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SHORT COMMUNICATION

Mfu16 is an unstable fire blight resistance QTL on linkage group 16 of *Malus fusca* MAL0045

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Abstract

A strong fire blight resistance QTL (*Mfu10*) was previously detected on linkage group 10 (LG10) of *Malus fusca* accession MAL0045, using several strains of the causative bacterium, *Erwinia amylovora*. As no strain capable of breaking the resistance of MAL0045 has been found, we hypothesized that another locus contributes to its fire blight resistance. However, none was detected with strains previously tested on the progeny. Here, an *avrRpt2*$_{EA}$ mutant strain (Ea1038) with the chromosomal S-allele deleted and complemented with the less aggressive C-allele, was used to phenotype MAL0045 × 'Idared' progeny. We performed phenotype-genotype analyses using the first genetic map of MAL0045, which is scarcely dense, and a recently constructed saturated map. As expected, *Mfu10* was detected on LG10 with Ea1038, as was previously with other strains. Interestingly, a QTL with a logarithm of odds (LOD) thresholds of 5.5 and 2.9, significant at the genome-wide and chromosome levels, respectively, was detected with Ea1038 on LG16 (*Mfu16*) in a subset of 76 individuals, but only using the saturated map. Progenies carrying both *Mfu10* and *Mfu16* were significantly more resistant than progenies carrying only *Mfu10*. However, the LOD of *Mfu16* diminished to 2.6 in a larger subset of individuals. We hypothesize that *Mfu16* is present in the genome of MAL0045 albeit unstable in the progeny.

Keywords *Erwinia amylovora* strains · MAL0045 · *Mfu10* · *Mfu16* · *Malus fusca* fire blight resistance loci

The bacterium, *Erwinia amylovora*, causes fire blight – a devastating disease of the domesticated apple (*Malus domestica* Borkh.) and related species (*Malus* spp.) (Norelli et al. 2003; Peil et al. 2020; Emeriewen et al. 2019). The mechanisms by which the pathogen invades and causes disease in susceptible hosts have been extensively reviewed (Malnoy et al. 2012; Yuan et al. 2020). Similarly, the molecular strategies employed by resistant hosts for the recognition of *E. amylovora* elicitors are well documented (Khan et al. 2012; Emeriewen et al. 2019). *Malus* host resistance is mostly quantitative, evidenced by the distribution of resistant and susceptible phenotypes in *Malus* populations (Calenge et al. 2005; Durel et al. 2009; Emeriewen et al. 2014; Peil et al. 2007). Quantitative trait loci (QTLs) for fire blight resistance have been detected in apple cultivars and wild apple species accessions (reviewed in Emeriewen et al. 2019; Peil et al. 2020). Although most apple cultivars are more susceptible to the disease, their QTLs are faster to introgress but not sufficient to provide strong resistance as those of their wild relatives (Durel et al. 2009; Peil et al. 2007, 2019; Emeriewen et al. 2017a, 2021b). Moreover, fire blight resistance candidate genes have been proposed only in wild apple genotypes (Parravicini et al. 2011; Fahrentrapp et al. 2013; Emeriewen et al. 2018, 2021a, 2022) and, in one instance, functionally proven via transgenic and cisgenic approaches (Broggini et al. 2014; Kost et al. 2015).

A strong fire blight resistance QTL (*Mfu10*) was detected on linkage group (LG) 10 of the wild apple accession, *Malus fusca* MAL0045 (Emeriewen et al. 2014). The stability of *Mfu10* has been demonstrated using several strains of *E. amylovora* differing in virulence/aggressiveness (Emeriewen et al. 2020). In particular, the highly aggressive Canadian

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strain Ea3049, and the mutant strain ZYRKD3-1, both of which break down the resistance locus of another wild *Malus* genotype, *Malus*×*robusta* 5 (Mr5) (Peil et al. 2011; Vogt et al. 2013), could not breakdown *Mfu10* (Emeriewen et al. 2015, 2017b). However, unlike in Mr5, where minor QTLs were detected following inoculation with the aforementioned strains (Wöhner et al. 2014), no minor QTL was detected in MAL0045 following inoculations with four different strains (Emeriewen et al. 2020). It was previously reported that an amino acid switch from cysteine (C-allele) to serine (S-allele) in the avrRpt2$_{EA}$ effector protein sequence of *E. amylovora* at position 156 is responsible for virulence and resistance breakdown in Mr5 (Vogt et al. 2013), but also for increased aggressiveness in other *Malus* hosts (Emeriewen et al. 2019). The highly aggressive Canadian strain Ea3049 possesses the S-allele contributing to the high virulence/aggressiveness of this strain.

