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QTL mapping of lentil anthracnose (*Colletotrichum lentis*) resistance from *Lens ervoides* accession IG 72815 in an interspecific RIL population

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Abstract Anthracnose, caused by *Colletotrichum lentis,* is one of the most damaging diseases of lentil (*L. culinaris*) in western Canada. *Lens ervoides* accession IG 72815 exhibits high levels of resistance to the pathogenic races 0 and 1. The objective of this study was to identify quantitative trait loci (QTL) associated with anthracnose resistance in lentil using a recombinant inbred line (RIL) population from the interspecific cross between IG 72815 and the susceptible cultivar Eston. A total of 168 RILs were genotyped and evaluated for anthracnose race 0 and race 1 reaction in the growth chamber and polyhouse. QTL analysis identified major resistance loci on chromosomes 3 and 7 for both races, accounting for 20.1–31.2% and 8.3–18.4%, respectively, of the total phenotypic variation for anthracnose resistance. Multiple classes of putative defense-related genes are located within both loci. Further characterization of these regions will facilitate the introgression of anthracnose resistance from *Lens ervoides* into elite lentil cultivars via marker-assisted selection.

Keywords Disease resistance · Pathogenic races · Quantitative trait loci · Wild lentil

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Introduction

Lentil (*Lens culinaris* Medik.) is an economically important pulse crop on a global scale that is consumed for its high levels of protein, micronutrients, vitamins, and dietary fiber (Kissinger 2016; Raghuvanshi and Singh 2009). The crop is cultivated in more than 70 countries, and western Canada accounted for 46% of the world's lentil production from 2013 to 2017 (FAOSTAT 2017). Lentil productivity in western Canada is challenged primarily by four fungal diseases: ascochyta blight (*Ascochyta lentis)*, stemphylium blight (*Stemphylium botryosum)*, aphanomyces root rot *(Aphanomyces euteiches),* and anthracnose, caused by the fungal ascomycete pathogen *Colletotrichum lentis* (Damm) (Damm et al. 2014). Anthracnose can cause up to 70% yield loss under high disease pressure (Chongo et al. 1999; Morrall and Pedersen 1990). The first incidence of anthracnose in lentil was reported in the province of

Manitoba in 1987 (Morrall 1988) and is now widespread in western Canada.

The pathogen survives as microsclerotia on lentil debris and primarily spreads between fields by wind. The disease management options include 3–4 year crop rotations, foliar fungicide application and host-plant resistance (Buchwaldt et al. 2018). Two pathogenic races of *C. lentis* were previously identified (Buchwaldt et al. 2004) and re-designated as race 0 and race 1 (Banniza et al. 2018). Race 0 has high virulence, and no effective resistance has been found in *L. culinaris* accessions; and race 1 is less virulent, against which partial resistance was identified in a number of *L. culinaris* accessions (Buchwaldt et al. 2004, 2018; Gela et al. 2020).

Current germplasm collections of lentil exhibit low genetic diversity and a narrow genetic base for anthracnose resistance (Buchwaldt et al. 2004; Gela et al. 2020). Genetic diversity can be reintroduced using crop wild relatives (reviewed by Coyne et al. 2020; Dempewolf et al. 2017). These natural genetic reservoirs retain much of the genetic diversity lost during the process of domestication and/or deliberate selection for cultivar development (Tanksley and McCouch 1997). The species of the genus *Lens* are classified into four gene pools; the primary gene pool (*L. culinaris*, *L. orientalis* and *L. tomentosus*), secondary *(L. odemensis* and *L. lamottei*); tertiary (*L. ervoides*), and quaternary gene pool (*L. nigricans)* (*see* Cubero et al. 2009; Wong et al. 2015). All the species have seven chromosomes (2n = 14) and similar karyotypes (Ladizinsky et al. 1984; Van Oss et al. 1997). *Lens ervoides* exhibit resistance to many lentil diseases, including resistance to both anthracnose races (Tullu et al. 2006), ascochyta blight (Tullu et al. 2010), and stemphylium blight (Podder et al. 2013). High level of resistance to anthracnose race 0 has not been found in other wild relatives (Barilli et al. 2020). Resistance breeding against the highly virulent race 0 of *C. lentis* is therefore especially dependent on the use of resistant germplasm from *L. ervoides*. Initial screening of *L. ervoides* identified two accessions, L-01-827A and IG 72815 with superior resistance to both races (Tullu et al. 2006; Fiala et al. 2009). Embryo rescue was used to create F_1 hybrids that were advanced to develop interspecific RIL population, such as LR-26 (*L. culinaris* Eston × *L. ervoides* IG 72815) (Tullu et al. 2013).

