Mapping and Analysis of Genetic Loci Conferring Resistance to Anthracnose in Lentil

A Thesis Submitted to the
College of Graduate and Postdoctoral Studies
In Partial Fulfillment of the Requirements
For the Degree of Doctor of Philosophy
In the Department of Plant Sciences
University of Saskatchewan
Saskatoon

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ABSTRACT

Anthracnose, caused by *Colletotrichum lentis*, is an important fungal disease of lentil in western Canada. Two pathogenic races, race 0 and race 1, have been characterized. Sources of resistance to race 1 are available in the primary gene pool (*Lens culinaris*), but not for the more virulent race 0. A high level of resistance to race 0 is restricted to *Lens ervoides*, a wild lentil species in the tertiary gene pool, thus current cultivars of lentil have little or no resistance to race 0. This study comprehensively examined the genetic sources of resistance to race 0 and race 1 in *L. culinaris* and *L. ervoides* germplasm. The aims of the thesis project were: 1) to evaluate promising sources of resistance to *C. lentis* race 0 previously identified in *L. culinaris* landrace accessions; 2) to identify quantitative trait loci (QTL) conferring resistance to anthracnose race 1 in two lentil biparental recombinant inbred lines (RIL) populations; 3) to perform marker-trait associations for race 1 resistance in lentil via genome-wide association study (GWAS); 4) to identify trait loci associated with both races of anthracnose resistance in *L. ervoides* accession IG 72815; 5) to create and characterize a BC_2-derived lentil advanced backcross (LABC-01) population in cv. CDC Redberry background for *L. ervoides* genes/alleles derived from the interspecific RIL LR-59-81, and thereby dissect QTL conferring resistance to anthracnose race 0 in the LABC-01 population.

The first study evaluated the reaction of 8 promising *L. culinaris* landrace accessions against race 0 relative to the resistant check LR-59-81. Results revealed lack of effective resistance to race 0 among the accessions tested compared to that of LR-59-81. QTL mapping of the two bi-parental populations identified a major-effect QTL (*qAnt*1.*Lc*-3; R^2 = 66.6 – 69.8%) that conferred resistance to race 1 on lentil chromosome 3. GWAS detected 14 significant SNPs associated with race 1 resistance on chromosomes 3, 4, 5, and 6. The most significant GWAS SNPs on chromosome 3 colocalized with *qAnt*1.*Lc*-3 and delineated a region of 1.6 Mb containing candidate disease resistance genes. A QTL analysis of an interspecific RIL population derived from accession IG 72815 identified major resistance loci on chromosomes 3 and 7 for both races, accounting for 50.2 to 73.3% of the total phenotypic variance. Multiple classes of candidate disease resistance and defense-related genes were uncovered in the intervals of both loci. The LABC-01 population displayed genetic variation for resistance to race 0 and transfer of resistance alleles into the elite cultivar was also evident. A marker-trait association analysis identified a resistance locus (*qAnt*0.*Le*-3) on chromosome 3, accounting for 12.5 to 20.7% of the phenotypic variation conferring resistance to race 0. Overall, the research study provides new insights into the

inheritance and positions of loci underlying resistance to anthracnose in lentil and lays out an important foundation for marker-assisted introgression of anthracnose resistance from *L. ervoides* accessions into elite lentil cultivars.

ACKNOWLEDGEMENTS

I would like to express my sincere and heartfelt thanks to my supervisor, Dr. Albert Vandenberg for his support and guidance over the course of my graduate research. His ideas, encouragement, and mentoring have helped me to develop both personally and professionally. I thank my advisory committee members: Dr. Kirstin E. Bett, Dr. Randy Kutcher, Dr. Eric Bishop von Wettberg (Department of Plant and Soil Science, University of Vermont, USA), and Dr. Ken Wilson (Department of Biology, University of Saskatchewan) for their suggestions and guidance. I thank Dr. Yuguang Bai for serving as my committee chair. I would also like to acknowledge Dr. Sabine Banniza for many thoughtful discussions.

This work could not have been accomplished without the help of many talented and knowledgeable individuals at pulse crop breeding and research programs of the Crop Development Centre, University of Saskatchewan, particularly: Pulse Pathology Lab members, Stephanie and Pathirannehelage for their help in disease phenotyping of the landrace accessions; Pulse Molecular Breeding Lab, Robert and Akiko for their support in exome capture library preparation; Pulse Bioinformatic Team, Larissa, Lacey, Carolyn and Chu Shin (Kevin) for their assistance in handling the genotyping data; Crop Science Field Lab crew including, Brent, Alison, Stacey, Yong, Kevin, and Devini for technical assistance with various aspects of the project.

I am also grateful to the good friends and peers Dr. Rajib Podder, Dr. Hamid Khazaei, Dr. Endale Tafese, Dr. Teketel Haile, Derek Wright and my fellow grad students for their encouragement, support and warm friendship. I want to express my special thanks to graduate student Stanley Adobor for his technical support and friendship. I thank Erin Daniels Jones for her wonderful support during my research work.

The author would like to acknowledge financial assistance received from the Natural Sciences and Engineering Research Council of Canada (NSERC) Industrial Research Chair Program, and the Saskatchewan Pulse Growers for their funding support throughout my Ph.D. program. I would also like to acknowledge funding assistance from Saskatchewan innovation and opportunity scholarship, and Gerhard Rakow Legacy Award.

Finally, I would like to extend my heartfelt thanks to my wife Emebet A. Ayano and my sons Monenus and Yerosen for their sacrifice and moral support for completion of the degree.

