Article

Mapping of QTL in the Mutus#1 wheat line resistant to leaf spot

Mariel del Rosario Sánchez-Vidaña[1, 2]
Mateo Vargas-Hernández[3]
Xinyao He[4]
Carolina Paola Sansaloni[4]
Pawan Kumar-Singh[4]
Ana María Hernández-Anguiano[1§]

[1]Postgraduate in Phytosanitary-Phytopathology-Postgraduate College. Mexico-Texcoco Highway km 36.5, Montecillo, Texcoco, State of Mexico. CP. 56230. [2]Division of Life Sciences-University of Guanajuato. Highway Irapuato-Silao km 9, Ex-Hacienda El Copal, Irapuato, Guanajuato. CP. 36500. (sanchez.mdr@ugto.mx). [3]Chapingo Autonomous University. Mexico-Texcoco Highway km 38.5, Texcoco, State of Mexico. CP. 56230. (vargas_mateo@hotmail.com). [4]International Maize and Wheat Improvement Center. Mexico-Veracruz highway km 45, El Batán, Texcoco, State of Mexico. CP. 56237. (x.he@cgiar.org; c.sansaloni@cgiar.org; pk.singh@cgiar.org).

[§]Corresponding author: ahernandez@colpos.mx.

Abstract

Wheat leaf spot caused by *Zymoseptoria tritici* is a devastating disease in wheat cultivation internationally. Chemical control and the use of resistant varieties are the main control strategies. The International Maize and Wheat Improvement Center (CIMMYT) has wheat lines with quantitative resistance to the disease, so the objective was to map quantitative trait loci (QTL) associated with genetic resistance to leaf spot in the elite line Mutus#1 (resistant), in a population of 275 recombinant inbred lines (RILs) derived from the cross of Mutus#1 with the elite line Huirivis#1 (susceptible). In 2018 and 2019, a field experiment was established at the CIMMYT-Toluca station under an Alpha Lattice experimental design. An artificial epidemic was generated with *Z. tritici* and the area under the disease progress curve (AUDPC) was calculated. The 275 RILs and parents were sequenced using the DArTSeq platform. The linkage maps were constructed with the IciMapping program using phenotype and genotype information. Five minor-effect QTLs were identified, three located on chromosomes 1B, 4A and 4B and two on chromosome 5B, which explained less of the symptoms and production of pycnidia in adult plants carried by Mutus#1, they can be used with other resistance genes or QTLs to reduce the selection of new pathogenic strains of *Z. tritici*.

Keywords: *Mycosphaerella graminicola*, *Triticum aestivum*, *Zymoseptoria tritici*.

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Introduction

Leaf spot (Septoria leaf spot or leaf blight) due to *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous (syn. *Septoria tritici*, *Mycosphaerella graminicola*) (Mirdita *et al*., 2015) is a disease that negatively affects the yield and quality of wheat grain (*Triticum aestivum* L.) (Castro *et al*., 2015; Torriani *et al*., 2015). It is considered a devastating foliar disease in the high-rainfall wheat-growing regions of Africa, the United States of America, Europe, the Mediterranean and South America, where it can generate significant losses in yield, depending on the year and the variety of wheat (Mehta, 2014).

Among the strategies to control leaf spot are cultural practices, biological control, and chemical control (Mehta, 2014; Ghaffary *et al*., 2018; Sánchez-Vidaña *et al*., 2020). However, these strategies have proved complicated for the control of the disease due to the high genetic variability and biological adaptability to the selective pressure of fungicides presented by *Z. tritici* (Estep *et al*., 2015; Ghaffary *et al*., 2018), which allows it to overcome adverse conditions during the development of the crop. In addition to the above, the chemical control of the disease is expensive.

For example, in Europe approximately 70% of the resources of the cost of production are allocated only for its control (Torriani *et al*., 2015). Therefore, the use of wheat varieties with resistance to leaf spot is considered the best viable alternative to control the disease (Brown *et al*., 2015; Sánchez-Vidaña *et al*., 2020).