Of the available management strategies for anthracnose, the use of genetic sources of host resistance may be the best approach to control the disease. Little is known, however, about the genomic regions and molecular markers associated with anthracnose resistance. Therefore, more precise localization of QTL/genes is critical to the development and application of marker assisted breeding and develop strategies for pyramiding anthracnose resistance genes from multiple resistance sources into elite cultivars. Both dominant and recessive sources of genetic resistance to anthracnose race 1 are reported in *L. culinaris* intraspecific populations (Tullu et al. 2003; Buchwaldt et al. 2013). In *L. culinaris* × *L. ervoides* interspecific populations double recessive genetic inheritance of resistance to race 0 and race 1 are reported (Fiala et al. 2009; Tullu et al. 2013). Bhadauria et al. (2017) mapped QTLs conferring resistance to race 0 and race 1 on chromosomes 2, 3, 5, and 7 using an *L. ervoides* intraspecific population (L-01-827A × IG 72815). However, these QTLs are not yet mapped in interspecific populations.

In the current study we tested the hypothesis that regions of the *L. ervoides* genome that are associated with anthracnose resistance will continue to confer resistance following hybridization with *L. culinaris*. The objective was to identify the regions of the *L. ervoides* genome associated with anthracnose resistance in the LR-26 interspecific RIL population.

Material and methods

Plant materials

Evaluation of genetic resistance to *C. lentis* was conducted using 168 RILs of the LR-26 interspecific mapping population derived from a cross between *L. culinaris* Eston × *L. ervoides* IG 72815 (Tullu et al. 2013). The RILs had been advanced using single seed descent to the F_7 generation. Then the F_7-derived bulked seed of the RILs were selfed for at least three additional generations. The *L. ervoides* accession IG 72815 is from Turkey and has resistance to both anthracnose races (Tullu et al. 2006). Lentil cultivar Eston is a small seeded, yellow cotyledon, green seed coat, early maturing line released in Canada in 1980 (Slinkard 1981); it is susceptible to both races of anthracnose.

Inoculation and phenotyping for anthracnose reactions

Colletotrichum lentis isolates CT-30 (race 0) and CT-21 (race 1) (Banniza et al. 2018) were used to inoculate the LR-26 RIL population in two environments: in growth chambers at the University of Saskatchewan (USask) College of Agriculture and Bioresources phytotron facility, Saskatoon, Canada, and in an outdoor polyhouse. Fungal inoculum production and inoculation in the growth chamber was done according to methods described by Gela et al. (2020). In the growth chambers, the parents and LR-26 RILs were evaluated (inoculated with race 0 isolate CT-30 and race 1 isolate CT-21, separately) for resistance to *C. lentis*; under polyhouse conditions they were evaluated for resistance to race 0 isolate CT-30. In the growth chamber experiments, plants were grown in 38-cell cone trays (26.8 × 53.5 cm) filled with Sun Gro Horticulture Sunshine Mix LA4 (Sun Gro Horticulture, Bellevue, USA) and perlite (Specialty Vermiculite Canada, Winnipeg, MB) in a 3:1 ratio (v/v). The LR-26 RILs and the parents were randomized in a set of trays per replicate. Four-week-old seedlings were inoculated with a spore suspension (5×10^4 spores mL^{-1}) of *C. lentis* race 0 and 1 at 3 mL per plant using an airbrush. Plants were placed in an incubation chamber (relative humidity 90–100%) for 48 h before being moved to misting benches (*see* Gela et al. 2020). The disease severity data were collected per individual plant (an experimental unit). The experiment was repeated eight times for each race separately (blocked over time) and analyzed as a randomized complete block design with eight replications. Individual plants were scored for anthracnose severity at 8–10 days post-inoculation (dpi).