DEDICATION

This thesis/dissertation is dedicated to my wife, Emebet Alemu Ayano for her support and unconditional love; and to my mom, Zeritu Birmechu Sutta for the understanding of the values of education.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
ANOVA	Analysis of variance
CDC	Crop Development Centre, University of Saskatchewan
CIM	Composite interval mapping
cM	CentiMorgam
DArT	Diversity array technology
DNA	Deoxyribonucleic acid
DPI	Days post inoculation
DS	disease severity
FDSS	Fungicide decision support system
GBS	Genotyping-by-sequencing
GWAS	Genome-wide association study
H^2	Narrow sense Heritability
LD	Linkage disequilibrium
LG	Linkage group LOD:
LOD	Logarithm of odds ratio
LSD	Fisher's least significant difference
Lsmeans	Least square means
Mb	Megabase
MAF	Minor allele frequency
MAS	Marker assisted selection
MLM	Mixed linear model
NIL	Near-isogenic line
NBS-LRR	Nucleotide binding site leucine rich repeat protein
NGS	Next generation sequencing
PCA	Principal Component analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA

RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
SAS	Statistical analysis software
SD	Standard deviation
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
X^2	Chi-square

CHAPTER 1

1. Introduction

Lentil (*Lens culinaris* Medik.) is a diploid (2n=2x=14), self-pollinating annual cool season legume crop, with a genome size of approximately 4 Gb (Arumuganathan and Earle, 1991). The crop is cultivated in more than 70 countries, and it is the world's fourth largest pulse crop with production from Canada, India, and Australia providing most of the international supply (FAOSTAT, 2018). Lentil provides an affordable source of dietary proteins, minerals, fiber, and carbohydrates and plays a vital role in alleviating malnutrition and micronutrient deficiencies (Srivastava and Vasishtha, 2012). Compared to other major pulses, lentil production and demand has been increasing quickly for the past 50 years (Khazaei et al., 2019). In parallel with the increasing global awareness of, and demand for, new plant-based protein foods and animal feeds, lentil has the potential to be an important protein source for food processing applications (Khazaei et al., 2019). Thus, breeding strategies focus on the introduction of new genetic resources for yield, quality traits, biotic and abiotic stress resistance are required to keep the lentil industry sustainable.

The introduction of the lentil crop into western Canadian production systems in 1969 began with relatively disease-free fields (Morrall et al., 1972; McKenzie and Morrall, 1973; McKenzie and Morrall, 1975). The area cropped to lentil has increased annually due to its profitability (Morrall, 1997). In 1978 ascochyta blight (*Ascochyta lentis*) was reported from Saskatchewan (Morrall and Sheppard, 1981) and nine years later anthracnose [*Colletotrichum truncatum* - reclassified as *Colletotrichum lentis* (Damm) (Damm et al., 2014)], was reported as a new disease in Manitoba with severe yield loss and rapid shoot dieback (Morrall, 1988). Currently, lentil productivity in western Canada is challenged by more diseases, including stemphylium blight (*Stemphylium botryosum)* and aphanomyces root rot (*Aphanomyces euteiches).*

Anthracnose, caused by the fungal ascomycete pathogen *Colletotrichum lentis*, has become the most important foliar fungal disease of lentil in western Canada and can cause up to 70% yield loss under high disease pressure (Chongo et al., 1999; Morrall and Pedersen, 1990). The disease is considered of minor importance in other parts of the world, and has been reported from Bangladesh, Bulgaria, Brazil, Ethiopia, Morocco, Pakistan, Syria and USA (Bellar and Kebabeh, 1983; Bayaa and Erskine, 1997; Morrall, 1997; Kaiser et al., 1998). The pathogen survives as microsclerotia on lentil debris and spreads among fields by wind. 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). Race1 is less virulent, and partial resistance was found in a number of *L. culinaris* accessions (Buchwaldt et al., 2004, 2018). Resistance to race 1 was effectively transferred into elite lentil breeding lines and resulted in the release of several cultivars with partial resistance to race 1 in lentil production (Vandenberg et al., 2002, 2006; Government of Saskatchewan, 2019), which probably contributed to the decline in the proportion of race 1 isolates in the pathogen population, now dominated by race 0 isolates in Saskatchewan fields (Durkin et al. 2015; Menat et al., 2016). However, breeding for resistance to the highly virulent race 0 of *C. lentis* is dependent on the use of resistant germplasm from the crop's wild relative species *L. ervoides,* the tertiary genepool (Tullu et al., 2006). The lentil breeding program at the Crop Development Centre (CDC), at the University of Saskatchewan (U of S) has identified wild species accessions with resistance to both races that can be transferred to *L. culinaris* germplasm after interspecific hybridization using embryo rescue techniques (Fiala et al., 2009; Tullu et al., 2013).

More precise localization of QTL/genes along with identification of linked molecular markers is an important step in the development of effective marker assisted selection (MAS) in lentil breeding. Targeted MAS could accelerate the introgression of anthracnose resistance genes from both *L. culinaris* and *L. ervoides* accessions and facilitate pyramiding of resistance genes into lentil cultivars to achieve high levels of resistance against both races of anthracnose. The projects discussed in this thesis are part of the ongoing efforts in the CDC lentil breeding program to improve and understand the genetic mechanism(s) of resistance to anthracnose in lentil. This has been made possible through the availability of genetic and genomic resources at CDC. Therefore, this study involved linkage analysis of two intraspecific and two interspecies mapping populations, and GWAS of one diversity panel to identify potential QTLs for anthracnose resistance. Prior to this thesis project, promising sources of resistance to *C. lentis* race 0 in *L. culinaris* landrace accessions were reported (Shaikh et al., 2013) and these were also evaluated in this body of work.

