003066	12	48.54	7.24	16.69	2.129 BM24



**Figure 2** QTLs detected rice yellow mottle virus (RYMV) resistance and agronomical traits

## DISCUSSION

The present study was undertaken to identify QTL-trait association using F<sub>2</sub> mapping population derived from

resistant (BM24) and susceptible (IR64) parents for *Rice yellow mottle virus* (RYMV). The F<sub>2</sub> population was phenotyped for RYMV resistance related traits and for five (5) agronomical traits followed by genotyping with SNP markers and subsequent identification of potential QTLs linked to RYMV tolerance under RYMV effect. Tolerance to RYMV in our experiments is estimated by the ability of lines to limit symptom appearance. The first symptom was observed 9 DAI in parent IR64. Also IR64 recorded 7 as disease score at very early stage and confirmed its susceptibility to RYMV. Similar results were previously reported by Ghesquière *et al.* (1997), Albar *et al.* (2003), Ventelon-Debout *et al.* (2008), Kam (2011) and Orjuela *et al.* (2013). The susceptible parent IR64 recorded zero values for number of fertile panicle, panicle weight and single plant yield, indicating the importance of RYMV on reducing rice yield on susceptible varieties. Early studies reported also yield losses in highly susceptible varieties (Fomba, 1988 and Abo *et al.*, 1998). However BM24 recorded 1 as disease score and the first symptom was observed on it from 35 DAI. This found indicate that BM24 could be use as tolerant parent in breeding RYMV resistant genotypes. The difference between the two parents decreased with time, and an average of 15 DAI was recorded for F<sub>2</sub> population, could be considered optimal for assessing the response to RYMV infection in the progenies.

Polymorphism is a measure of genetic diversity and varies with the parental combinations used (Reddy *et al.*, 2005). So a total of one hundred and ninety one SNPs markers were used to screen the two parental lines (IR64 and BM24). One hundred eighty markers (94.24%) showed polymorphism between the parents. High polymorphism between two parental lines included IR64 were early reported by several studies (Albar *et al.*, 1998; Septiningsih *et al.*, 2003). However, this result is not in accordance with those of Reddy *et al.* (2005) and Swamy *et al.* (2014) who reported lower polymorphism. The high percentage of polymorphism may be due to a higher degree of genetic diversity between the two parents used in this study.

Boisnard *et al.* (2007) reported that their QTL on chromosome 12 is close to the *indica / japonica* zone of differentiation and bracketed in an interval of 2.23 Mb containing the marker RM101. This interval is relatively large and makes the tagging and the fine mapping of the QTL12 implicated in the partial resistance of Azucena difficult. In our study, the SNP markers between IR64 and BM24 permitted to identify QTLs close to SNPs (K\_id12006801 and K\_id12003066) on chromosome 12. This result is a break-through to the identification and the cloning of the gene(s) involve(s).

Among the QTLs identified, *qDiScore* and *qDSym* associated to RYMV resistance explained phenotypic variance more than 10%. Also, all QTLs identified had a positive additive effect. In the present study, we used composite interval mapping (CIM) method to map QTLs associated to RYMV resistance and agro-morphological traits under RYMV effect. Two QTLs were identified for the two RYMV resistance related traits (*qDiScore* and *qDSym*) located in chromosome 12. The contribution of these QTLs to phenotypic variation was 14.75% and 16.67% respectively for *qDiScore* and *qDSym* and located at same position on the chromosome 12. According to Collar *et al.* (2005), these QTLs could be considered as major because, they explained coefficient of

phenotypic variation superior to 10%. These results are in accordance to those of Ghesquière *et al.* (1997), Albar *et al.* (1998); Boisnard *et al.* (2007) and Pidon (2016) who reported a major QTL located at the same position on chromosome 12. In addition the two QTLs identified for RYMV resistance were located in the same position on chromosome 12, indicating that these traits (day to first symptom and disease score) could be positively correlated. Thus, this region might contain one or more key genes for RYMV resistance. Albar *et al.* (1998) found that the symptoms and virus content were highly correlated. This could explain the closeness of the two QTLs found in our study on chromosome 12 for the appearance of the symptoms and the disease score. These two QTLs could be the same given their adjacency. This corroborate the preliminary study by Ghesquière *et al.* (1997) who identified only a single Quantitative Trait Locus (QTL) on chromosome 12 to be implicated in the resistance of Azucena.

In addition two QTLs were identified for number of fertile panicle (FertPan) under RYMV effect. These QTLs (*qFertPan1* and *qFertPan2*) are located on chromosome 4 and 12, and contribute a total of 11.86% to phenotypic variation. The complementary effect of these two QTLs could be the major genetic factor controlling fertility or sterility under RYMV to BM24 contrary to Azucena where QTLs on chromosomes 12 and 7 were evidenced as genetic factors controlling resistance (Ahmadi *et al.*, 2001; Pressoir *et al.*, 1998).

QTLs associated with number of fertile panicle were also detected in similar regions as previous reported by Reddy *et al.* (2005), Zhou *et al.* (2013), Swamy *et al.* (2014) using wilds species, Yue *et al.* (2015) under low Nitrogen level, Konate *et al.* (2016) under drought condition and Singh *et al.* (2017) under stagnant flooding condition.

All QTLs obtained through this study recorded positive values of additive effect, indicating that favorable alleles are from the resistant parent (BM24). Also, all were consistent as reported earlier, indicating that these QTLs were reliable because of the repeatability in different studies with various segregation populations.

In our study, QTLs were not detected for plant height, tiller numbers, panicle weight and single plant yield at a threshold of 3.107 whereas in Albar *et al.* (1998), QTLs were detected for plant height and grain yield. However, a QTL was detected on chromosome 4 at a threshold of 3.0 at position 103.4 (data not shown). This could be explained by the high threshold used in our study (above 3). The decrease of the threshold (below 3) could have help to identify a QTL for plant height.

Pyramiding the QTLs found in BM24 with the major genes and their different alleles found in *O. glaberrima* and *O. sativa* (Ndjiondjop *et al.*, 1999; Albar *et al.*, 2003; Albar *et al.*, 2006; Rakotomalala *et al.*, 2008; Thiémélé *et al.*, 2010; Pidon *et al.*, 2017) could help to have a durable and sustainable resistance against RYMV in new varieties.

## CONCLUSION

*Rice yellow mottle virus* continues to be one of the most devastating diseases in rice production in Africa. Breeding of resistant cultivars is one of the major objectives of current

breeding programs. In this study, 190 F<sub>2</sub> lines from IR64 X BM24 were screened for RYMV resistance and used for QTLs analysis. The study identified two major QTLs associated to RYMV resistance mapped on chromosome 12. In addition two other QTLs associated with number of fertile panicle under RYMV effect were identified on chromosome 4 and 12. QTLs identified in this study can be employed in marker assisted breeding for selection of RYMV resistant genotypes in rice breeding.

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