The polyhouse experiment was conducted at the Department of Plant Sciences field laboratory at the USask. Four seeds of each genotype, and two seeds of cultivar Eston (susceptible control), were sown in individual 1-gallon pots (15.5 cm diameter) containing a soilless mixture (Sunshine Mix No. 4, Sun Grow Horticulture® Ltd., Vancouver, BC, Canada). The plants were grown under open field conditions for 6 weeks (early flowering stage). Then a polyhouse tunnel cover of translucent thin plastic sheeting was installed by suspending it 1.5 m above the ground over the pots immediately before inoculation. The area under the cover was equipped with a misting irrigation system. Each pot was sprayed with approximately 36 ml (6 ml $plant^{-1}$) of aqueous spore suspension (5×10^4 spores mL^{-1}) of race 0 isolate CT-30 until runoff using a pressurized knapsack sprayer. The inoculations were performed in the evening to avoid high temperature conditions and to facilitate the germination of spores on the leaves. After inoculation, misting irrigation was applied starting from early morning to evening for 30 s every 15 min to promote disease development. The experiment was conducted in a randomized complete block design (RCBD) with four replications. Disease severity data were collected 14 d after inoculation using a 0 to 10 rating scale with 10% increments. Data were converted to percentage disease severity using the class midpoints for data analysis.

Statistical analysis

Disease scores data were analyzed using SAS v.9.4 (SAS Institute, Cary, USA). Normality and variance homogeneity of the residuals were tested using the Shapiro–Wilk normality test and Levene's test for homogeneity, respectively. The data did not fit the assumptions of a Gaussian distribution. As a result, a generalized linear mixed model with a binomial distribution function was fitted to the data using PROC GLIMMIX with the LOGIT link function (SAS 9.4). Means of the disease reaction scores were compared for post hoc comparison using Tukey's Honestly Significant Difference at $\alpha = 0.05$. The mean disease score data were transformed using log transformation for QTL analysis to reduce the skewness. Spearman's rank correlation of disease severity between races and test environments was performed using the CORR procedure in SAS.

Genotyping and linkage mapping

Genotyping was done as described in Chen (2018). Briefly, total genomic DNA (gDNA) from bulk leaf tissue of parents and LR-26 RILs was extracted using the DNeasy® 96 Plant Kit (Qiagen, Germany). The LR-26 RILs were genotyped following the two-enzyme (*Pst*I-*Msp*I)-based GBS protocol of Poland et al. (2012) as described in Wong et al. (2015) for lentil. The gDNA quality was checked on a 1% agarose gel, quantified using PicoGreen, normalized to a concentration of 20 ng/µl per RIL, a total of 200 ng

of gDNA per RIL was digested with *Pst*I-HF and *Msp*I, and ligated to barcoded adapters using T4 DNA ligase (New England Biolabs, Inc., Ipswich, USA). Individual libraries were then pooled (43-plexed library), bead-cleaned, PCR amplified, and bead-cleaned again. Average size and concentration of pooled libraries were estimated using a DNA2100 chip on an Agilent Bioanalyzer, and libraries were sequenced on an Illumina HiSeq.2000 instrument at the Génome Québec Innovation Centre, McGill University, Montréal Canada.

The GBS reads were processed using an open source GBS pipeline written in Perl and developed by the Pulse Bioinformatics group in the Department of Plant Sciences, USask, Canada (https://knowpulse.usask.ca/software/GBS-Pipeline; Wong et al. 2015). The pipeline demultiplexes raw reads and removes barcode sequences prior to trimming them with the recommended parameters for paired-end reads using Trimmomatic-0.38 (Bolger et al. 2014). The pipeline then aligns the trimmed reads to the reference genome, in this case *L. culinaris* cultivar CDC Redberry genome v2.0 (Ramsay et al. 2019), using Bowtie2-2.2.9 (Langmead and Salzberg 2012). The parameters for maximum fragment length were set to 11,000, maximum reseed rate was set to 5, and reads were allowed multimap up to three times before using a custom script to filter for the best hit of three. The final step in the pipeline was variant calling of the combined samples using SAMtools-1.9 and BCFtools-1.6 (Li et al. 2009) using the multiallelic calling model, respectively. Overall, a total of 833,041,263 raw reads of the GBS library were processed and resulted in detection of 167,102 raw SNPs that passed all quality controls. These were further filtered using VCFtools (Danecek et al. 2011) to retain SNPs with less than 35% missing allele calls (minimum read depth = 5) and SNPs with minor allele frequency greater than 25%. Linkage analysis was performed using ASMap (Taylor and Butler 2017) with the parameters: segregation ratio = 75:25 and p value = 10^{-5}; and treating heterozygous calls as missing values.