1.1. Research hypotheses

1. The source of resistance to anthracnose race 0 in previously identified *L. culinaris* landrace accessions (Shaikh et al., 2013) is comparable to the resistance of line LR-59-81, the interspecific resistant check for race 0.
2. Genomic regions controlling resistance to lentil anthracnose race 1 can be identified through linkage analysis and genome-wide association mapping in *L. culinaris* genotypes.
3. Regions of the *L. ervoides* genome that are associated with anthracnose resistance will continue to confer resistance following hybridization with *L. culinaris*.
4. The introgression of *L. ervoides* derived genes/alleles creates genetic variation for anthracnose race 0 resistance in *L. culinaris* background that can be mapped using an advanced backcross population.

1.2. Experimental objectives

The objectives of the thesis project were:

1. To evaluate promising sources of resistance to anthracnose race 0 identified in *L. culinaris* landrace accessions in relation to the resistance of line LR-59-81, a *L. culinaris* × *L. ervoides* interspecific recombinant inbred line (RIL).
2. To identify QTLs for anthracnose race 1 resistance in two lentil biparental RIL populations.
3. To conduct an association mapping study using genome-wide SNP markers and identify chromosomal regions associated with race 1 resistance, and thereby to cross-validate the QTL detected in biparental populations.
4. To identify trait loci associated with anthracnose resistance in *L. ervoides* accession IG 72815 and the underlying candidate genes involved in disease resistance.
5. To develop a lentil advanced backcross population and identify QTL associated with race 0 resistance derived from *L. ervoides* accession L-01-827A.

CHAPTER 2

2. Literature Review

2.1. Lentil: brief domestication and production

Lentil (*Lens culinaris* Medik.) is an annual self-pollinating, diploid (2n=2x=14) plant with genome size of approximately 4 Gb (Arumuganathan and Earle, 1991). Lentil is one of the oldest crops grown and domesticated by man, but its time and place of domestication is debatable. Much research has been conducted regarding the domestication history of modern crop species. The archaeological evidence supports that domestication of lentil dates back to the Fertile Crescent era (Cubero et al., 2009; Coyney et al., 2020) and originated in the Near East along with other pulses and cereals (Cubero et al., 2009; Ladizinsky, 1993). Its ability to adapt to poor soil conditions, pod dehiscence and seed shattering are considered key factors for the spread of lentil to new climatic zones (Erskine, 1997; Erskine, 2009; Ljuština and Mikić, 2010). Currently, lentil is grown in more than 70 countries world-wide with Canada, India, Australia, Turkey and Nepal being the top five producers (FAO, 2017). Major lentil growing areas are grouped into three agro-ecological zones: Mediterranean, South Asia (sub-tropical savannah) and Northern temperate (Khazaei et al., 2016; Tullu et al., 2011).

In the last five decades, the global lentil production has increased more than seven-fold from 1.0 Mt to 7.6 Mt (FAO, 2017). This coincides with the introduction of the lentil to the western Canadian prairie production area in early 1970s and Australia in the 1990s. Canada grows 46% of the world lentil production, making Canada the largest global producer and exporter of lentil from 2013-2017 (FOA, 2017). More than 90% of the production in Canada is from the province of Saskatchewan (Canadian Grain Commission, 2018). The adoption of lentil production in Saskatchewan might be attributed to success in developing high yielding and better adapted varieties at the CDC that led to the inclusion of lentil in cropping systems for crop diversification, extension of crop rotations, reduction of nitrogen fertilizer requirements in succeeding crops because of nitrogen fixation, and development of an export market that improved economic returns to the growers.

Lentil provides an affordable source of dietary proteins, carbohydrates and micronutrients (DellaValle et al., 2013) and is largely consumed in developing countries (Sarker and Erskine,

2006). Lentil has a variety of seed coat colours (brown, gray, green, tan, black, and white) and seed coat patterns (dotted, spotted, marbled, complex, and unpatterned) (Vandenberg and Slinkard, 1990). The market classes are mainly based on seed size (large, medium or small) and cotyledon colour (yellow, red, or green). The small red lentil market class is based on red cotyledon colour of dehulled seeds, and the large green lentil class is characterized by green seed coat colour and yellow cotyledons - these are the major commercial market classes of Canadian lentil production. The other specialty market classes include small green, French green, medium green, Spanish brown and different seed sizes of red cotyledon lentil. Red lentil is mainly consumed in the Indian subcontinent and eastern Mediterranean regions as split cotyledons of the dehulled seed (Vandenberg, 2009). Large green lentils are consumed as whole seeds and mostly marketed in Europe, Middle East, and South America (Muehlbauer, 2009).

2.2. The genus *Lens* and gene pools

The word *Lens* is a Latin word designated to a specific genus that describes the seed shape of cultivated lentil. The genus *Lens* Miller consists of seven taxa, all with the same number of chromosomes (2n=14) and have similar karyotypes (Ladizinsky et al., 1984; Van Oss et al., 1997). Many studies of taxonomic classification among *Lens* species, based on morphological, cytological, and cytogenetic observation, isozyme and molecular markers do not agree with each other (Havey and Muehlbauer, 1989; Abo-Elwafa et al., 1995; Ahmad and McNeil, 1996; Sonnante et al., 2003; Cubero et al., 2009). Presently, the seven taxa of the genus *Lens* are classified into four species: *Lens culinaris* (including subsp. *culinaris*, *orientalis*, *tomentosus* and *odemensis*), *Lens lamottei*, *Lens ervoides* and *Lens nigricans* (Cubero et al., 2009; Ferguson et al., 2000). However, based on their hybridization barriers (Cubero et al., 2009), genome similarity studies using the two-enzyme GBS approach (Wong et al., 2015) and exome capture arrays (Ogutcen et al., 2018), *Lens* species can be classified into four gene pools of which the primary gene pool (*L. culinaris*, *L. orientalis*, and *L. tomentosus*) can be easily crossed with cultivated lentil and produce fertile progeny. The other four species are classified as the secondary (*L. lamottei* and *L. odemensis*), tertiary (*L. ervoides*) and quaternary gene pools (*L. nigricans*). For these, embryo/ovule rescue techniques are required to overcome hybridization barriers with the primary gene pool.