In wheat, two types of resistance to *Z. tritici* can be found. Qualitative (monogenic or vertical) resistance and quantitative (polygenic, horizontal, or partial) resistance (Orton *et al*., 2011; Dreisigacker *et al*., 2015). In the last decade, 21 genes (*Stb*) that give qualitative resistance and 167 quantitative character loci (QTL) in 19 biparental mapping populations, which give quantitative resistance, have been identified (Gurung *et al*., 2014; Brown *et al*., 2015).

That is why working is being done on the search for new sources of resistance with desirable agronomic characteristics (Braun and Payne 2013; Villaseñor-Mir, 2015; Piñera-Chávez *et al*., 2017), through the use of efficient improvement techniques, such as molecular marker-assisted selection, pyramiding of resistance genes, and identification of minor QTLs (Chartrain *et al*., 2004; Torriani *et al*., 2015).

Currently, although there is a range of wheat lines with acceptable agronomic yield and with at least one *Stb* gene or QTL that gives resistance to leaf spot (Simón *et al*., 2016), there is a lack of lines with durable, complete and broad-spectrum resistance (Raman and Milgate, 2012). The elite line Mutus#1 of CIMMYT has high resistance, so the objective of the study was to map QTLs associated with genetic resistance to leaf spot in the elite wheat line Mutus#1.

Materials and methods

Genetic material and field trials

Two hundred seventy-five $F_{2:7}$ RILs were established during the spring-summer (May-September) cycle of 2018 and 2019, at the CIMMYT-Toluca experimental station, State of Mexico (19° 23' north latitude and 99° 54' west longitude at an altitude of 2 600 m), with temperate subhumid climate, summer rains (Cwb) (García, 2004), minimum average temperature of 11 °C and maximum of 22 °C and 800 mm of average annual rainfall. The RILs, derived from the cross between the elite lines Huirivis#1 (susceptible female parent) and Mutus#1 (resistant male parent), were generated in 2008, using the single seed descent method (Knott and Kumar, 1974).

These parents carry the following information. Selection history: Huirivis#1, CMSS97M03642T-040Y-020Y-030M-020Y-040M-7Y-3M-0Y and Mutus#1, CMSS97M03689T-040Y-030M-020Y-030M-015Y-38M-1Y-1M-0Y. Genealogy: Huirivis#1, Hoopoe/Tanager//Veery/3/2∗Papago-89M86/4/Milan/5/Super-Seri#1 and Mutus#1, MILAN/S87230/4/BOW/NAC//VEE/3/BJY/COC (Osman *et al*., 2015). The RILs and their parents were sown in 65 m long furrows divided into plots of 0.75 m (sowing area), with spacing between plots of 0.5 m. The experimental unit consisted of two furrows where 4 g of seed was sown to steady flow at a depth of 6 cm.

According to Eyal *et al*. (1987), a mixture was prepared with six pathogenic strains of *Z. tritici*: St1 (B1), St2 (P8), St5 (OT), St6 (KK), 64 (St 81.1) and 86 (St 133.4), isolated from wheat leaves with leaf spot, to be used as an inoculum (1×10^7 spores ml^{-1}) in the inoculation of the plants. At 40 days after sowing, a first inoculation was carried out followed by two more inoculations (at seven and 14 days after the first inoculation). At each inoculation, 2.11 ml of spore suspension per wheat line was sprayed with a portable sprayer for low-volume application ©Micron Ulva+ sprayers (Herefordshire, UK, England).

Phenotypic evaluation

The development of leaf spot severity was assessed with the scales of Saari-Prescott 0-9, and double digit 00-99 (Eyal *et al*., 1987) at 21 days after the last inoculation (dai) and thereafter, every week for 21 days. Severity was estimated with the formula: severity (%)= (D1/9)×(D2/9)×100. Where: the first digit (D1) represents the height of the vertical dispersion of the disease and the second digit (D2), the severity as a function of the area of the symptomatic leaf.