QTL mapping of disease resistance

Multiple QTL mapping (Manichaikul et al. 2009) and composite interval mapping (Zeng 1994) run in R/qtl software (Broman et al. 2003) were used to detect QTL. The Haley-Knott regression (Haley and Knott 1992) was used for both methods to confirm the consistency of the QTL detected due to the non-normal distribution of the phenotype. The regression-based QTL mapping methods are robust against non-Gaussian trait distribution (Rebai 1997). Multiple QTL mapping was completed with the *stepwiseqtl* function (Broman et al. 2003) and the optimal QTL model was chosen based on the highest penalized LOD score (Manichaikul et al. 2009) after forward and backward selection and elimination modelling. Penalties for model selection and genome-wide significance threshold ($\alpha = 0.05$) were determined by 1000 permutations with *scantwo* function for two-dimensional QTL scan. For composite interval mapping (CIM), five markers were selected as cofactors by forward selection to control genomic background effects. Thresholds for declaring QTL were determined by 1000 permutations at a significance of $\alpha = 0.05$. The percentage of the phenotypic variance explained (PVE) and effects of QTL were obtained by fitting a mixed linear model using the "*fitqtl*" function. The confidence intervals for each QTL were estimated using the "*lodint*" function that calculates the 1.5 LOD support intervals. The QTL intervals on the genetic map were compared against their physical positions on the CDC Redberry genome assembly v.2.0 (Ramsay et al. 2019) for the identification candidate genes associated with disease resistance. All reported disease resistance (R-) or defense-related genes in plants were considered for selection of candidate genes.

Results

Reactions of RILs to *C. lentis* infection

The resistant parent IG 72815 showed moderate to high levels of resistance to both race 0 and race 1 (35% mean disease severity), whereas the susceptible parent Eston showed susceptible reactions to both races (95% mean disease severity) in all assays. Significant variation in disease reaction was observed among the RILs for both races ($p < 0.001$). A high proportion of the lines in the population showed susceptible reactions to race 0 and race 1 under growth chamber inoculations (Fig. 1a, b). Disease severity scores ranged from 8.33 to 95% and 5 to 95%, with a mean of 79.4% and 76.0% for race 0 and race 1, respectively.

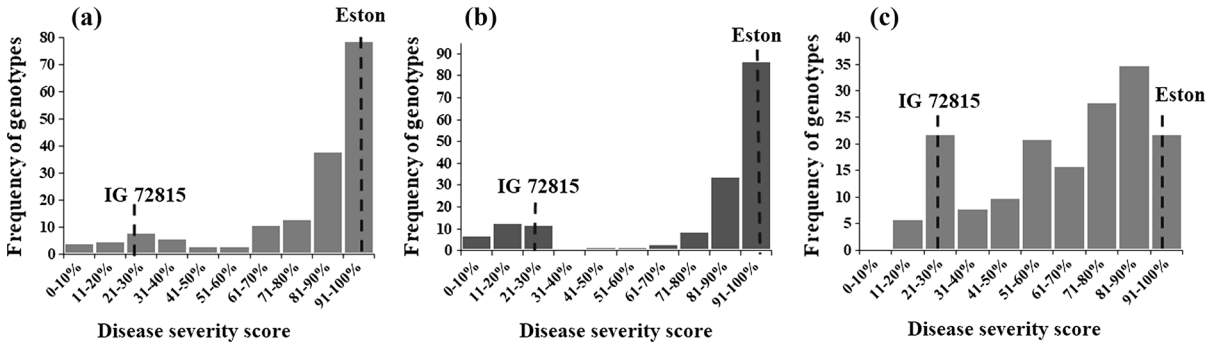

Fig. 1 Frequency distributions of percent anthracnose severity for 168 members of the interspecific RIL population LR-26 derived from the interspecific cross *Lens culinaris* Eston × *L. ervoides* IG 72815 following inoculation with: **a**) race 0, and **b**) race 1 of *C. lentis* under growth chamber (phytotron) conditions, and **c**) race 0 in a polyhouse. Disease severity was rated on a 0–10 scale, increasing in 10% increments. Data were converted to % disease severity using the class midpoints for data analysis

The polyhouse ratings of the disease reactions were continuously distributed, with a skew toward the higher level of disease severity, ranging from 12.5 to 95%, with a mean of 65.7% (Fig. 1c). Correlation analysis indicated a significant positive relationship between the results from the two races and test environments (Table 1), suggesting the resistance to both races derived from IG 72815 may be controlled by the same gene or by tightly linked genes. Chi-squared analysis of the combined data suggested that the best-fitting segregation ratio was 1 resistant: 3 susceptible (χ^2 = 1.115 at 1 d.f.; p = 0.291), supporting the presence of two complementary resistance genes that confer resistance to anthracnose.