2.3. Anthracnose in lentil

The first report of a *Colletotrichum* species, a fungal pathogen causing characteristic anthracnose symptoms in lentil was originated in 1987 in the province of Manitoba, Canada (Morrall, 1988). The pathogen was originally described as *Colletotrichum truncatum* (Schwein.) Andrus & W.D., Moore; however, it was re-classified as *Colletotrichum lentis* (Damm) in 2014 (Damm et al., 2014). The disease is either not mentioned or listed as a disease of minor importance in other parts of the world, although it has occasionally been reported from Bulgaria, Bangladesh, Pakistan, Ethiopia and Syria (Kaiser et al., 1998; Morrall, 1997). The pathogen has a relatively narrow host range that is restricted to species in the tribe Fabeae of the Fabaceae such as faba bean (*Vicia faba* L.) and common vetch (*Vicia sativa* L.) (Banniza et al., 2018). It has been speculated that *C. lentis* presumably evolved as a host shift from another *Colletotrichum* species, most likely from local wild vetches or faba bean fields in Manitoba (Morrall, 1997; Buchwaldt et al., 2018). Plant pathogen host shifts are known to occur when crop species are introduced into new geographical areas (Silva et al., 2012).

Anthracnose has continued to be the major foliar disease of lentil in western Canada. Dokken-Bouchard et al. (2016) reported 60-83% of lentil fields scouted in Saskatchewan showed anthracnose during the years 2012-2015. Yield losses of susceptible cultivars have been documented to be up to 70% under high disease pressure (Chongo et al., 1999; Morrall and Pedersen, 1990).

2.3.1. Disease cycle and symptoms of anthracnose

The microsclerotia of the pathogen survive on lentil debris or stubble *in situ* and serves as a primary source of inoculum in subsequent lentil crops. Long distance dispersal of the inoculum to neighboring fields is aided by wind during combine harvest operations (Buchwaldt et al., 1996). It is unlikely that seed-to-seedling transmission of anthracnose is important, as relatively low seed infection are detected, even in highly affected lentil crops (Gibson, 1993). Therefore, anthracnose of lentil is not considered as seed transmitted.

Initial symptoms of the disease appear as superficial lesions, tiny yellow spots that enlarge into tan-colored lesions on young leaves that results in premature leaflet abscission. On stems, lesions start at the stem base and gradually move upwards. Stem lesions can girdle stems and cause

the plant to wilt and die (Buchwaldt et al., 1996; Chongo & Bernier, 2000). Excessive moisture that prolongs growth and delays harvest into late summer increases disease severity (Morrall et al., 2008). Moreover, high temperatures above the optimal for disease development (20 to 24ºC) coupled with humid conditions also increase the disease transmission (Chongo and Bernier, 2000).

2.3.2. Infection process of *Colletotrichum lentis*

Colletotrichum lentis is a hemibiotrophic pathogen that undergoes two infection stages, a short symptomless biotrophic phase followed by a switch to destructive necrotrophic growth. The biotrophy-necrotrophy switch is necessary for disease development. Conidia of *C. lentis* inoculated onto detached leaflets germinate within 3-6 h post inoculation (hpi) at 20°C, and melanized appressoria can be differentiated at the end of germ tubes within 6-12 hpi (Chongo et al., 2002). Penetration pegs of *C. lentis* generated from appressoria in the contact zone with the epidermis pierce the cuticle, and by 20 hpi, infection vesicles appear in the apoplastic space of epidermal cells underneath the penetration sites (Armstrong-Cho et al., 2012), which develop into large unbranched or multi-lobed primary hyphae. The biotrophic phase of *C. lentis* is symptomless and ends with the appearance of thin secondary hyphae 48-68 hpi, signaling the start of a destructive phase during which the fungus acts as a necrotrophic organism by invading and killing host cells (Bhadauria et al., 2011; Armstrong-Cho et al., 2012).

2.3.3. Pathogenic races of *C. lentis*

After screening 1701 lentil accessions for anthracnose resistance under field and controlled conditions, Buchwaldt et al. (2004) identified seven differential lentil genotypes for pathogenic race identification. Based on the significant difference in disease severity of the seven selected host differential lentil genotypes, 50 isolates of *C. lentis* collected from Manitoba and Saskatchewan were characterized. As a result, two pathogenic races of *C. lentis*, Ct0 and Ct1 were described for the first time (Buchwaldt et al., 2004). Ct0 was recently re-designated as race 0 and Ct1 as race 1 (Banniza et al., 2018). Race 0 is a more virulent race to which little or no resistance has been found in *L. culinaris* accessions. Race 1 is less virulent race, against which partial resistance was identified in a number of *L. culinaris* germplasm (Buchwaldt et al., 2004).