Likewise, the area under the disease progress curve (AUDPC) was calculated with the equation proposed by Shaner and Finney (1977). $AUDPC= \sum_{i=1}^{n}\left[\left\{\left(Y_i+Y_{(i+1)}\right)/2\times\left(t_{(i+1)}-t_i\right)\right]\right.$. Where: Y_i = severity of the disease in time t_i; $t_{(i+1)}-t_i$= time of the interval in days between two evaluations of the disease; n= total number of evaluations of the disease; i= i-th observation. The following agronomic variables were also recorded: days to heading, when more than 50% of the plants had more than 50% of the spikes outside the flag leaf, plant height, length in cm between the base of the soil and the tip of the spike, excluding the awns, and weight of a thousand grains (g), measured in an OHAUS® triple beam digital balance, Model CT200 (Florham Park, New Jersey, USA).

Experimental design and statistical analysis

Sowing was carried out according to an Alpha Lattice design (Incomplete Blocks) (Patterson and Williams, 1976) with two repetitions; each block consisted of 20 experimental units. The data obtained were calculated the best linear unbiased estimators or means adjusted by least squares (BLUEs), the components of variance, the average standard error of the differences, the grand mean (GMean), the least significant difference and the coefficient of variation (%).

For this, the mixed model corresponding to the experimental design and spatial analysis were used using an autoregressive covariance structure of order 1, in both directions, rows and columns. The Pearson correlation coefficient between the AUDPC and the variables of days to heading, height and weight of a thousand grains was calculated with the PROC CORR procedure. All analyses were done with SAS® software (Version 9.4).

DNA extraction

The collection and preparation of samples was in accordance with the methodology proposed by Dreisigacker *et al*. (2016). DNA was extracted from leaves of 10-day-old plants grown in greenhouses using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Delobel *et al*., 2012). DNA was recovered in 100 µl of TE buffer (0.01 M of Tris-Base and 0.001 M EDTA, at pH 8) (Brunner *et al*., 2009) and adjusted to a concentration of 50 ng μl^{-1} for sequencing.

Genotyping for QTL analysis

The genotype of the mapping population was obtained with the platform of genotyping by sequencing DArTSeq (Diversity Arrays Technology), with markers of the type GBS (Genotyping by sequencing) (Li *et al*., 2015) generated in the laboratory of the Genetic Analysis Service for Agriculture (SAGA, for its acronym in Spanish) located at CIMMYT-El Batán, Texcoco, State of Mexico. The sequences of the markers were not edited for analysis. By means of the BIN function of IciMapping version 4.1, redundant and low-quality markers were removed; that is, markers with more than 30% of lost data and with high distortion in segregation. The results obtained with the GBS-type markers and markers of the 21 known *Stb* genes (Brown *et al*., 2015) were combined for QTL analysis.

Construction of linkage maps

The linkage maps were built with the lciMapping program version 4.1 (Meng *et al*., 2015). QTLs related to resistance to leaf spot (*Z. tritici*) were detected and mapped. For this, the mean of the AUDPC registered in the evaluation years 2018 and 2019 was calculated. The means of days to heading and plant height were included in the analysis to determine the relationship between the QTLs that give resistance and those that determine these variables.

The linkage groups were assigned according to the linkage likelihood logarithms (LOD) with a value of 15 and in all of them, the recombination values were converted to centimorgans (cM) with the Kosambi function. The size of the maps was estimated with the two-point mapping function (Valverde, 2007). The percentages of phenotypic variance and the additive effect were obtained by gradual regression with the QTL IciMapping program. The inclusive composite interval mapping method for QTL with additive effects (ICIM-ADD) was used, with a threshold of LOD= 2 to declare a QTL as significant.

Projection of QTL into the IWGSC reference genome

To compare the QTLs mapped with those reported in other studies, the physical positions of the markers that flanked each QTL were obtained in mega base pairs (Mb). The genome of the Chinese spring wheats RefSeq version 1.0 of the International Wheat Genome Sequencing Consortium (IWGSC) was used as a reference by means of the T3/Wheat (https://triticeaetoolbox.org/wheat/) platform with the BLAST search function.

Markers reported in the literature but not found on that platform were searched in the database for *Triticeae* and GrainGenes oats of the Agricultural Research Service of the United States Department of Agriculture (https://wheat.pw.usda.gov/cgi-bin/GG3/browse.cgi?class=marker) or in the next-generation genome navigator JBrowse (http://202.194.139.32/jbrowse-1.12.3-release/?data=Chinese_Spring&loc=chr5B%3A507727349..507728005&tracks=wheat_90K%2CPCR_markers%2CIWGSC_v1.1_HC_gene&highlight=).