Linkage map construction

A genetic map was constructed from 5455 SNP markers that mapped to 7 linkage groups (LGs) (Table 2). The LGs were numbered to match the respective chromosomes where markers lie in the lentil reference genome (v2.0; Ramsay et al. 2019). The linkage map spanned a total genetic distance of 3252.8 cM with an average marker interval at 0.6 cM. The genetic distance within LGs varied from 363.5 cM for LG 3 to 627.4 cM for LG 5. LG 5 contained the smallest number of SNPs, whereas LG 2 and LG 4 contained the highest number of SNPs (Table 2).

QTL for anthracnose resistance

We detected two large effect QTLs associated with anthracnose resistance (Fig. 2); one on chromosome 3, and another on chromosome 7. A genome-wide view of the QTL detected with individual data sets across the chromosomes (LGs) and the details of each QTL identified are presented in Table 3 and Fig. S2. The results were consistent across test conditions and were not sensitive to the QTL analysis methods used. QTL on a chromosome were considered the same QTL when their 1.5-LOD intervals overlapped, although

Table 1 Analysis of variance and Spearman's rank correlation of disease severity for growth chamber and polyhouse evaluations of the 168 interspecific RILs of the LR-26 lentil population inoculated with race 0 and race 1 of *C. lentis*

Tests	Analysis of variance		Correlation	
	df[a]	F value	Growth chamber race 0	Growth chamber race 1
Growth chamber race 0	167	13.5***	–	0.9***
Growth chamber race 1	167	11.4***	0.9***	–
Polyhouse race 0	167	6.0***	0.8***	0.8***

[a]Degrees of freedom, ***Significant at the 0.001 probability level

Table 2 Summary statistics of genetic linkage map of the LR-26 interspecific population derived from a cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815

Linkage groups	Number of markers	Map length (cM)	Average marker interval (cM)	Maximum gap (cM)
LG1	667	603.3	0.9	40.8
LG2	1002	443.3	0.4	19.9
LG3	783	363.5	0.5	21.2
LG4	1002	387.6	0.4	11.9
LG5	342	627.4	1.8	21.3
LG6	790	399.9	0.5	14.3
LG7	869	428.0	0.5	11.2
Total	5455	3252.8	0.6	

