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Identification of the Loci Associated with Resistance to Banana *Xanthomonas* Wilt (*Xanthomonas vasicola* pv. *musacearum*) Using DArTSeq Markers and Continuous Mapping

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Abstract: Banana *Xanthomonas* wilt, caused by *Xanthomonas vasicola* pv. *musacearum* (*Xvm*), is a devastating disease that results in total yield loss of affected plants. Resistance to the disease is limited in *Musa acuminata*, but it has been identified so far in the *zebrina* subspecies. This study identified markers associated with tolerance to *Xvm* in Monyet, a tetraploid banana from the *zebrina* subspecies which was identified to be partially resistant to the bacterium. We used a triploid progeny of 135 F_1 hybrids resulting from a cross between Monyet (*Xvm* partially resistant) and Kokopo (diploid and *Xvm* susceptible). The F_1 hybrids were screened in pots for resistance to *Xvm*. The population was genotyped using the genotyping-by-sequencing platform of Diversity Array Technology (DArTSeq). The adjusted means of the phenotypic data were combined with the allele frequencies of the genotypic data in continuous mapping. We identified 25 SNPs associated with resistance to *Xvm*, and these were grouped into five quantitative traits loci (QTL) on chromosomes 2, 3, 6, and 7. For each marker, we identified the favorable allele and the additive effect of replacing the reference allele with the alternative allele. The comparison between weevil borer (*Cosmopolites sordidus* (Germar)) and *Xvm* QTL revealed one QTL shared between the two biotic stresses at the distal end of chromosome 6 but with a repulsion linkage. This linkage should be broken down by generating more recombinants in the region. We also identified 18 putative alleles in the vicinity of the SNPs associated with resistance to *Xvm*. Among the 18 putative genes, two particularly putative genes, namely, *Ma06_g13550* and *Ma06_g36840*, are most likely linked to disease resistance. This study is a basis for marker-assisted selection to improve banana resistance to banana Xanthomonas wilt, especially in East and Central Africa where the disease is still devastating the crop.

Keywords: banana; banana bacterial wilt; banana *Xanthomonas* wilt; *Musa*; *Xanthomonas vasicola* pv. *musacearum*; *Xvm*

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1. Introduction

Banana *Xanthomonas* wilt (BXW) is one of the devastating diseases of bananas (*Musa* spp.). The disease is caused by the bacterium *Xanthomonas vasicola* pv. *musacearum* (*Xvm*, formerly known as *Xanthomonas campestris* pv. *musacearum*), and results in yield losses of up to 80 to 100% [1]. The disease was first reported in Ethiopia in the 1960s, on ensete, a close relative of bananas [2]. It was first reported outside Ethiopia in Uganda in 2001 [3]. Since then, it has spread to the rest of the Great Lakes Region of East and Central Africa

and is now a banana production constraint in Uganda, the eastern Democratic Republic of Congo, western Kenya, northwest and western Tanzania, and the whole of Rwanda and Burundi [4–6]. Since its first occurrence, the disease has swiftly and negatively impacted the livelihoods of the people in the region, in terms of income and food security [7,8].

The Gram-negative bacterium enters the plant through flower scars and especially contaminated cutting tools used in field management. The first symptoms of the disease include yellowing and wilting of plants, discoloration at the tip of the flower, withering of the flower bracts, premature ripening and rotting of the fruits, and a distinctive yellowish liquid that oozes out of the pseudostem upon cutting [9–11]. The plant dies within a month from the first appearance of the symptoms [12].

There are no known chemical or biological control measures for BXW [11,13]. Cultural management is based on minimizing the spread of the disease. It includes the destruction of affected plants within a mat, disinfection of the tools used in the plantation, the use of clean planting materials, early removal of male buds, and quarantine measures [9,13,14]. With proper deployment, these methods can significantly reduce the incidence of the disease, as exemplified in Uganda [8,15]. However, their sustainability requires keeping awareness high and active, and it comes with a sizable financial investment [16,17]. Therefore, complete eradication of the disease cannot be achieved as the disease keeps reoccurring and spreading anew.

Resistance to BXW is limited in bananas. All the banana cultivars in the Great Lakes region of East and Central Africa are susceptible [8,10]. These include the East African highland cooking banana (EAHB-AAAh, $2n = 3x = 33$ and EAHB-AA ($2n = 2x = 22$), a main source of livelihood for smallholder farmers in the region. When no resistance is identified in cultivars, crop improvement turns to the wild relatives of the species to identify resistant genotypes and use them to develop resistant varieties. So far, wild banana, *Musa balbisiana*, one of the two progenitors of cultivated bananas, has been found to be resistant [10,18]. However, its use in cross-breeding as a source of resistance is restricted due to the endogenous Banana Streak Virus (eBSV) [19]. *M. acuminata*, the second progenitor of cultivated bananas, is widely used in crossbreeding as a source of resistance to pests and diseases in bananas [20]. However, *Xvm* resistance in *M. acuminata* is limited. A recent study [18] identified and confirmed partial resistance in *M. acuminata* ssp. *zebrina*, in the genotypes ITC1179 Monyet, ITC1178 Buitenzorg, ITC1177 Zebrina, and ITC0728 Maia Oa. Partial resistance was also identified in ITC1224 Kikundi and ITC0019 I.C.2, while many other *M. acuminata* genotypes were susceptible, including ITC1233 Kokopo. Beyond crossbreeding, resistant genotypes have been successfully developed with genetic engineering using *Hrap* and *Pflp* genes from sweet pepper and *Xa2* from rice that confer resistance to BXW [21–23]. Although their adoption rate by end-users is potentially high, as estimated by Ainembabazi, et al. [24] in an ex-ante study, the release of the resistant genotypes from genetic engineering is still hampered by the lack of a regulation system for genetically modified organisms in most of sub-Saharan Africa. Gene editing has also given promising results, by targeting the *Musa* ortholog genes already known to confer susceptibility to bacterial diseases [25].