C. lentis race identification was also done at the genomic level using inter-genic spacer (IGS) minisatellite polymorphisms. Durkin et al. (2015) identified two minisatellite repeat-rich

regions of the IGS, containing 23 and 39 nucleotides in the ribosomal RNA genes. Variation exists within the 23 nucleotide minisatellite that separates the races. Race 1 isolates have 17 repeats of identical sequence while race 0 isolates have either 14 or 19 repeats of different sequence variations. The 39 nucleotide minisatellite differentiated race 1 isolates having seven or nine repeats from race 0 isolates, which have only two or four repeats (Durkin et al., 2015).

In many biotrophic pathogens with physiological races, virulence is a simply inherited trait (Caten, 1987). This concept is related to the host-pathogen interaction hypothesis that known as the gene-for-gene model (Flor, 1946). In this model virulence in pathogens and resistance in their hosts are governed by single genes, and absence of either will result in a compatible interaction. However, in the case of *C. lentis*, a hemibiotrophic pathogen, the host-pathogen interaction is different from that of biotrophic species (Banniza et al., 2018). For example, the molecular interactions of *C. lentis* with susceptible lentil cultivar Eston that was studied using an expressed sequence tag (EST) library mined during the biotrophy-necrotrophy switch for a race 1 isolate, showed 39% of the ESTs were predicted to be of fungal origin and 61% were of lentil origin. For race 0, about 69% of transcripts were attributed to the pathogen. In both cases, the interaction with Eston was compatible, which resulted in high amount of fungal biomass (Bhadauria et al., 2011, 2015; Banniza et al., 2018).

2.3.4. Management of anthracnose

Integrated disease management (IDM) is recommended for western Canadian lentil growers (Tivoli et al., 2006). The control options for anthracnose include crop rotations that include at least two if not three or more other crop species with lentil, foliar fungicide applications and host-plant resistance. In the IDM practices, the use of resistant cultivars is an integral component to manage the disease as it is economical with the least environmental impact.

Anthracnose is often observed in lentil plants at the 10-12 node stage (about 6 weeks after seeding) or at early flowering (Chongo & Bernier, 1999). Therefore, the optimal time of fungicide application is between the 10-12 node stage to early flowering, when the lower leaflets infected with disease start falling to the soil surface (Buchwaldt et al., 1999). To reduce potential secondary spread due to the polycyclic nature of the pathogen, a second fungicide application might be considered at mid-flowering, about 10-14 days after the first application (Buchwaldt et al., 1999; Chongo et al., 1999). However, the second application has been found to be economically

beneficial only under high disease pressure. Thus, the balance between yield loss and the high cost of fungicide application created a need for development of a fungicide decision support system (FDSS). The FDSS has been used by lentil growers and staff in public and private extension services in western Canada and is demonstrated to be 85% accurate for assessing disease risk (Buchwaldt et al., 2018).

2.4. Anthracnose resistance in lentil

2.4.1. Anthracnose resistance in cultivated lentil (*L. culinaris*)

A search for resistance to anthracnose started in the early 1990s, a few years after the discovery of the disease (Bernier et al., 1992). The first identified source of resistance to race 1 of the pathogen was in the cultivar Indianhead, also known as plant introduction accession PI 320952 (Bernier et al., 1992; Gibson, 1993). Indianhead has black seed coats and was released mainly for use as a green manure but is now marketed for culinary use under the name "Beluga". The resistance of Indianhead was transferred to a small-seeded red cotyledon breeding line with marketable seed attributes and resulted in the release of cultivar CDC Robin with partial resistance to race 1 (Vandenberg et al., 2002).

As disease spread continued, anthracnose became a major disease across Western Canadian lentil production, and the quest for sources of resistance in the cultivated lentil germplasm pool was initiated through screening of the accessions acquired from global gene banks such as the Vavilov Institute of Plant Industry in St. Petersburg, Russia (with prefix VIR), the US Department of Agriculture-Agriculture Research Services (USDA) in Pullman, WA, USA (with prefix PI), the Institute for Plant Genetics and Plant Research in Gartersleben, Germany (with prefix LENS), and ICARDA (International Center for Agricultural Research in the Dry Areas) in Aleppo, Syria (with prefix ILL) (reviewed by Buchwaldt et al., 2018).

So far, more than 2300 *L. culinaris* accessions originally collected from more than 50 counties were evaluated for resistance to both races of *C. lentis*. Among this group, partial resistance to race 1 has been documented in 49 accessions (Buchwaldt, 2018, 2004; Shaikh et al., 2013). However, none were identified with resistance to the more virulent race 0, except for eight promising accessions reported by Shaikh et al. (2013). These were further evaluated as part of this thesis project. Since the release of the cultivar CDC Robin, a number of cultivars with partial

resistance to race 1 have been released including CDC Redberry (Vandenberg et al., 2006), a lentil reference genome, and have been deployed in Saskatchewan lentil production (Government of Saskatchewan, 2018).

2.4.2. Anthracnose resistance in wild lentil species

As described previously, the cultivated lentil gene pool has a narrow genetic base for resistance to the more virulent race 0. Although some germplasm with partial resistance to race 1 has been reported in the cultivated gene pool, the frequency of allelic diversity for race 1 (less virulent) resistance is very low (~2% of the 2300 accessions). To broaden the genetic base of anthracnose resistance in lentil breeding programs, it is necessary to identify effective resistance genes from the crop's wild relatives. Wild relatives of crop species are a genetic reservoir that provide a tractable source of resistance in many pathosystems (Hajjar and Hodgkin, 2007; Coyne et al., 2020). An effort was made at the CDC, U of S to identify novel resistance sources from wild lentil species for various lentil diseases, including, ascochyta blight (Tullu et al., 2010), stemphylium blight (Podder et al., 2013), and anthracnose (Tullu et al., 2006). Tullu et al. (2006) evaluated wild lentil accessions assembled from all *Lens* species under greenhouse (574 accessions) and field (484 accessions) conditions for resistance to anthracnose. Among the *Lens* species evaluated, *L. ervoides*, *L. lamottei*, and *L. nigricans* show resistance to race 0 and race 1 (Tullu et al., 2006). However, the highest frequency of resistance sources to both races, particularly to the more virulent race 0, was identified in *L. ervoides* of the tertiary gene pool.