The fact that MAL0045 itself is highly resistant to Ea3049 but the average percent lesion length (PLL) of the progeny increased to 62.4 compared to 22.6 after inoculation with C-allele strain Ea222 (Emeriewen et al. 2015) led us to speculate that there might be a second factor contributing to the resistance of MAL0045, and/or that the SNP in the *avrRpt2$_{EA}$* effector of Ea3049 (Vogt et al. 2013) might not be the only virulence factor of this strain. We inoculated the F1 mapping population derived from MAL0045 × 'Idared' cross (Emeriewen et al. 2014), with a mutant strain of Ea3049. In the meantime, we developed a dense genetic map of MAL0045 using 560 genotyping-by-sequencing-generated SNPs incorporated with 53 microsatellite markers (SSRs) for MAL0045-derived F1 progeny (Emeriewen et al. 2020).

The strain used in this study, Ea1038, is a derivative of Ea3049 with the chromosomal S-allele of the *avrRpt2*$_{EA}$ effector deleted and complemented with the C-allele on an expression vector. Artificial shoot inoculation was performed on scions of up to 10 replicates of each progeny individual grafted onto rootstock M9, by cutting the youngest leaves with a pair of scissors dipped into bacterial inoculum (10^9 cfu/ml). Disease necrosis was measured in centimeters 27 days post inoculation (dpi) and converted to PLL by dividing the necrotic shoot by the total shoot length and multiplying by 100. Preliminary QTL mapping analysis was done with the phenotypic data of 76 inoculated individuals. Subsequently, the number of phenotyped individuals were increased to 121 individuals. These data were used for subsequent QTL mapping. The calculated average of PLL of all replicates of each individual was used for Kruskal–Wallis analysis and interval mapping using MapQTL 5.0 (Van Ooijen 2004). The first incomplete genetic map of MAL0045 (Emeriewen et al. 2014) and the recently developed dense map (Emeriewen et al. 2020) were used for QTL analysis. This map was established using 148 F1 individuals in total. SAS (SAS Institute) GLIMMIX (generalized linear mixed model) analysis was performed to determine whether the effects of detected loci were significantly different. For this analysis, phenotypic values (PLL) of each progeny individual as well as their marker alleles were employed.

It was possible to phenotype only 76 individuals in 2017 by artificial shoot inoculation with Ea1038. For these individuals, 61.1 and 65.7 were the mean and median PLLs, respectively. Only five individuals recorded PLLs below 10.0 with 3.3 the lowest. Fifty-two individuals recorded PLLs over 50 with 100 recorded as the highest for twelve individuals. For the parents, whilst a PLL of 9.6 was recorded for MAL0045, 100 was recorded for 'Idared'. Between 2018 and 2022, we have phenotyped 121 F1 individuals in total. The overall mean and median PLLs of these individuals are 55.3 and 59.4, respectively. The distribution of the PLLs of these individuals is shown in Fig. 1. Nine individuals recorded PLLs below 10.0 with 1.09 being the lowest in only one individual. Seventy individuals recorded PLLs over 50 with 100 recorded as the highest for five individuals. The averages of all replicates for each of the first phenotyped 76 individuals as well as the entire 121 individuals were respectively used for Kruskal–Wallis analyses and interval mapping with the latest developed MAL0045 genetic map (Emeriewen et al. 2020) as template, but also with the first genetic map (Emeriewen et al.