Results and discussion

Phenotypic evaluation

The interpretation of the data on the phenotypic variables evaluated in this study was made considering the temperature and precipitation conditions recorded during the development of the crop in the field (Table 1). Data on the phenotypic variables showed that only the calculated means of the AUDPC and those of the weight of 1 000 grains had any important difference between the years of evaluation 2018 and 2019 (Table 2). In 2018, RILs had high AUDPC values, which was reflected in a lower weight of 1 000 grains, with 3 g less than that of 2019.

Table 1. Environmental conditions recorded at the CIMMYT-Toluca experimental station from May to September 2018 and 2019.

Year	Temperature (°C)			Average rainfall (mm)
	Minimun	Maximum	Average	
2018	6.8	22.8	14.8	163
2019	8.2	22.6	15.4	80

Table 2. Means and statistics of the phenotypic variables of the RILs derived from the cross of Huirivis#1 × Mutus#1, inoculated with *Z. tritici*, in the spring-summer cycle of 2018 and 2019, in Toluca, Mexico.

	AUDPC		Heading (days)		Height (cm)		Weight of 1 000 (g)	
	2018	2019	2018	2019	2018	2019	2018	2019
GMean	381.3	263.9	72.4	75.4	101.5	98.7	33.8	36.8
LSD	160.8	133.9	3.5	4.5	6	9.7	5.4	5
CV (%)	21.4	25.7	2.5	3	3	4.9	8.1	6.8

AUDPC= area under the disease progress curve; GMean= grand mean; LSD= least significant difference (Fisher, 0.05); CV= coefficient of variation.

These results suggest that the warmer and drier environmental conditions recorded in 2019 may not have been favorable for the development of leaf spot, resulting in a lower AUDPC and thus, greater weight of a thousand grains. Moderate temperatures, from 15 to 25 °C, with alternating wet and dry periods, promote the production of pycnidiospores and ascospores, in wheat genotypes with different degrees of susceptibility to *Z. tritici*. The highest production of pycnidiospores occurs during the heading and maturity of plants, a period in which the greatest precipitation normally occurs, and temperatures are favorable for the development of the disease (Cordo *et al*., 2017).

In this study, in 2018 there were periods of two to eight consecutive rainy days followed by one or two days without rain and average mean temperature of 15 °C. This suggests that the environmental conditions that occurred in 2018 favored the production, release and dispersion of pycnidiospores throughout the canopy of plants and led to the development of secondary infections during much of the crop cycle, as reported by (Morais *et al*., 2015).

In contrast, in 2019 there were prolonged periods of drought and erratic rains after inoculations, with periods of two to 10 days without rain from July to September, conditions that could affect the development of severe secondary infections and therefore the development of the leaf spot in the crop. The AUDPC variable is evaluated to analyze the development of foliar diseases caused by polycyclic pathogens such as *Z. tritici* (Jeger and Viljanen-Rollinson, 2001) and to estimate the relationship between disease progress and loss of the photosynthetically active area (Waggoner, 1986).

When the leaf area decreases, the number of spikes per m^2, the number of grains per spike and the weight of 1 000 grains decrease, thus affecting the yield, as mentioned by Castro *et al*. (2015). In this study, the Pearson correlation coefficient indicated that there is a negative correlation between the AUDPC and the variables height, heading and weight of 1 000 grains recorded in the RILs, in both years of evaluation (Table 3). That is, the higher the value of the AUDPC, the lower the value of the agronomic variables indicated.

Table 3. Pearson correlation coefficient between AUDPC and the variables heading, height and weight of a thousand grains, recorded in the RILs derived from the cross of Huirivis#1 × Mutus#1 in two years of evaluation.

Year	Heading	Height	Weight of a thousand grains
2018	-0.38***	-0.34***	-0.12***
2019	-0.23***	-0.3***	-0.28***

***= Highly significant correlation $p< 0.0001$.

In 2018, 26% of the RILs presented AUDPC values lower or similar to those registered by the resistant parent Mutus#1, while in 2019, it was 74% of these lines (Figure 1).