Subsequently, the two *L. ervoides* accessions (L-01-827A and IG 72815) with the highest level of resistance to both races of *C. lentis*, were successfully crossed with *L. culinaris* cultivar Eston using ovule and embryo rescue techniques (Fiala et al., 2009; Tullu et al., 2013). Eston is a small-seeded, yellow cotyledon lentil with green seed coat, an early maturing cultivar released in Canada in 1980 (Slinkard, 1981), and commonly used as a susceptible check for both race 0 and race1 at the CDC, U of S (Banniza et al., 2018). The resulting interspecific RIL populations were named LR-59 (Eston × L-01-827A; Fiala et al., 2009) and LR-26 (Eston × IG 72815; Tullu et al., 2013), and both showed wide variation for anthracnose resistance (Fiala et al., 2009, Tullu et al., 2013). In both LR-59 and LR-26 populations, reduction in population size during population advancement from F_2 to F_7 generation was reported, most likely due to variable levels of fertility.

2.5. Genetic control of anthracnose resistance

In resistance breeding, an understanding of the genetic inheritance of resistance is a critical step to extract maximum benefit from the available resistance sources in the breeding program. Accordingly, previous studies of *C. lentis* resistance suggested that resistance to race 1 was conditioned by either a dominant or a recessive gene and one closely linked dominant gene (Buchwaldt et al., 2013; Tullu et al., 2003). Tullu et al. (2003) mapped a QTL controlling race 1 resistance in *L. culinaris* accession PI 320937 using a biparental RIL population derived from a cross with cultivar Eston (susceptible) and identified associated RAPD markers. They reported the genetic control of resistance in PI 320937 was governed by a major dominant gene and several minor genes. This major dominant gene was later confirmed by Buchwaldt et al. (2013), who also studied the inheritance of anthracnose race 1 resistance in Indianhead and PI 345629 and proposed that it was conferred by a combination of recessive and dominant genes. The study suggested that these resistance genes are closely linked and most likely were different alleles at a single locus.

The examination of the resulting interspecific populations of LR-59 and LR-26 (discussed in section 2.4.2) suggested that both race 0 and race 1 resistance derived from *L. ervoides* accessions L-01-827A and IG 72815 were controlled by two recessive genes. It was, however, suspected that the results were skewed due to segregation distortion (Fiala et al., 2009; Tullu et al., 2013). Moreover, QTL analysis conducted by Bhadauria et al. (2017) using an intraspecific RIL population developed from a cross between *L. ervoides* accessions L01-827A and IG 72815, revealed five QTL associated with resistance to race 0 and six with resistance to race 1 distributed across four of the seven chromosomes of *L. ervoides*.

2.6. Molecular research in lentil breeding

2.6.1. Molecular markers and genetic linkage map in lentil

Molecular markers are segments of DNA or known sequences of DNA that represent variation among individuals at the genome level (Collard and Mackill 2008). Plant breeders can increase the rate and accuracy of their selection processes by implementing molecular marker technology in their breeding programs in a variety of ways. For example, detection of the allelic variations that exist for a gene and are responsible for expression of the traits due to the presence of genetic linkage (Collard and Mackill, 2008), can lead to development of marker-assisted

procedures for germplasm improvement and varietal development by incorporating multiple genes (gene pyramiding) for resistance to biotic and abiotic stresses into an elite cultivar (Varshney et al., 2007) and for germplasm characterization, characterization of transformants, and the study of phylogenetic relationships (Varshney et al. 2007).

Molecular markers are classified into two categories based on basic techniques of their development; hybridization-based markers like restriction fragment length polymorphisms (RFLP) and PCR (polymerase chain reaction) based markers. In lentil, the first linkage map involving molecular markers was developed by Havey and Muehlbauer (1989) using RFLP, isozyme and morphological markers. However, significant progress has been made after the development of PCR-based markers in lentil, which increased the number of the available markers for genetic map construction in lentil. Thereby, Eujayl et al. (1998) developed a comprehensive linkage map consisting of PCR-based markers, such as random amplified polymorphic DNA (RAPD) markers and amplified fragment length polymorphism (AFLP) markers, along with RFLP and morphological markers. Subsequently, lentil linkage maps comprised of RAPD, AFLP, inter simple sequence repeat (ISSR) and resistance gene analog (RGA) markers were developed (Rubeena et al., 2003, 2006; Tullu et al., 2003; Duran et al., 2004).

Among the PCR-based markers, simple sequence repeats (SSR) or microsatellites (Akkaya et al. 1992), are widely used genetic markers in many of the crop breeding programs because of their co-dominant inheritance, abundance, reproducibility, extent of allelic diversity, and the ease of assessing allelic size variation by PCR with pairs of flanking primers (Agarwal et al., 2008). Lentil genetic linkage maps involving SSR markers were generated by many lentil research groups (Duran et al., 2004; Hamwieh et al., 2005, Phan et al., 2007; Tullu et al., 2008; Ford et al., 2009; Saha et al., 2013; Fedoruk et al., 2013; Verma et al., 2015). Moreover, Gupta et al. (2012) developed expressed sequence tag (EST) derived SSR sequences from the model legume species *Medicago truncatula* to enrich an existing intraspecific lentil linkage map developed by Phan et al. (2007). This linkage map was initially developed from gene-based markers, intron targeted amplified polymorphism (ITAP) markers generated through the synteny between lentils and *M. truncatula*.