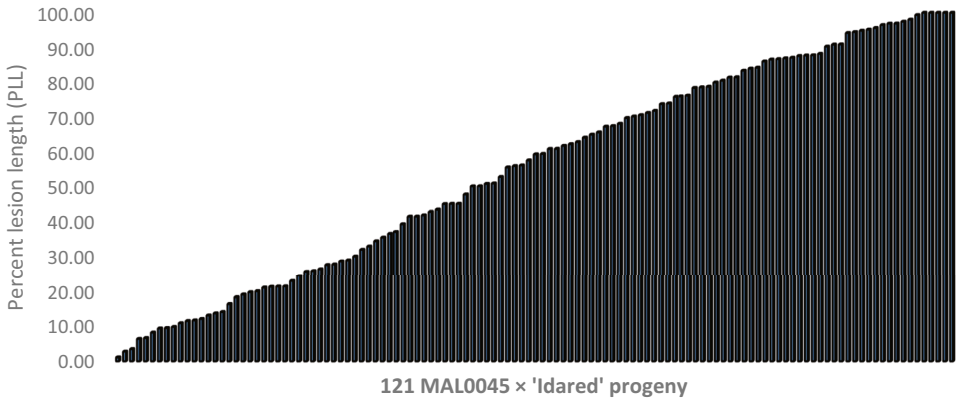

Fig. 1 Distribution of PLL of 121 individuals of MAL0045 × 'Idared' progeny phenotyped with Ea1038 over three years

2014). Kruskal–Wallis analysis with the PLL data of the 76 individuals showed that markers on LG10 (highest *K* value = 37.2), LG16 (highest *K* = 13.0) and LG17 (highest *K* = 11.6) correlated with fire blight resistance (Table 1). The most significant correlation with resistance was observed in LG10 where individuals inheriting the resistant allele of the marker with the highest *K* value (Sca_304010_602250) possessed 43.8% less necrosis than individuals inheriting the susceptible allele. For LG16 and LG17, the differences were 28.1% (Sca_304000_613165) and 22.1% (CH05b06_L4), respectively (Table 1). With the first incomplete map, only markers on LG10 showed correlation with fire blight resistance (data not shown). Furthermore, with the entire 121 individuals, the highest significant association was observed again with markers on LG10 using both maps, and the significance of markers on LG16 and LG17 diminished.

Interval mapping using the 2017 data with a genome wide (GW) threshold of 4.9 identified two QTLs of significant LOD scores on LG10 and LG16. No significant QTL was found on LG17. The QTL detected on LG10 is *Mfu10*, previously detected with other strains of *E. amylovora* since it is located in the same interval between CH03d11 and FR149B with markers possessing highly significant *K* values ($P = 0.0001$) (Table 1). However, the QTL on LG16 (*Mfu16*) is a novel minor QTL (Fig. 2a) never previously detected with any strain, which is located between 10 and 16 cM on LG16 (Fig. 2b). *Mfu16* was not detected with the initial genetic map using Ea1038. Interval mapping results was in agreement with Kruskal–Wallis analysis as SNP markers on LG16 with highly significant *K* values (Table 1) possessed the highest LODs and appear underneath the QTL plot (Fig. 2a). The significance and interaction between *Mfu10* and *Mfu16* were determined using SAS GLIMMIX analysis. Fire blight resistance was significantly stronger when resistance alleles of both loci were present in individuals compared to when individuals possessed only *Mfu10* or *Mfu16* resistance alleles (Fig. 3). Further, the resistance level of *Mfu10* alone was significantly stronger than the resistance level of *Mfu16* alone. QTL analysis with the data of the entire 121 individuals resulted in the detection of *Mfu10* (data not shown) but the significance of *Mfu16* diminished from a LOD score of 5.5 (Fig. 2a) to 2.6 (data not shown).

We are studying the interaction of *M. fusca* (MAL0045) and derived progeny with different strains of *E. amylovora* through artificial inoculation and QTL mapping. Through this process, *Mfu10* was first identified on LG10 using Ea222 (Emeriewen et al. 2014). The highly virulent Canadian strain, Ea3049, diminished the significance of *Mfu10* but did not overcome it, although this strain was not aggressive on MAL0045 (Emeriewen et al. 2015). We therefore hypothesized a second putative resistance factor, possibly another locus, might be involved in the resistance of MAL0045. The failure to detect another locus was partly attributed to the fact that the first genetic map of MAL0045 was only scarce and did not represent the whole genome. This hypothesis was predicated on the situation in Mr5 where, although one minor QTL was detected on LG5 after inoculation with Ea3049 (Peil et al. 2011), a few more minor QTLs were detected following the development of a more saturated genetic map and inoculation with different strains (Wöhner et al. 2014). The first map of MAL0045 (Emeriewen et al. 2014) consists of 213 loci made up of DArT markers, a few *M. domestica* SNPs and SSRs developed from the apple genome (Velasco

Table 1 Kruskal–Wallis analysis results for some markers on the three linkage groups of the dense genetic map showing strong correlation with fire blight resistance following inoculation with Ea1038