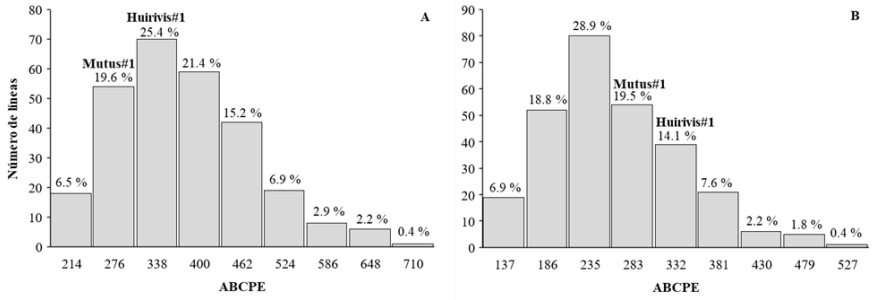

Figure 1. Frequency distribution and relative frequency (%) of the AUDPC in the RILs derived from the cross of Huirivis#1 × Mutus#1, inoculated with *Z. tritici* during the spring-summer cycle, 2018 (A) and 2019 (B), in Toluca, Mexico. Note the bar where Mutus#1 is and the lines with a value of AUDPC similar or less than this parent; these lines are considered resistant to the disease.

The results of the evaluation of the variable days to heading indicated that most of the RILs behaved as early (Table 2). The correlation between AUDPC and the days to heading was significant and negative, indicating that lines with long cycles correspond to lines with lower AUDPC value (Table 3). While those of the height variable indicated that the RILs behaved as semi-dwarf lines (with 70 and 120 cm in height) (Torres and Pietragalla, 2013).

The correlation between AUDPC and height was highly significant and negative in RILs (Table 3), which suggests, according to Rodríguez-Contreras *et al*. (2010), that tall lines had a lower degree of disease due to the lower number of secondary infections associated with leaf proximity and contact. In general, the agronomic characteristics of days to heading, height and weight of 1 000 grains, both of the resistant parent Mutus#1, and of its progeny, were stable between the years of evaluation and similar to those shown by commercial varieties and other elite lines of CIMMYT and of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP, for its acronym in Spanish) (Ramírez *et al*., 2016).

Genotyping and construction of linkage maps

The genetic analysis made with 839 polymorphic markers of high quality, with only 11% of data lost, allowed generating a total of 34 linkage groups with the ICIM-ADD mapping function. These groups represented the 21 chromosomes of the entire wheat genome. In the RIL population, the map obtained had a length of 2 169 cM with an average genetic distance between markers of 2.6 cM.

In general, in this population, chromosomes 4D, 5D and 2D had poor coverage with three (5 cM), five (28 cM) and six (39 cM) markers, respectively; in contrast, the rest of the chromosomes had better coverage by having a minimum length of 60 cM and an average length of 145 cM. Although there is no specific number of DNA markers to make linkage maps, because the number of markers depends on the number and length of the chromosomes of each organism, in preliminary genetic mapping studies, it is common to use 100 to 200 markers (Collard *et al*., 2005). However, in future studies with the lines studied here, it is suggested to try with a greater number of markers for better coverage of chromosomes 2D, 4D and 5D.

QTL analysis

In the RIL population, five minor-effect QTLs were identified, three located on chromosomes 1B, 4A, 4B and two on chromosome 5B (Figure 2), which explained less than 8.5% of the variance of the phenotype. Three of these QTLs were donated by Mutus#1, a resistant parent, and two by Huirivis#1, a susceptible parent (Table 4). This result indicates that Huirivis#1 has in its genealogy some resistance to leaf spot in adult plants and that it also represents a source of resistance to the disease, as indicated by (He *et al*., 2021).