We build on a study that screened 72 *Musa* accessions for resistance to *Xvm* [18] to identify loci associated with resistance to BXW using F_1 progenies from a cross between Monyet, a BXW partially resistant genotype, and Kokopo, a highly susceptible genotype. We used the continuous mapping method. This method uses allele-frequency estimates instead of genotype calls to counter the low sequencing depth of genotype by sequencing data, which results in miscalls, especially in polyploid populations [26,27].

2. Materials and Methods

2.1. Plant Material

This study used F_1 progeny from a cross between Monyet (ITC1179) and Kokopo (ITC1233) [28]. The female parent, Monyet, a tetraploid ($2n = 4x = 44$) genotype of the *zebrina* subspecies that is also resistant to weevils (*Cosmopolites sordidus* (Germar)) and

fusarium wilt race 1 [28,29], was one of the few *M. acuminata* genotypes identified as mildly resistant to BXW [18]. Monyet is recorded in the *Musa* International Transit Center as a diploid (https://www.crop-diversity.org/mgis/accession/01BEL0841179 (accessed on 6 November 2023)), but the ploidy analysis of the Monyet plants at the International Institute of Tropical Agriculture (IITA) in Uganda found the plants to be tetraploid (4*x*) [28,29]. Kokopo (ITC1233), a diploid (2*n* = 2*x* = 22) *M. acuminata* ssp. *banksii* genotype, which is highly susceptible to BXW [18], was used as the male parent for this population. We attempted a reciprocal cross of the two parents with Monyet as the male parent and Kokopo as the female parent. The cross did not produce any seeds because of the low pollen count of Monyet. The ploidy level of the progeny was confirmed as 3*x* for 94.3% of the population, with the rest being 2*x* (2.58%) and 4*x* (3.09%) [28]. This study used only the 3*x* progeny from the population.

2.2. Phenotyping

Phenotyping was carried out at IITA, Sendusu station in Uganda (0°31′30″ N; 32°36′54″ E, 1260 m above sea level), in a confined pot trial. Because of the low suckering behavior of the population, which resulted in a limited availability of the planting material at a given time, phenotyping was carried out in three separate experiments, where each experiment was laid out as a completely randomized block design with three blocks. Each block contained four plants per genotype. The parental genotypes were repeated in each experiment as controls and seven F_1 hybrids were repeated in two experiments. The first experiment had 122 F_1 hybrids and was evaluated from April to July 2019. The second experiment had 26 F_1 hybrids and was evaluated from September to December 2020, and the third experiment had 41 F_1 hybrids and was evaluated from October 2021 to February 2022. For each genotype, 12 plants were raised from healthy disease-free corms that were pared and treated with Dursban (chlorpyrifos, belonging to the phosphonothioate group of organophosphorus pesticides, Dow AgroSciences LLC) for 20 min to eliminate nematodes and weevils prior to planting. The plants were established in 13 L containers containing sterilized top forest soil, manure, and sawdust at a ratio of 3:1:1. In total, 182 F_1 hybrids were screened, together with the two parents.

The plants were inoculated three months after planting. Screening was performed using U40L2, the *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) isolate from Uganda. The isolate was confirmed to be *Xvm* using *Xvm*-specific primers [30] and characterized as sublineage II [31,32]. To prepare the inoculum, U40L2 isolate previously stored at −80 °C was multiplied in a Yeast Peptone Dextrose (YPD) broth medium and incubated at 28 °C for 48 h. The inoculum was adjusted to 1×10^8 colony forming units per mL (~0.5 OD_{600}) using a spectrophotometer (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA) before inoculation. Nine of the twelve plants for each genotype, or three plants per genotype per block per batch, were inoculated with *Xvm* [18]. The remaining three plants served as controls and were inoculated with sterile distilled water without *Xvm*.

Data were collected weekly for 105 days from the day of inoculation (dpi). The parameters were the number of functional leaves, the number of leaves wilting, and the death of the entire plant. The time interval between inoculation and the appearance of disease symptoms and complete wilting (as dpi) was computed by counting the number of days from inoculation to symptom development. The time to complete wilting of the plant was computed by counting the number of days from inoculation to the death of the entire plant. A disease severity scale of 1 to 3 was used to compute the disease index using Equation (1).

$$DI = (((1 \times A) + (2 \times B) + (3 \times C))/\text{total number of plants}) \times 100 \quad (1)$$

where DI = disease index;

A = number of plants with an inoculated leaf showing symptoms;

B = number of plants with uninoculated leaves showing symptoms;

C = number of wilted (dead) plants [18].

Average data for each weekly score per hybrid was used to compute the area under the disease progress curve (AUDPC) to quantitatively summarize disease intensity over time and to analyze differences among hybrids. The calculation was performed using Equation (2) by Ceballos, et al. [33] and Forbes, et al. [34]:

$$f(\text{AUDPC}) = \sum_{i=1}^{N_i-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} + t_i) \quad (2)$$

where t = time in weeks of each reading;
Y = percentage of affected plants at each reading;
N = number of observations;
i = reading.

2.3. Analysis of the Phenotypic Data

An exponential model relating AUDPC to dpi for each individual plant was fitted using exponential or asymptotic regression as implemented in Genstat 22nd Edition [35]. The model is:

$$\text{AUDPC} = \text{A} + \text{B} \times \text{R}^{\text{X}}, \quad (3)$$

which represents a curve rising to a plateau or asymptote at the value defined by the parameter A (=maxAUDPC). The direction of the response was selected as "right", corresponding to a value of R greater than 0 but less than 1. Parameter B represents the growth rate.