Marker	LG	Position (cM)	*K*	PLLs of plants with	
				Susceptible alleles	Resistance alleles
CH03d11	LG10	38.127	35.6******	85.1	42.2
Sca_313304_278642	LG10	39.404	36.2******	84.9	41.8
FR481A	LG10	41.421	31.3******	84.2	44.4
Sca_304010_602250	LG10	43.152	37.2******	84.7	40.9
FR149B	LG10	48.740	27.8******	81.7	44.7
Sca_300922_5406043	LG16	12.568	11.6****	76.8	50.3
Sca_315074_14818	LG16	14.715	12.9****	76.8	49.1
Sca_304000_613165	LG16	16.650	13.0****	76.8	48.7
Sca_300922_6119061	LG16	16.013	12.8****	76.5	48.7
Sca_315325_42556	LG17	0.000	11.2****	72.8	50.1
Sca_313414_17371	LG17	2.172	10.9****	73.2	51.2
Sca_307499_834166	LG17	2.204	10.9****	72.9	51.2
CH05b06_L4	LG17	2.808	11.6****	72.1	50.0

K Value of Kruskal–Wallis analysis (significance levels: ** = 0.05, **** = 0.005, ****** = 0.0001), *PLL* percentage lesion length

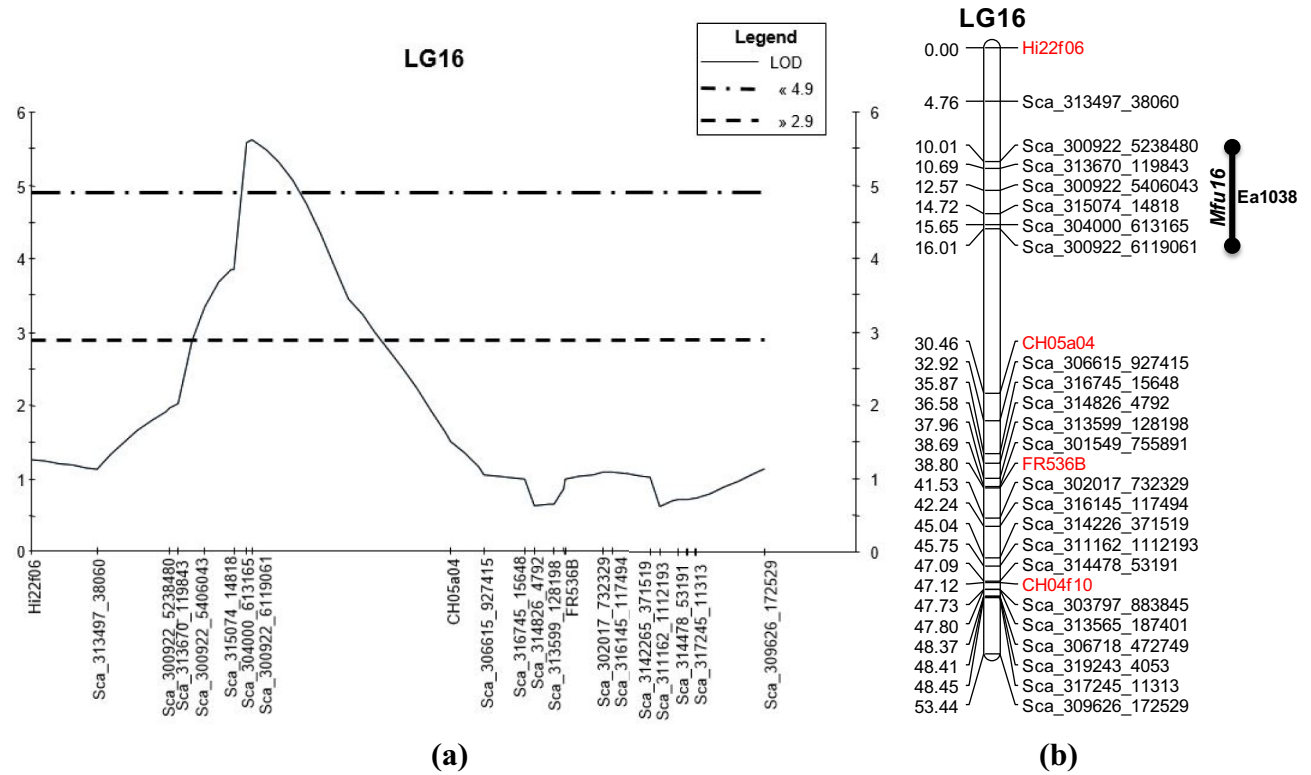

Fig. 2 LOD plot of interval mapping for the detected QTL on LG16 showing the significance at the chromosome level threshold of 2.9 and genome wide threshold of 4.9, with the markers **(a)** and the genetic map of LG16 showing the QTL region represented with a black bar **(b)**, SSR markers are highlighted in red