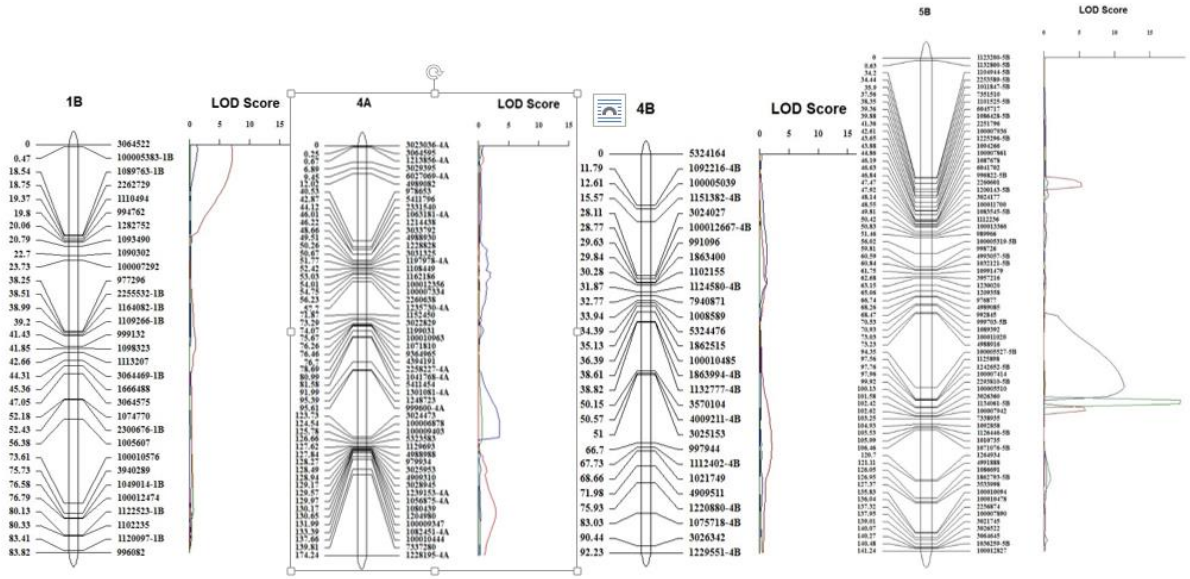

Figure 2. LOD curves of QTL on chromosomes 1B, 4A, 4B and 5B in the RILs (Huirivis#1 × Mutus#1) for resistance to leaf spot by *Z. tritici* in adult wheat plant.

Table 4. Summary of the QTL analysis for resistance to leaf spot, based on the mean AUDPC, recorded in the RILs during the spring-summer cycle of 2018 and 2019.

Chromosome†	Position‡	Marker		LOD	PVE (%)	Add	Source of resistance
		Left	Right				
1B	1	100005383	1089763	7.1	6.4	24.8	Mutus#1
4A	156	7337280	1228195	2.8	8.4	28.4	Mutus#1
4B	68	1112402	1021749	2.1	1.7	-12.9	Huirivis#1
5B.1	37	1011847	7351510	5.3	4.6	21.2	Mutus#1
5B.2	101	100005510	3026360	5.8	5.1	-22.5	Huirivis#1

†= chromosome where the QTL is. The numbers 1 and 2 placed next to the chromosome name were used to distinguish QTLs mapped in the same linkage group; ‡= maximum position in cM to the first marker linked to the linkage group of interest. LOD= score of the logarithm of odds based on 1 000 permutations; PVE= percentage of phenotypic variance explained by the QTL; Add= additive effect of phenotypic variance for each QTL.

The numbers to the left of the chromosome indicate the genetic distance in cM of the markers and those on the right, the name of the molecular marker. The graph depicts significant QTLs with LOD value >2. The AUDPC is indicated with a red line, the heading with green and the height with blue. It is common for the estimation of QTL in wheat to be based on theoretical assumptions of quantitative inheritance, so the effect of QTLs can vary in phenotype and not necessarily every estimated QTL contributes equally to the phenotype (Lan *et al*., 2015). The five QTLs mapped on chromosomes 1B, 4A, 4B and 5B in the RILs were in physical position close to some QTL associated with previously reported leaf spot resistance.