In the next step, two linear mixed models (Equations (4) and (5), where the random terms are underlined) were fitted to the individual maxAUDPC values as follows:

$$y_{ijk} = u_0 + Exp_k + \underline{Exp{\cdot}Block_{j(k)}} + \underline{Hybrids_i} + Parents_i + error_{ijk} \quad (4)$$

$$y_{ijk} = u_0 + Exp_k + \underline{Exp{\cdot}Block_{j(k)}} + Hybrids_i + Parents_i + error_{ijk} \quad (5)$$

Both models are similar with fixed experiment and parent effects and random block effects nested within experiment. The only difference between models 4 and 5 is that in model 4, the F_1 hybrids were modeled as random effects to allow the estimation of the genetic variance component and heritability according to Cullis et al. [36], and in model 5, the F_1 hybrids were modeled as fixed effects to produce adjusted means (Best Linear Unbiased Estimates, BLUEs), which were used in the subsequent mapping. The fitting of both models was performed using the preliminary single environment analysis menu, selected from the Stats > QTLs (linkage/association) > Phenotypic Analysis menu in Genstat. The homogeneity in the residual variance and the normality of the residuals were checked visually using residual plots.

2.4. Genotyping

The genotyping procedure and the genotypic data for this population are described in Uwimana, Mwanje, Batte, Akech, Shah, Vuylsteke, and Swennen [28]. Briefly, genomic DNA was extracted from cigar leaves using the CTAB method as described by Das, et al. [37]. The DNA concentration was estimated using a NanoDropTM 2000 spectrometer (ThermoFisher Scientific), and 50 µL per sample at a concentration of 75 ng/µL was sent to Biosciences Eastern and Central Africa–International Livestock Research Institute (BecA–ILRI) for genotyping using DArTSeq under the Integrated Genotyping Service and Support (IGSS). Genome complexity reduction was performed using the enzyme *PstI* for library preparation. The data were received as fastq files. After trimming the adapters using the FASTX-Toolkit version 0.0.132, the sequences in the fastq files were aligned to the sequence of the second version of the double haploid (DH) Pahang banana reference genome [38] using Bowtie2 version 2.3.4.1 [39]. The data were filtered for a minor allele frequency of 0.02. This genotyping resulted in 18,009 segregating SNPs, with 14,254 SNPs segregating in Kokopo only, 3067 SNPs segregating in Monyet only, and 688 SNPs segregating in both parents [28].

2.5. Continuous Mapping

Continuous mapping was performed in 135 F_1 hybrids that were genotyped and phenotyped. Because of the limited resources to construct linkage maps and perform QTL analyses in odd ploidy levels such as triploids, and the low sequencing depth associated with DArTSeq, which is often associated with genotype miscalling in polyploids, genotype calling was not carried out for the F_1 hybrids. Instead, continuous mapping [27,28] was performed by regressing the adjusted means of maxAUDPC on the allele frequencies per SNP marker locus, followed by calculating *p*-values to assess the significance of the phenotype-SNP association and the estimation of the additive effects. The allele frequency bias was corrected using the formula in Equation (6).

$$\text{Correction factor} = \frac{1}{1 + {}^{2}/_{S_T}} \tag{6}$$

where S_T represents the average sequencing depth across all samples [27,28].

Because continuous mapping involves conducting multiple significance tests along the genome, it needs adjustment for multiple comparisons. Assuming an independent test at every marker is a very conservative assumption, particularly when large LD blocks are present, as is the case in an F_1 mapping population in general. We assumed, therefore, that the number of LD blocks = 50 and calculated the threshold as $-\log10(\alpha/\#\text{LD blocks}) = 3$, where the parameter α is the genome-wide Type I error rate (default 0.05).

2.6. Identification of Putative Genes Linked with the Resistance Loci

The genes in the vicinity ($\leq$2.5 Mbp) of the identified loci linked with *Xvm* were identified using the annotated file of the DH Pahang reference genome version 2 [38]. ClosestBed function of bedtools (v2.29.0) was used to query the annotation file based on the physical positions of the SNPs [40]. The attributes of the identified putative genes were searched in the gene list of the protein analysis using evolutionary relationships gene ontology (PANTHER 18.0) with the *M. acuminata* spp. *malaccensis* database as the reference [41].

3. Results

3.1. Phenotypic Variation and Heritability

Symptoms started to appear in the inoculated susceptible parent Kokopo as early as 21 days post-inoculation (dpi), with progressive yellowing and wilting of the inoculated leaves. The uninoculated leaves of Kokopo started showing symptoms at 35 dpi, indicating a fast progression of the disease within the plant. By 42 dpi, all nine *Xvm*-inoculated plants of Kokopo were dead. In Monyet, symptoms on inoculated leaves were first observed at 28 dpi. Uninoculated leaves of this genotype started showing symptoms by 58 dpi. In contrast to Kokopo, none of the Monyet plants died. At the end of the experiment (105 dpi), Monyet was significantly more resistant than Kokopo, having a maxAUDPC of 201.17 compared with 321.93 (Figure 1).

The F_1 hybrids started showing symptoms in the uninoculated leaves as early as 7 dpi in the most susceptible genotype and at 63 dpi in the most resistant genotype. Fitting model 1 showed a relatively large genetic variance component in comparison with the other sources of random variation, resulting in a broad sense heritability of 0.62 for maxAUDPC. Fitting model 2 produced the adjusted means for each of the F_1 hybrids. For both models, residuals were consistent with an independent sample from a normal distribution with constant variance. The histogram of residuals was reasonably symmetrical, and there was no evidence of variance changing in relation to the fitted values. The normal plots were reasonably close to a straight line, giving no evidence of departure from the model assumptions of normality and constant variance. The adjusted maxAUDPC means for the F_1 hybrids ranged from 14.59, for genotype F1MK-108, to 394.52, for genotype F1MK-144.

The population showed transgressive segregation, with 17 F_1 hybrids being more resistant than Monyet and 9 F_1 hybrids being more susceptible than Kokopo.