et al. 2010). On the other hand, using tunable genotyping-by-sequencing technology (tGBS), thousands of *de novo* SNP markers were developed for MAL0045, of which 560 SNPs were mapped including 53 SSR markers (Emeriewen et al. 2020). Thus, the dense map has 400 markers more than the initial map and correctly represents the genome of MAL0045. However, no minor fire blight locus was detected with this map (Emeriewen et al. 2020). Since fire blight resistance is strain-dependent (Vogt et al. 2013), we therefore speculated that the failure to detect a minor locus is not only dependent on the marker density of the genetic map, but it also depends on the interaction with the given *E. amylovora* strain used for inoculation.

Ea1038 phenotypic results showed that this mutant strain was as virulent as the wild type, Ea3049 (Emeriewen et al. 2015) with more than half of the entire 121 individuals having PLLs above 50. The results are also similar to the effect of Ea3049 on another wild apple *M.*×*arnoldiana* – MAL0004 (Emeriewen et al. 2017a), where the mean PLL recorded for 87 individuals was 65.9. It is interesting to note that although this mutant is complemented with the C-allele, it was still very aggressive to the individuals inoculated. This suggests that the amino acid switch from cysteine (C-allele) to serine (S-allele) at position 156 of $AvrRpt2_{EA}$ amino acid sequence (Vogt et al. 2013) is not the only factor that contributes to the pathogenicity of S-allele strains. Nevertheless, we detected two QTLs of significant LODs on two different linkage groups, LG10 and LG16. It was quite clear that *Mfu10* is the QTL detected on LG10, however, a novel minor fire blight QTL, never previously detected with any strain, was located on LG16 (*Mfu16*). We propose *Mfu16* as a minor unstable fire blight resistance QTL due to its fluctuating significance. Although, *Mfu16* was significant only in a subset of 76 individuals, and independently did not contribute significantly to resistance levels, it positively affects *Mfu10*, as the effect of both loci is significantly stronger than *Mfu10* alone in the subset of individuals. Minor fire blight QTLs were detected on LGs 5, 7, 11, and 14 of Mr5 (Wöhner et al. 2014), however, only the minor QTL on LG7 was found to contribute to resistance in addition to the major QTL on LG3.

Both the strain and the dense map were important factors in detecting *Mfu16* in this study. That this mutant strain and not the wild type Ea3049 led to the detection of *Mfu16* is indicative of a strong incompatible interaction between *M. fusca* and the C-allele of the $avrRpt2_{EA}$ effector of *E. amylovora*. In addition, the failure to detect