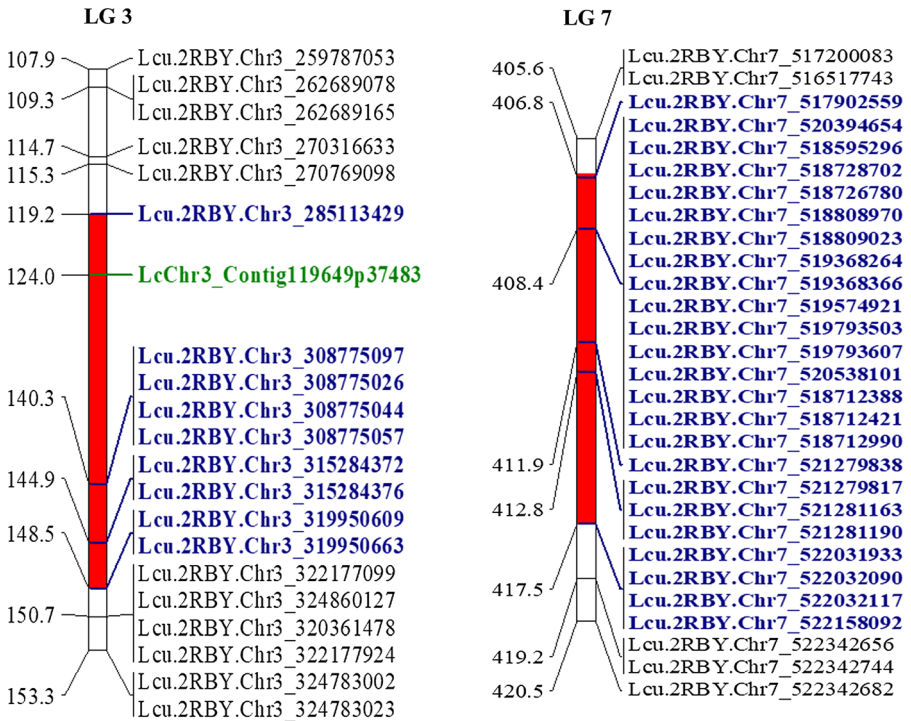

Fig. 2 Location of anthracnose resistance QTL in IG 72815 on linkage groups (LG) 3 and 7; the linkage map was constructed from an interspecific LR-26 recombinant inbred line (RIL) population derived from a cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815. The QTL positions are shown with a red bar and the loci within the QTL regions are colored with blue. The green locus indicates the position of a significant marker from Bhadauria et al. (2017). Only portions of the linkage map related to the QTL positions are displayed

the position of the peaks varied slightly. The QTL on chromosome 3 (qANTH-3) was consistently found in both environments and co-localized in the physical interval of 285.1–322.2 Mb for both races of *C. lentis*. This QTL explained 20.1 to 31.2% of the phenotypic variation. Similarly, the QTL on chromosome 7 (qANTH-7) was detected in both environments and found to overlap for both races in the interval of 518.7–522.5 Mb and explained 8.3 to 18.4% of the phenotypic variation. As expected, the resistance allele for both qANTH-3 and qANTH-7 were contributed from the wild parent IG 72815, and their attribution to both race 0 and 1 resistance also explained the high correlation observed

Table 3 Quantitative trait loci (QTL) for resistance to anthracnose races 0 and 1 in the LR-26 RIL population derived from a cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815

Environment	Race[a]	QTL	LG	Peak LOD	Position (cM)	1.5 LOD interval (cM)		PVE[b] (%)	Add[c]	Total PVE (%)
						Left	Right			
Phytotron	Race 0	qANTH.3	3	19.9	141.0	119.2	144.9	20.1	− 0.14	62.3
		qANTH.7	7	14.2	411.9	408.3	417.5	18.4	− 0.29	
	Race 1	qANTH.3	3	31.5	140.0	119.2	144.9	31.2	− 0.25	73.3
		qANTH.7	7	12.9	412.0	408.3	417.5	12.1	− 0.30	
Polyhouse	Race 0	qANTH.3	3	14.1	138.0	119.2	148.5	26.5	− 0.15	50.2
		qANTH.7	7	7.2	411.0	406.7	417.5	8.3	− 0.07	

[a]Inoculation with race 0 & race 1 of *C. lentis*, [b]Percentage of phenotypic variance explained by QTL, [c]Additive effect

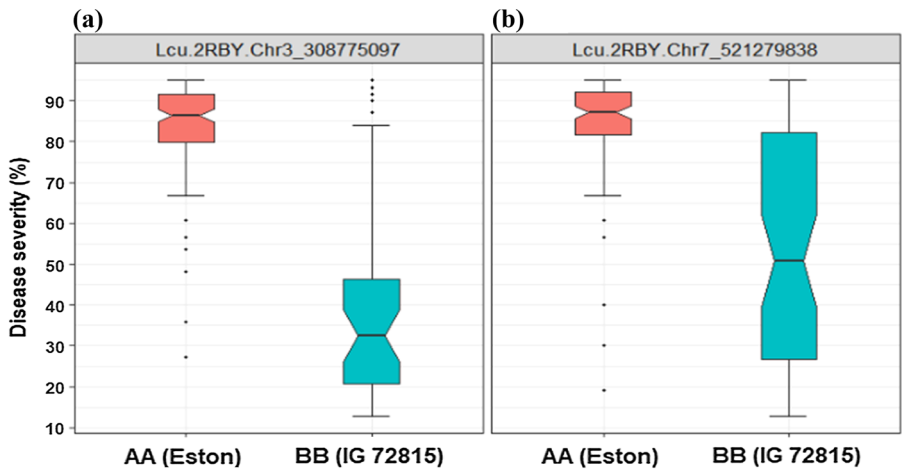

Fig. 3 Box plots showing percent anthracnose severity values of the allele classes of tightly linked SNP markers to the identified QTLs on linkage groups (LG) 3 (**a**) and 7 (**b**); the QTLs were mapped using an interspecific LR-26 recombinant inbred line (RIL) population derived from a cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815. The black horizontal lines in the middle of the boxes are the median values. The vertical size of the boxes represents the inter-quantile range. The upper and lower whiskers represent the minimum and maximum values of data