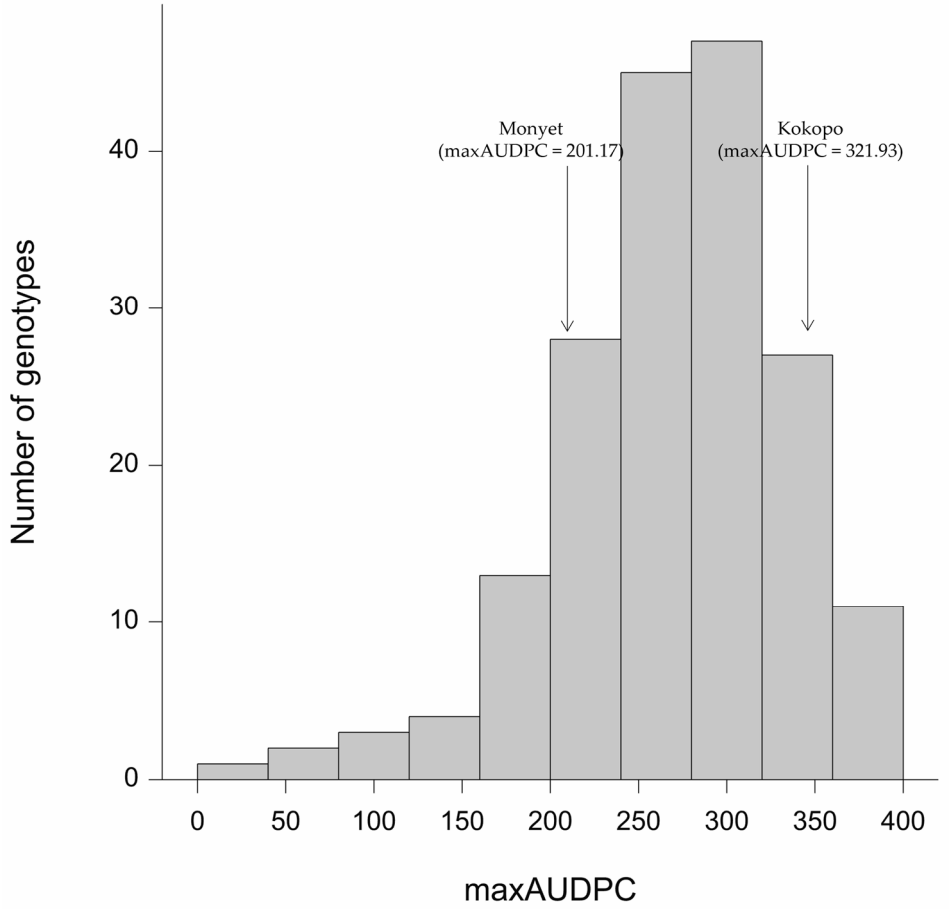

Figure 1. Distribution of the adjusted means of maxAUDPC for 182 F_1 hybrids and the parents at 105 dpi. The positions of the parents (Monyet and Kokopo) are indicated in the figure.

3.2. Continuous Mapping

Continuous QTL mapping identified 25 markers significantly ($p \leq 0.001$, equivalent to $-\log(p) \geq 3$) associated with resistance to *Xvm* (Table 1, Figure 2). Sixteen markers (64%) were located on chromosome 6 where they clustered into two distinct QTL at the two distal ends of the chromosome (Figure 2). Chromosome 7 also had a QTL that contained six markers that were significantly associated with the trait. The remaining markers were located on chromosomes 2 (two markers) and 3 (one marker) (Table 1, Figure 2). Considering the five QTLs (Figure 2), the additive explained variance sums up to 44.5% (Table 1), with every QTL represented by the most significant marker. Continuous mapping also estimated the effect of replacing the reference allele with the alternative allele at each locus (Table 1). The reference allele was linked to a positive effect for 15 markers, meaning that whenever the reference allele was replaced with the alternative allele, maxAUDPC (susceptibility) increased (Table 1). For the remaining 11 markers, the favorable allele was the alternative allele (negative effect, Table 1). At every marker, Monyet had two to four counts of the favorable allele, resulting in 87.5% frequency for the favorable alleles. Kokopo, the susceptible parent also carried favorable alleles, mostly in a heterozygous state, for 20 out of 26 markers, resulting in a frequency of the favorable alleles of 54% (Table 1).

Table 1. SNP markers associated with resistance to *Xvm* using maxAUDPC as the trait in the Monyet × Kokopo F_1 population.