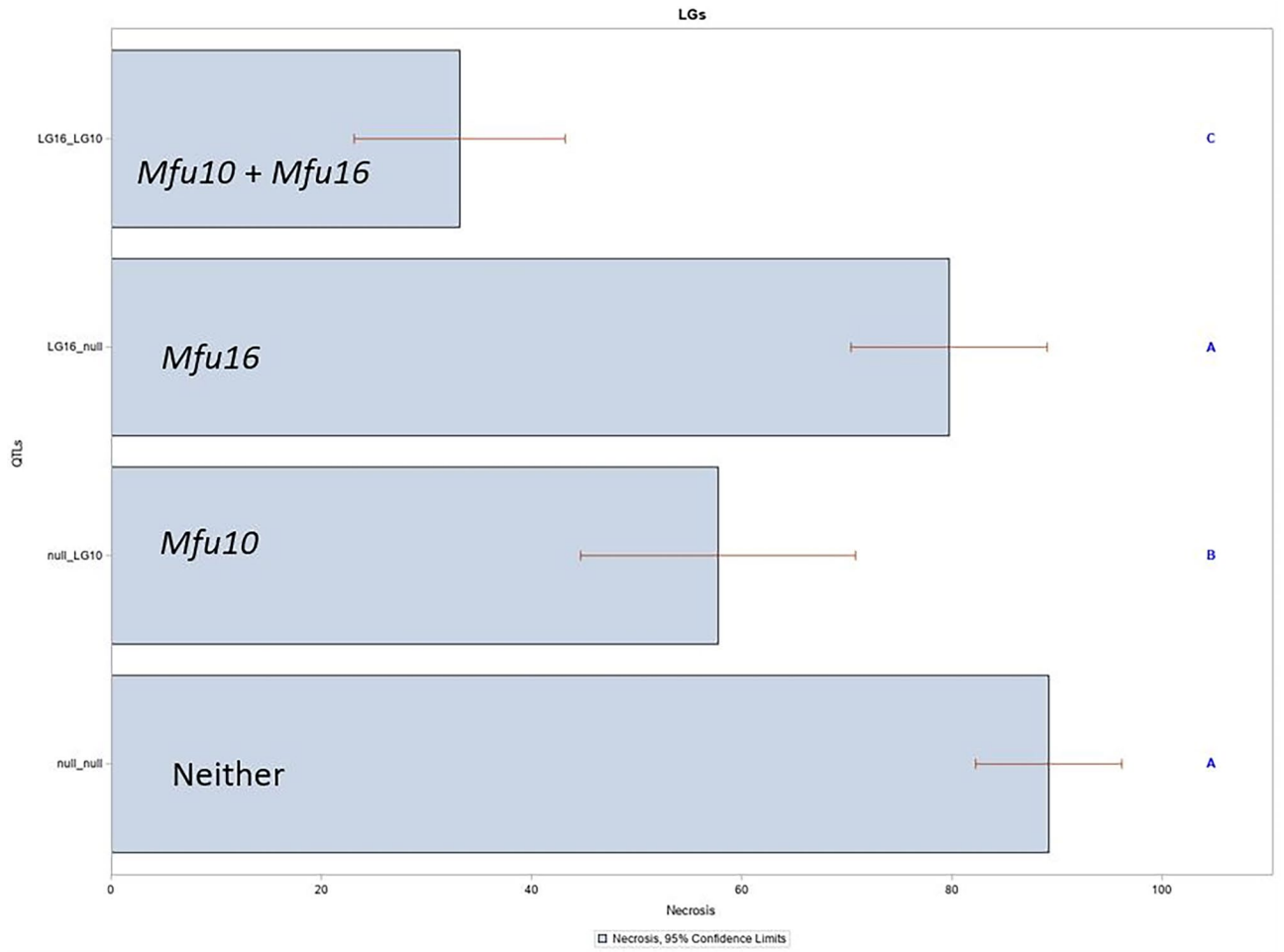

Fig. 3 Significance and interaction between *Mfu10* and *Mfu16* determined by SAS GLIMMIX analysis. LG16_LG10: both QTLs are present in these individuals; LG16_null: only LG16 QTL is present in these individuals; null_LG10: only LG10 QTL is present in these individuals; null_null: both QTLs on LG16 and LG10 are absent in these individuals

Mfu16 in the initially developed map is indicative of the important role dense genetic maps play in molecular genetics studies in *Malus* species and other plant species.

The diminished significance of *Mfu16* following the addition of more phenotypic data is unexplainable, as the results suggest that this locus is present in the MAL0045 genome otherwise it would not be detected in the first instance. Since the publication of this results as a preprint (Emeriewen et al. 2021c), we have proven this result by reevaluating the progeny by further artificial shoot inoculations, and the result has remained the same. Therefore we can conclude that this locus, though present, is not stable. However, 27 individuals of the 148 used to develop the genetic map of MAL0045 are still without phenotypic data and this could also affect the outcome of the analyses. Nevertheless, it will be worth selecting individuals carrying favourable alleles of both *Mfu16* and *Mfu10* in future pre-breeding programmes.

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Authors' contributions AP, OFE and MM for concept of the research, AP and OFE established the populations, AW developed the mutant strain, KR performed inoculations, AP and OFE performed mapping analyses and data interpretation, OFE and AP prepared the manuscript, and all authors read and approved the manuscript.

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Data availability Data generated from this study are published within this article. Further materials can be provided on request from the corresponding author.

Declarations

Competing interests The authors' declare no competing interests.

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