(r^2 = 0.8–0.9) between race 0 and race 1 phenotypes in the LR-26 population (Table 1). The marker effect of tightly linked SNP marker to qANTH-3 (Lcu.2RBY.Chr3_308775097) and qANTH-7 (Lcu.2RBY.Chr7_521279838) were compared with the resistance levels of the LR-26 population (Fig. 3). The homozygous resistant genotype BB (wild parent) is associated with enhanced resistance compared to the homozygous susceptible genotype AA for the combined mean disease scores.

Identification of putative candidate genes

Scanning of the two QTL regions on the annotated reference lentil genome within a 1.5-LOD interval revealed more than 290 and 140 genes of known functions on chromosomes 3 and 7, respectively. Further analysis of these candidate genes revealed 22 genes on chromosome 3 and 26 genes on chromosome 7 that were annotated as possibly associated with plant disease resistance and/or defense-related genes. Genes within these QTL intervals that may condition anthracnose resistance include NB-ARC domain disease resistance genes, LRR receptor-like kinases

(LRR-RLK), transmembrane proteins (TM), pentatricopeptide repeats proteins (PPRP), cellulose synthase proteins, ring figure proteins, serine/threonine kinase family proteins, peroxidases, ABC-transporters, and F-box proteins (Table S1).

Discussion

The absence of allelic diversity in the cultivated lentil gene pool for anthracnose race 0 resistance necessitated the introduction of resistance gene from *L. ervoides* which is in the tertiary gene pool of *Lens.* Interspecific introgression of anthracnose resistance from this source into elite cultivars could be facilitated using marker-assisted selection if there were linked markers. To detect the QTLs conditioning anthracnose resistance for race 0 and race 1 of the pathogen, we used an interspecific RIL population derived from a resistant *L. ervoides* accession, IG 72815 (Tullu et al. 2013). The RIL population showed significant variation in disease reaction for both races, conferred by resistance genes/alleles. A high positive correlation for disease reaction was found between race 0 and race 1, supporting the hypothesis that the resistance loci inherited from IG 72815 for both races can be co-localized. Similar results were reported for both races in another *L. culinaris* x *L. ervoides* interspecific population (Fiala et al. 2009), and in a *L. ervoides* intraspecific RIL population (Bhadauria et al. 2017).

The GBS-based linkage map generated for the interspecific LR-26 RIL population covered the seven chromosomes of lentil with 5455 SNP markers. The genetic linkage map spanned 3252.8 cM with an average marker density of 0.6 cM. Stange et al. (2013) reported that an increase in marker density from 5 to 1 cM could increase the power sufficiently to precisely localize and resolve closely linked QTL. Although the map size is inflated, the LR-26 genetic map developed in this study has sufficient marker density to provide adequate power for QTL mapping. Using this genetic map and the anthracnose phenotypic data of LR-26 populations, we mapped a major QTL on linkage group 3, and also on the distal end of linkage group 7. The major QTL for anthracnose resistance on chromosome 3 (qANTH-3) was derived from IG 72815, with an explained phenotypic variation ranging from 20.1 to 31.2%, conferring resistance to both races. The SNP marker previously reported in the *L. ervoides* genome by Bhadauria et al. (2017) for both races was located in the same region as this QTL (Fig. 2) and is quite likely the same QTL. Another QTL, with moderate effect, was detected on chromosome 7 (qANTH-7) and also conferred resistance to both races. Bhadauria et al. (2017) identified a QTL conferring resistance to race 0 on chromosome 7 using the intraspecific RIL population (LR-66) derived from race 0 and race 1 resistant *L. ervoides* parents (L-01-827A × IG 72815). The qANTH-7 identified in IG 72815 in this study conferred resistance to both races, possibly because accessions L-01-827A and IG 72815 carry the same race 1 resistance gene in this region of chromosome 7 and thus was not segregating in LR-66. Alternatively, this could be a different locus nearby. Murube et al. (2019) reported a co-occurring QTL conferring anthracnose resistance in common bean (*Phaseolus vulgaris* L.) to multiple races of *C. lindemuthianum*, in a region where multiple race-specific genes are found in clusters. Thus, the race 0 and race 1 resistances in accession IG 72815 could possibly be controlled by tightly clustered genes that are co-inherited.