sn	SNP ID	Chrom [i]	Position [i]	−LOG(*p*)	Genotype of the Parents		Effect [ii]	R [iii]	A [iii]	expl_var. [iv]
					Monyet	Kokopo				
1	chr02_23268545	2	3,268,545	3.04	RRAA	RR	−29.88	G	**A**	7.22
2	chr02_23894612	2	3,894,612	3.14	RRAA	RR	−30.89	A	**G**	7.53
3	chr03_25228251	3	5,228,251	3.15	AAAA	RA	−37.80	A	**G**	7.54
4	chr06_9287277	6	9,287,277	3.16	AAAA	RA	−40.01	A	**G**	7.58
5	chr06_10081954	6	10,081,954	3.66	RRRR	RA	36.90	**T**	A	9.18
6	chr06_10081996	6	10,081,996	3.72	RRRR	RA	37.22	**G**	T	9.36
7	chr06_11466145	6	11,466,145	3.25	RRRR	RA	34.98	**A**	G	8.20
8	chr06_11997622	6	11,997,622	3.12	RRRR	RA	40.21	**G**	T	7.52
9	chr06_31895299	6	31,895,299	3.53	RRAA	RR	30.47	**G**	T	8.66
10	chr06_35307807	6	35,307,807	4.49	RRRR	RA	41.98	**T**	C	11.55
11	chr06_35399010	6	35,399,010	3.08	AAAA	RA	−33.41	A	**T**	7.35
12	chr06_35734979	6	35,734,979	3.96	AAAA	RA	−35.05	G	**A**	10.00
13	chr06_35735014	6	35,735,014	3.74	RRRR	RA	33.67	**A**	G	9.36
14	chr06_35773242	6	35,773,242	3.11	RRRR	RA	38.85	**G**	C	7.53
15	chr06_36095415	6	36,095,415	3.13	RRRR	RA	43.69	**T**	C	7.47
16	chr06_36497284	6	36,497,284	3.24	AAAA	RA	−38.84	G	**T**	8.18
17	chr06_36497290	6	36,497,290	3.22	AAAA	RA	−38.89	G	**T**	8.17
18	chr06_36497314	6	36,497,314	3.24	RRRR	RA	41.56	**T**	C	8.15
19	chr06_37429651	6	37,429,651	3.22	AAAA	RA	−38.61	A	**G**	7.86
20	chr07_2838116	7	2,838,116	3.38	RRRA	RR	42.82	**C**	T	8.21
21	chr07_2838135	7	2,838,135	3.47	RRRA	RR	43.45	**G**	C	8.49
22	chr07_2838162	7	2,838,162	3.45	RRRA	RR	43.26	**T**	A	8.43
23	chr07_5962374	7	5,962,374	3.02	AAAA	RA	−39.48	A	**G**	7.42
24	chr07_6863391	7	6,863,391	3.11	RRRR	RA	45.63	**C**	A	7.49
25	chr07_6863416	7	6,863,416	3.25	RRRR	RA	46.44	**A**	C	7.90

[i] Chromosome and position in bp for the markers significantly associated with maxAUDPC based on the physical map of the DH Pahang reference genome version 2; [ii] additive effects of replacing the reference allele with the alternative allele at each locus; [iii] R: reference allele, A: alternative allele, positive for the trait at each marker is in bold, based on the additive effect of replacing the reference allele with the alternative allele; [iv] percentage of the phenotype explained by the marker. The grey and white colors indicate different QTLs.

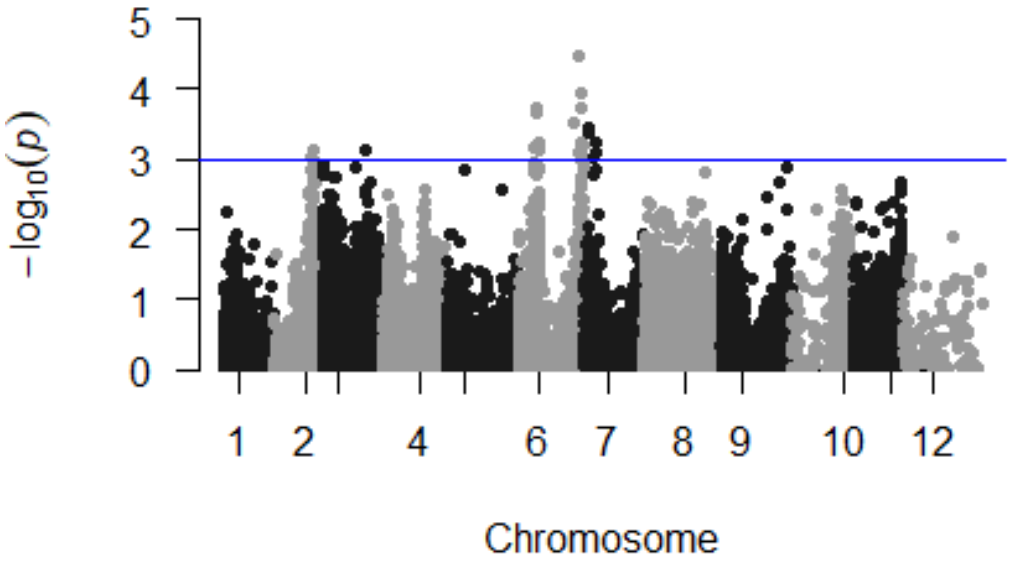

Figure 2. Manhattan plot showing the significance of the association between *Xvm* resistance (measured as maxAUDPC) and 18,009 DArTSeq SNPs, identified with the continuous mapping method using 135 F_1 progeny from the Monyet × Kokopo mapping population. Chromosome 12 represents the unanchored contigs of the DH Pahang reference genome.

3.3. Comparison between Weevil and BXW Resistance

As mentioned above, the same Monyet × Kokopo population was used to identify the loci associated with weevil (*Cosmopolites sordidus* (Germar)) resistance (Section 2.1) using peripheral damage (PD) and total cross-section damage (TXD) of the corm as traits [28]. The correlation analysis of adjusted F_1 means showed a low negative but significant correlation

between the two weevil resistance traits (PD and TXD) with maxAUDPC ($r = -0.21$ and -0.22 and $p \leq 0.05$, respectively, Figure 3). We also compared the positions and allelic effect of the loci identified for resistance to *Xvm* with those associated with weevil resistance based on the physical map of the DH Pahang reference genome version 2 using MapChart 2.32 [42]. There were three QTLs unique to banana resistance to weevils on chromosomes (two for peripheral damage on chromosomes 5 and 8, and one for total cross-section damage at the beginning of chromosome 6) and four QTLs unique to banana resistance to *Xvm*. One QTL was shared between weevil and *Xvm* resistance at the distal end of chromosome 6 (Figure 4). While PD and maxAUDPC had many shared markers, which allowed us to compare the two, TXD had only one marker in the region. The allelic effects of the shared markers and those in the vicinity between peripheral damage and maxAUDPC show an antagonistic effect (repulsion linkage) between the two traits, with Monyet carrying four copies (homozygous) of the favorable alleles for resistance to *Xvm* but the same alleles being unfavorable for resistance to weevil damage as peripheral damage. In contrast, Kokopo is heterozygous for the favorable and unfavorable alleles.