The meta-QTL of major effect MQTL3, close to the markers wPt-6975 (632.9 Mb) and wPt-5281 (644.4 Mb), located on chromosome 1B and donated by the FD3/Robigus line, explained 11% of the phenotype variance (Goudemand *et al*., 2013). In this study, the QTL mapped on chromosome 1B was found in physical position from 40.8 to 563 Mb, close to that meta-QTL MQTL3. Meanwhile, QTL on chromosome 4A (39.5-378.5 Mb) was mapped close to the major-effect QTL7 (662.7-662.7 Mb), and the QTL located on chromosome 4B (96.2-652.9 Mb) closed to those with a major effect MQTL16 (544.6 Mb) and MQTL17 (549.1 Mb), associated with the percentage of symptoms in adult plants (Goudemand *et al*., 2013).

Two QTLs were on chromosome 5B, one from 47.4 to 604 Mb and another from 489.8 to 583.6 Mb, donated by Mutus#1 (5B.1) and Huirivis#1 (5B.2), respectively (Table 4). Both QTLs were found close to the major-effect QTL 5BL (511.8 Mb) related to the severity of symptoms of leaf spot in seedling (Mergoum *et al*., 2013) and those with a minor effect QStb.lsa_af-5B (411 Mb) and QStb.lsa_fb-5B (474.2 Mb) associated with the percentage of pycnidia in adult wheat plant (Risser *et al*., 2011; Miedaner *et al*., 2012).

In the RIL population, some QTLs associated with plant height and days to heading close to some QTL that gives resistance to the disease were also detected. The QTL (39.5-378.5 Mb) on chromosome 4A was found close to a QTL (38.1-594.6 Mb) of minor effect (3.4% of the variance of the phenotype) that determined height (Figure 2) and the QTL (489.8-583.6 Mb) on chromosome 5B.2 close to two QTL: one (537.7-562.9 Mb) of minor effect related to height that explained 9% of the variance of the phenotype; and another (571.7-577.2 Mb) of major effect related to heading with 25.9% of the phenotype (Figure 2).

These results coincide with the Pearson correlation analysis (Table 3), where height turned out to have a negative correlation with AUDPC, which may be related to minor-effect QTLs close to QTLs that give resistance. Whereas the correlation between heading and AUDPC was highly significant, which coincides with the presence of the major-effect QTL related to the days to heading in the RILs.

Overall, in this study, it was found that QTLs associated with leaf spot resistance in adult wheat plants are located close to QTLs reported in recombinant populations donated by various sources of resistance, both in seedling and adult plant stage. Therefore, the results suggest that the resistance to leaf spot in the mapping population of RIL, derived from the cross of Huirivis#1 × Mutus#1, is given by the quantitative resistance controlled by the sum of effects of several minor-effect QTLs distributed in the wheat genome (Arraiano and Brown, 2017).

Based on the results obtained in this study, it is recommended to use Mutus#1 as a source of leaf spot resistance in future improvement programs, with the aim of introgressing the QTLs reported here to wheat materials of interest and of using the pyramiding with other *Stb* resistance genes to maximize the effectiveness and duration of the resistance to the disease in improved wheats as indicated by Dreisigacker *et al*. (2015).

Likewise, it is recommended to include the RILs that showed a higher level of resistance to the leaf spot than their resistant parent Mutus#1 as parents in these programs, especially the RILs corresponding to 26% of 2018. Collard *et al*. (2005) point out that RILs are homogeneous and have unique combinations of chromosome segments of the original parents, resulting from genetic recombination and Bonnett (2013) indicates that the use of RIL allows preserving polygenic traits of low heritability, and the identification of QTL of interest in early stages of improving.

Conclusions

Five minor-effect QTLs were identified, three located on chromosomes 1B, 4A and 4B and two on chromosome 5B, which explained less than 8.5% of the AUDPC variance. Specifically, QTLs on chromosomes 1B, 4A and 5B, related to the development of symptoms and production of pycnidia in adult plants, carried by the elite wheat line Mutus#1, can be used with other resistance genes or QTL to reduce the selection of new pathogenic strains of *Z. tritici*. Some RILs showed desirable agronomic characteristics and high resistance to leaf spot so they should also be considered in programs for the development of resistant varieties.

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