The peak locus for qANTH-3 is 1 cM away from a large gap (21.2 cM) close to the middle of linkage group 3. This locus most likely includes the centromere; thus, the large gap interval could be due to low SNP density around the centromeric region. Underrepresentation of SNPs in pericentromeric regions were reported when using methylation-sensitive enzyme based-GBS in sorghum (*Sorghum bicolor*) (Patil et al. 2017). Moreover, Felderhoff et al. (2016) reported a locus spanning 48.7 Mb that includes the centromere, when mapping QTL for sorghum anthracnose resistance using GBS.

In this study we found a wealth of candidate genes that may play a role in disease resistance and plant defense-related genes in the two QTL regions. The molecular basis of disease resistance in plants is mediated through a suite of cellular receptors that perform direct detection of pathogenic molecules (reviewed by Andersen et al. 2018). This relies on the recognition of conserved pathogen-associated or microbe-associated molecular patterns (PAMPs or MAMPs) or effectors from pathogens (Martin et al. 2003). We identified candidate genes encoding for LRR-receptor-like kinase (LRR-RLK) and transmembrane proteins (TM), which are associated with the PAMPs response mechanisms, underlying the two

QTL. The LRR-RLK constitutes a diverse group of proteins (also called pattern recognition receptors) allowing the cell to recognize and elicit defense responses (Torii 2004). Burt et al. (2015) reported 27 LRR-RLK related candidate genes associated within a physical region of 936.46 kb for anthracnose resistance in common bean.

We also identified a NB-ARC domain disease resistance gene under both QTL. NB-ARC class genes typically encode R genes that usually detect the pathogen and activate downstream signaling, leading to pathogen resistance (Dodds and Rathjen 2010). Receptor-like serine/threonine kinases are also associated with defense mechanisms and play a vital role in the signal transduction pathway in plants (Zhou et al. 1995). The expression of genes encoding peroxidase (Almagro et al. 2009), wall-associated receptor kinase (Delteil et al. 2016) and cellulose synthase (Douchkov et al. 2016), occurs in response to attack by pathogens, resulting in the strengthening the plant cell wall, an important first line of defense.

Genes belonging to the pentatricopeptide repeat protein (PPRP) family, which are considered resistance-related genes (Sekhwal et al. 2015), were also identified. PPRP are known to affect post-transcriptional regulations such as RNA editing, splicing and translation modification (Schmitz-Linneweber and Small 2008) and are involved in plant disease resistance (Garcia-Andrade et al. 2013). Other candidate plant defense-related genes found in the QTL regions (Table S1), include zinc-finger proteins (Shi et al. 2014). Ogutcen et al. (2018) found copy number variation between *L. ervoides* and *L. culinaris* accessions in the coding regions of a zinc-finger transcription factor gene.

Conclusion

The aim of this study was to identify QTL associated with anthracnose resistance in *L. ervoides* accession IG 72815 using an interspecific RIL population. The source of resistance in *L. ervoides* accession IG 72815 appears to be derived from resistance loci on chromosomes 3 and 7 for both races, an indication that resistance is possibly controlled by tightly clustered genes that are co-inherited. The SNP markers linked to these QTL will be useful in the breeding program for marker-assisted introgression of anthracnose resistance into cultivated lentil after their validation in appropriate segregating populations. Multiple classes of candidate genes that encode plant disease resistance are identified within the QTL regions that will need to be considered in follow-up validation studies.

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Author contributions TSG generated phenotypic data, preformed data analyses and wrote the manuscript; LC performed the genotyping of the mapping population; CC carried out SNP calling; CSK constructed the genetic map, AV and KEB conceived the experiment and critically reviewed the manuscript. All authors read and approved the final version.

Data availability The raw genotypic data supporting this study are available at https:// knowpulse.usask.ca/ Geneticmap/ 2691115 upon request from the authors.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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