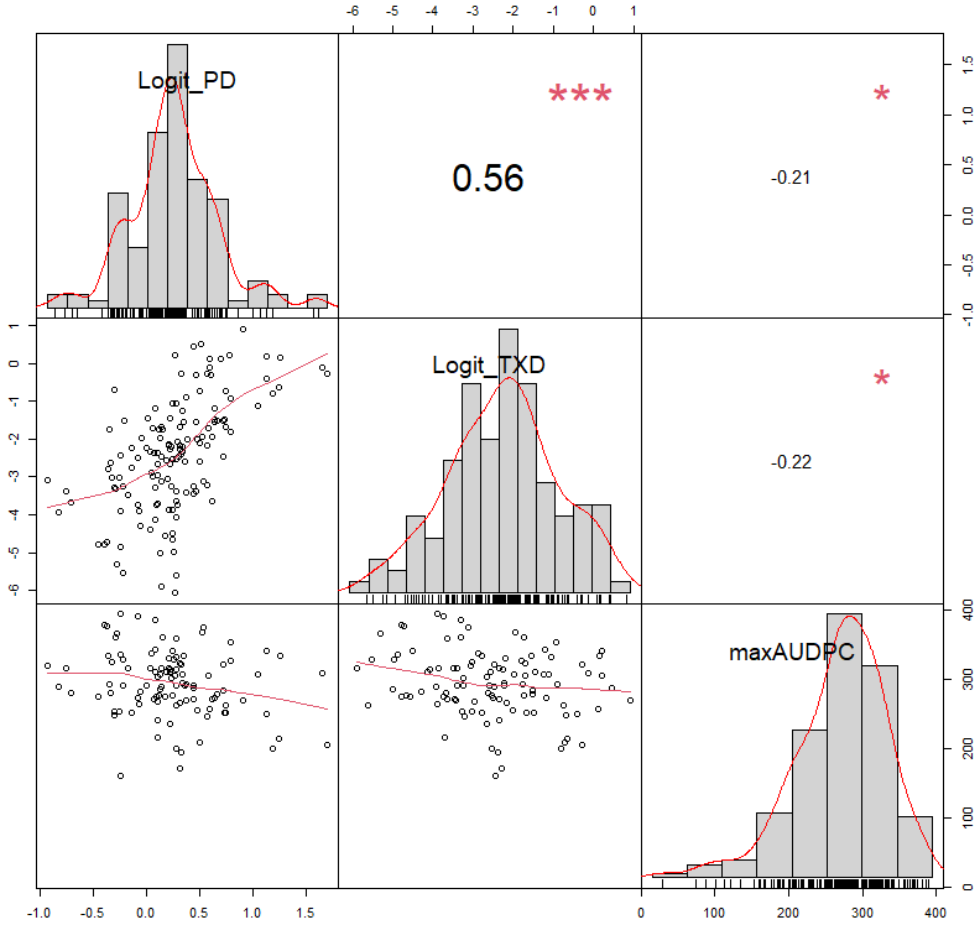

Figure 3. Pearson's correlation between the F_1 adjusted means of the weevil traits (logit_PD and logit_TXD [28]) and the *Xvm* trait (maxAUDPC). *** $p \leq 0.001$, * $p \leq 0.05$.

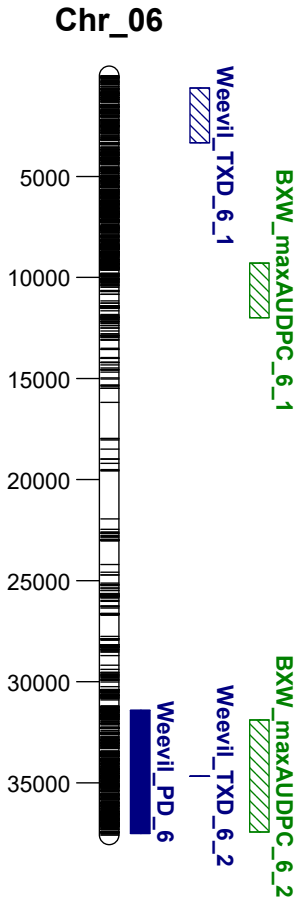

Figure 4. Chromosome 6 of the markers in the Monyet × Kokopo population based on the physical map of DH Pahang reference genome version 2 showing the QTL for resistance to weevils (blue) and BXW (green) on the right side of the bar. The beginning and the end of each QTL are represented by the first and the last significant marker position on the chromosome. Full QTL bar = Kokopo as the main source of resistance, barred bar = Monyet as the source of resistance. The left side of the bar shows the physical position of the markers in Mbp. PD = peripheral damage of the corm; TXD = total cross-section damage of the corm.

3.4. Putative Genes

The search for the putative genes returned 18 genes in the vicinity of the markers associated with resistance to *Xvm* with one to three markers per gene (Table 2). Ten of the genes had significant SNPs within the gene sequences, while the remaining eight were located at a maximum distance of 2.2 kbp. The PANTHER analysis returned 25 biological processes for the 18 putative genes. Thirteen of the processes fell under the unclassified category, while twelve were classified into six functional categories (Figure 5). Among the seven biological processes, three are most likely associated with disease resistance, namely, the biological process involved in interspecies interaction between organisms (GO:0044419), the immune system process (GO:0002376), and the response to stimulus (GO:0050896). Two putative genes were involved in the three above-mentioned biological functions, namely, *Ma06_g13550* (biological process involved in the interspecies interaction between organisms, the immune system, and the response to stimulus) and *Ma06_g36840* (the response to stimulus). The two putative genes were annotated as MACPF domain-containing protein At4g24290-like and transmembrane protein adipocyte-associated 1-like, respectively (Table 2). We also obtained the list of 1851 putative genes found in the four QTL regions but not in the vicinity of the significant SNPs (those with more than one significant marker). The list is provided as Supplementary Table S1.