Currently, single nucleotide polymorphism (SNP) markers have becoming the marker of choice, due to their abundance in nature and even distribution across the genome. The advancement in next-generation sequencing (NGS) technologies has enabled the detections of large-scale and

high-throughput SNP variations across the whole genome. In lentil, efforts have been made to discover high-density SNP markers for genetic linkage map construction using high-throughput genotyping technologies, such as 1,536-SNP Illumina GoldenGate assays (Kaur et al., 2014; Sharpe et al., 2013; Fedoruk et al., 2013) and diversity arrays technology (DArT) (Ates et al., 2016, 2018; Aldemir et al., 2017). In line with the affordability and flexibility of the NGS technology platforms, lentil researchers are currently using sequence-based marker technologies like genotyping by sequencing (GBS, Elshire et al., 2011) and exome capture sequencing (Hodges et al., 2007). The utility of the GBS approach was demonstrated in lentil to characterize *Lens* species (Wong et al., 2015) and to develop genetic linkage maps (Bhadauria et al., 2017). An exome capture sequencing array targeting 85 Mb of the protein-coding region of the lentil genome was developed (Ogutcen et al., 2018). This method produces a large number of high quality and informative SNP markers that are being used in different lentil molecular research activities including high density linkage map development.

2.6.2. Quantitative trait loci (QTL) mapping in lentil

Linkage analysis has been used to map genomic regions controlling the phenotypic variation of quantitative and qualitative traits, and thereby identify the markers linked to the trait that could be implemented in MAS (Collard and Mackill, 2008). In plants, linkage mapping is conducted by creating biparental mapping populations such as recombinant inbred lines (RILs), doubled haploids (DH), backcross populations, and F_2 populations (Collard et al., 2005), and then through development of linkage maps. A larger population size is often preferred (Collard et al. 2005), especially to detect QTL with small effects and precisely evaluate the target trait (Doerge 2002). QTL mapping in biparental populations uncovers only QTL that are segregation in the mapping population (Flint-Garcia et al., 2003), basically the genetic variability between the parental lines (Bernardo, 2008). In lentil, several QTL mappings studies have been conducted over the past 20 years using different types of molecular markers from low to high-fidelity (as described in section 2.5.1). These studies have identified molecular markers linked to desirable QTLs/genes that affect disease resistance, agronomic performance, seed quality traits, drought and boron tolerance, and seed iron concentration (reviewed and summarized in Kumar et al., 2015, 2019a). However, the majority of these QTL have not been deployed in MAS in lentil breeding due to several reasons, including: lack of high number of genome-wide molecular markers, poor linkage

between markers and traits, and low phenotypic predictive values (variation explained) of the markers (Kumar et al., 2019a). Likewise, Collard et al. (2005) emphasized the need for further testing and development of markers identified in preliminary genetic mapping studies before use in MAS. Therefore, to employ MAS in breeding programs, breeders should consider markers that are tightly linked to the trait loci of interest, highly polymorphic in the breeding material, cost-effective and high-throughput genotyping methods, and their predictiveness should be validated in different genetic backgrounds before applied in MAS (Collard and Mackill, 2008).

2.6.3. Genome-Wide Association Study (GWAS) in lentil

Association mapping (AM) has become a powerful tool for dissecting genomic regions associated with agronomically important traits in major crops, including lentil. Unlike linkage analysis, AM uses diverse accessions from germplasm collections of cultivars, natural populations, or elite breeding lines, referred to as a 'diversity panel' or 'association panel' to identify polymorphisms associated with phenotypic variation (Flint-Garcia et al., 2003). The association panel can also constitute of individuals of multiple biparental populations (NAM - nested association mapping), and/or multi-parent populations (MAGIC - multi-parent advanced generation inter-cross) (Yu and Buckler, 2006; Steinhoff et al., 2011; Wurschum, 2012; Gupta et al., 2014). Furthermore, association panels can be assembled from diverse advanced breeding lines (Gupta et al., 2014). This type of association panel is more useful compared to other diversity panels for mapping favorable traits in breeding programs and marker-QTL associations identified can be immediately used in MAS (Kumar et al., 2017).

The AM method relies on the principle of linkage disequilibrium (LD) that tends to be accumulated over many generations between loci that are genetically associated to one another (Neumann et al., 2011). LD, known as gametic phase disequilibrium, is the non-random association of alleles at different loci controlling particular genetic variations in a population. In random mating populations, LD is created by genetic drift and mutation, and decays by recombination (Breseghello and Sorrells, 2006). Marker-trait associations are identified based on historical recombination events between markers (mainly SNPs) and loci at the population level (Myles et al., 2009).