Table 2. Putative genes in the vicinity of the loci that are associated with resistance to *Xvm*.

sn	Gene_ID	Chrom	Gene Position (bp)	Strand	Linked Marker	Distance (bp)	Annotation
1	*Ma02_g15610*	chr02	23,270,730–23,272,339	+	chr02_23268545	2185	Remorin_C domain-containing protein
2	*Ma02_g16600*	chr02	23,890,072–23,894,146	+	chr02_23894612	466	Lipoyl synthase, mitochondrial
3	*Ma03_g19990*	chr03	25,228,695–25,230,557	+	chr03_25228251	444	Abscisic stress-ripening protein 5
4	*Ma06_g13550*	chr06	9,286,287–9,294,510	–	chr06_9287277	0	MACPF domain-containing protein At4g24290-like
5	*Ma06_g14760*	chr06	10,081,045–10,082,123	+	chr06_10081954 and chr06_10081996	0	Conserved hypothetical protein
6	*Ma06_g16910*	chr06	11,467,120–11,467,747	–	chr06_11466145	975	Zinc finger protein ZAT12-like
7	*Ma06_g17690*	chr06	11,985,303–12,004,743	+	chr06_11997622	0	HUA2-like protein 2
8	*Ma06_g30680*	chr06	31,884,480–31,898,896	+	chr06_31895299	0	Non-lysosomal glucosylceramidase-like
9	*Ma06_g35490*	chr06	35,304,891–35,315,265	+	chr06_35307807	0	Amino-acid N-acetyltransferase
10	*Ma06_g35620*	chr06	35,399,155–35,400,631	+	chr06_35399010	145	Transcription factor MYB44
11	*Ma06_g36200*	chr06	35,734,408–35,738,511	–	chr06_35734979 and chr06_35735014	0	RRM domain-containing protein
12	*Ma06_g36300*	chr06	35,774,897–357,76,327	+	chr06_35773242	1655	HMA domain-containing protein
13	*Ma06_g36840*	chr06	36,091,779–36,096,147	+	chr06_36095415	0	Transmembrane protein adipocyte-associated 1-like
14	*Ma06_g37450*	chr06	36,492,067–36,495,406	–	chr06_36497284, chr06_36497290, and chr06_36497314	1878	Clp R domain-containing protein
15	*Ma06_g38780*	chr06	37,415,987–37,427,552	–	chr06_37429651	2099	Oxysterol-binding protein-related protein 2A
16	*Ma07_g03700*	chr07	2,836,251–2,840,247	+	chr07_2838116, chr07_2838135, and chr07_2838162	0	M20_dimer domain-containing protein
17	*Ma07_g08000*	chr07	5,961,864–5,962,783	+	chr07_5962374	0	CASP-like protein
18	*Ma07_g09140*	chr07	6,857,345–6,870,535	–	chr07_6863391 and chr07_6863416	0	Na_H_Exchanger domain-containing protein

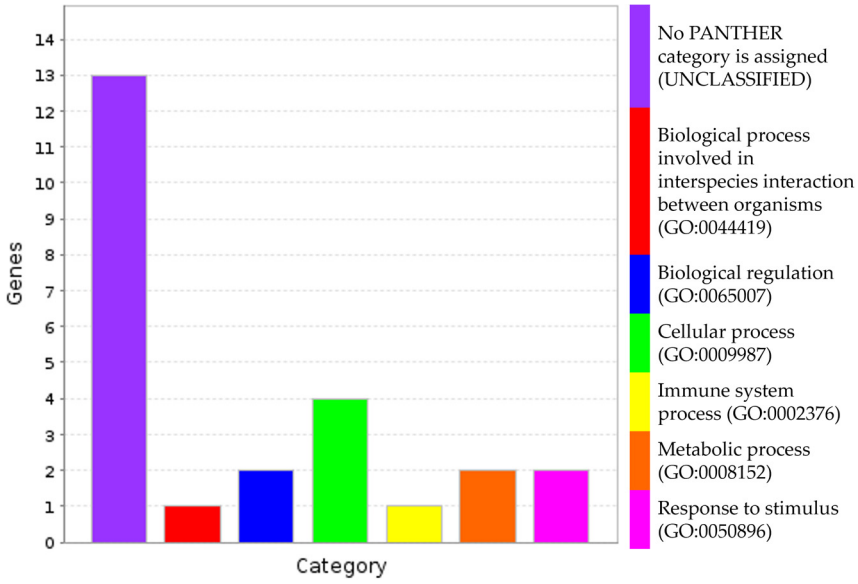

Figure 5. PANTHER GO-slim biological processes of the 18 putative genes returned 33 hits (https://pantherdb.org, (accessed on 9 November 2023)).

4. Discussion

Banana production is handicapped by many biotic and abiotic stresses that reduce yield. The particularity of *X. vasicola* pv. *musacearum* is that affected plants produce non-edible fruits, hence causing high yield and economic losses [1,7,8]. The screening of 72 accessions from the banana collection in Sendusu revealed limited resistance to the disease in *Musa acuminata,* mostly identified in the subspecies *zebrina* [18]. This study evaluated the response of triploid banana hybrids from a bi-parental population of 182 hybrids generated by crossing mildly resistant Monyet (4*x*) with highly susceptible Kokopo (2*x*).

The parents behaved as expected, with Kokopo being identified as highly susceptible and Monyet as moderately resistant [18]. The cross between the two parents produced highly resistant genotypes through transgressive segregation [43]. Although Kokopo is the susceptible genotype, it also carries alleles associated with resistance at a rate of 54% in a heterozygous state. Therefore, transgressive segregation could be a result of the dispersion of favorable alleles from the two parents [44]. Alternatively, the observed transgressive segregation could be a result of heterosis through hybrid vigor, as often observed among polyploid hybrids [45]. These hybrids constitute, so far, the most *Xvm*-resistant genotypes known from the *Musa acuminata* genome and are valuable in breeding for resistance to the disease. Given their triploid level [28], their resistance should be transferred into a diploid background to increase the seed set and the ease of crossing for an improvement in the parents. However, if the observed transgressive segregation is from heterosis, its exploitation might be difficult as hybrid vigor disappears with increased homozygosity and may result in inbreeding depression through introgression, especially in a cross-pollinated crop such as banana [46].

A number of studies have applied the continuous mapping method in genetic and genomic analyses with low sequencing depth data to counter the effect of low-sequencing depth on genotype calling in polyploids [26,28,47]. The major drawback of continuous mapping is that its results do not give any information about the genetic inheritance pattern of the trait, such as the effect of dominance, epistasis, and linkage disequilibrium as a result of linkage [28], which is not the case for genomic selection. The major strengths of continuous mapping, however, are its simple technique (i.e., simple linear regression), which achieves fast QTL results without a heavy computational load, and its ability to circumvent the accurate calling of the allele dosage genotypes and the lack of resources to construct linkage maps and perform QTL analyses in odd ploidy levels [48]. Continuous mapping identified 25 markers significantly associated with resistance to *Xvm*. The markers clustered into five main QTLs, which were in line with the medium broad-sense heritability of 0.62. The additive effect of replacing the reference allele with the alternative allele was determined at each marker point. This study complements the recent marker–trait association studies for pests and diseases in bananas for fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* [49–51] and weevils or *Cosmopolites sordidus* (Germar) [28]. We did not find any common loci between resistance to fusarium wilt and resistance to *Xvm*, neither due to the differences in the pathogens nor because the studies were performed in different genetic backgrounds (*M. acuminata* spp. *malaccensis* for fusarium wilt vs. *Musa acuminata* spp. *banksii* and *zebrina* in this study). We compared the results of resistance to weevils and resistance to *Xvm* since the two were performed in the same population. There were three QTLs unique to banana resistance to weevil and four QTLs unique to banana resistance to *Xvm*. We identified a common locus between the two biotic stresses at the distal end of chromosome 6 for the traits maxAUDPC (*Xvm*), peripheral damage (resistance to weevil), and total cross-section damage (resistance to weevil). The allelic effects suggest a repulsion linkage phase between the weevil (peripheral damage) and *Xvm* traits, which was corroborated by the negative correlation between the weevil and BXW traits. Repulsion linkage between traits is undesirable as it complicates selection. More recombinants in the region are needed to estimate the intensity and, if possible, to break down this antagonistic linkage [52–54].

We identified 18 putative genes in the vicinity of the markers associated with resistance to BXW. Thirteen out of twenty-five biological processes of the identified putative genes were unclassified. The two genes of interest, *Ma06_g13550* and *Ma06_g36840*, have been associated with resistance. *Ma06_g13550* belongs to the membrane attack complex component/perforin (MACPF) domain-containing protein *CAD1* (constitutively activated cell death 1) family, a domain operating at the plasma membrane level and involved in resistance through cellular response to stress, programmed cell death, and the innate immune system. In a model organism, *Arabidopsis thaliana*, *At4g24290* and *CAD1* from the same family and the closest orthologs to *Ma06_g13550* confer resistance to bacterial pathogens [55,56]. The gene is a close ortholog of *Zm00001eb241040* (protein C0PFJ5) in maize [57] and *Os02g0475300* (protein Q6K741) in rice [58], with speculated similar functions. Ma06_g36840 belongs to the transmembrane protein adipocyte-associated 1 (PTHR15876) family and operates at the plasma membrane level. It is involved in the G protein-coupled receptor signaling pathway and plays a role in plant–microbe interactions [59]. Among the monocot crops, it is closest to *Zm00001eb214610* (protein *B6T9J0*) [57] and *TraesCS5A02G393300* (protein *A0A3B6KPS1*) in wheat [60].

Zebrina is one of the three *M. acuminata* subspecies that are the main contributors to edible bananas, together with *banksii*, and *malaccensis* [61–64]. In the evaluation of various accessions for their response to *Xvm*, *zebrina* was found to be partially resistant, while *banksii* and *malaccensis* were found to be highly susceptible [18]. That study screened 72 wild and cultivated *Musa* spp., and so far, it is the most extensive evaluation of banana germplasm for *Xvm* resistance. There is a need to expand the search for resistance to *Xvm* in bananas by screening wild genotypes. The markers associated with resistance to *Xvm*, as identified in this study, will enable a fast genotyping-based screening of wild diploid accessions closely related to Monyet or belonging to the *zebrina* subspecies [63]. These, in contrast to 4x Monyet, will be readily usable for introgression in the improvement of diploid parents. They will also help to establish whether the other genotypes that are resistant or partially resistant to *Xvm*, namely, the *M. acuminata* spp. *zebrina* genotypes (ITC0728 Maia Oa, ITC1177 Zebrina, and ITC1178 Buitenzorg), the non-*zebrina M. acuminata* genotypes (ITC1224 Kikundi and ITC0019 I.C.2), and those containing the B-genome (MMC 192 *M.* balbisiana, ITC0116 Saba, ITC1120 Tani, ITC0396 Pelipita, ITC0246 Cameroun, and ITC0243 Pisang Raja) [18], share the same resistance alleles as Monyet or constitute other sources of resistance to Xvm.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10010087/s1, Table S1: List of all the genes in the QTL regions (chromosomes 2, 6 and 7) associated with resistance to banana Xanthomonas wilt based on the DH Pahang reference genome version 2.

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