In plants, a population size of 100 to 250 individuals can be used for initial AM studies (Collard et al., 2005), but a larger population size is required for detection of QTLs having minor

effects on the target trait. The advantage of AM over linkage analysis is that it uses the germplasm collections or a natural population. Therefore, it requires less time and resources because there is no need for the development of extensive biparental mapping populations. AM also opens the possibility of exploiting historically measured trait data within breeding programs for association, and the availability of broader genetic backgrounds in the panel could also provide an opportunity to map multiple traits simultaneously. In contrast to linkage mapping, AM uses populations that have undergone many generations of recombination since domestication, which increases the mapping resolution and power, and broadens alleles to be tested for association (Flint-Garcia et al., 2003; Zhu et al., 2008). Since the alleles with minor allele frequency (< 5%) are often filtered out in AM, rare alleles are usually not detectable. Alternatively, AM provides a way to identify QTL that have effects across a broader range of germplasm. Although the biparental linkage mapping approach detects only QTLs that differ between the two parental lines, it can lead to the discovery of rare alleles given that the donor parent carries it (Bernardo, 2008). Therefore, the biparental mapping and AM methods are complementary to each other and can be integrated as proposed by Wu and Zeng (2001) to overcome their limitations. On the other hand, a high rate of false positive associations may arise from AM studies due to population structure and genetic relatedness of the populations used in diversity panel (Yu and Buckler, 2006). Thus, statistical approaches such as mixed models that account for populations membership (Q) and kinship (K) need to be used to identify true associations (Yu and Buckler, 2006; Bradbury et al., 2007).

In lentil, compared to many efforts made to map QTL in the biparental population for different traits, limited studies have been conducted employing AM in the recent past due to the lack of the availability of genome-wide molecular markers like SNPs (Kumar et al., 2015). Fedoruk et al. (2013) conducted the first AM study using SNP markers to identify QTL for seed size and shape. Subsequently, AM analysis in lentil were conducted to identify markers associated with seed iron and zinc concentration (Khazaei et al., 2017; Kumar et la., 2019b), days to flowing (Kumar et al., 2018a), seed quality characteristics (Khazaei et al., 2018) and agronomic traits (Kumar et al., 2018b). Meanwhile, with the availability of the lentil reference genome, a draft assembly of cultivar CDC Redberry (Bett et al., 2016; https://knowpulse.usask.ca/genome-assembly), scientists have started using of a large number of genome-wide molecular markers in lentil molecular research. Therefore, reports on the GWAS approach and other related genomics studies will be available in near future.

2.6.4. Genomic-assisted introgression of exotic alleles

The improvement of cultivars using conventional breeding can be time consuming and laborious, especially if the breeding program incorporates exotic germplasm. This is mainly due to the inheritance of deleterious genes along with beneficial alleles that can mask the genetic variation of the desired trait, a challenge known as linkage drag (Tanksley and Nelson, 1996). To reduce the risk of linkage drag to a minimum, Tanksley and McCouch (1997) underlined the potential application of molecular markers for the efficient discovery and integration of the desired QTL/gene(s) from exotic germplasm into the elite cultivar. In line with this, the advances in next-generation sequencing technology and its ability to generate genome-wide molecular markers even in minor crop species (Varshney et al., 2012), has facilitated the tracking of the introgression of beneficial alleles in breeding programs (Dempewolf et al., 2017). This process greatly reduces time required to identify the cultivars with desired traits (Collard and Mackill, 2008).

Backcross populations, in which small chromosomal regions have segregated in a highly homogeneous background, provide an opportunity for efficiently locating QTL conferring the introgressions of favorable exotic traits (Paterson et al., 1990). The most commonly used mapping populations for dissecting genetic architecture of trait introgressed from exotic germplasm to the elite cultivar background, are advanced backcross (AB) populations such as introgression lines (ILs), chromosome segment substitution lines (CSSLs) and near-isogenic lines (NILs) (reviewed by Dempewolf et al., 2017). These populations are advanced through two or more backcrosses of an exotic donor to an adapted recurrent (usually elite) parent, and multiple rounds of selfing depending on the type of population. These populations have been used as useful genetic resources to identify a single or a few genomic segments that are associated with desired traits (Frischa et al., 1998), although precise genotyping and phenotyping information is required. Once the markers linked to QTLs/genes are identified, breeders can perform marker-assisted introgression to integrate favorable alleles into elite cultivars. This approach has been proven in many crop species for the transfer of novel alleles from exotic germplasm to improve disease resistance, crop yield, quality and nutritional value, and environmental adaptation of crops (Zamir, 2001; Zamir et al., 1994; Eshed and Zamir, 1994; Xiao et al., 1996; McCouch et al., 2007; Imai et al., 2013; Placido et al., 2013).

Furthermore, Tanksley and Nelson (1996) proposed the advanced backcross QTL (AB-QTL) mapping approach, which involves QTL analysis and the transfer of valuable QTL alleles

from exotic germplasm to elite cultivars in a single process. In this approach QTL analysis is performed at advanced generations such as BC_2 or BC_3 to minimize the genome of the donor in individual recombinants, allowing for a more accurate assessment of the QTL effect. Consequently, the frequencies of deleterious or undesirable alleles are reduced and individual lines can be evaluated for the desired trait in targeted environments. Since the genome of the AB population is more skewed towards the recurrent parent, the effect of background or epistatic interactions generated from an interspecific hybridization is reduced. Moreover, the AB-QTL method has been proposed to shorten the time required to introgress favorable alleles, since the introgression process has already started (Tanksley and Nelson, 1996). The utility of this technique has been used in many crop species to broaden the genetic base of crop species as reviewed by (Bhanu et al., 2017).

Prologue to Chapter 3

The information from the review of literature (Chapter 2) indicated that resistance to the more virulent race 0 of *C. lentis* within the primary gene pool (*L. culinaris*) is limited, and the tertiary gene pool, *L. ervoides* shows high frequency of resistance to race 0 and race 1. From a breeder's perspective, deploying resistance genes/alleles from the tertiary gene pool into elite cultivars is often challenging and time-consuming. Therefore, introgression of novel anthracnose resistance genes from the cultivated gene pool is often preferred if there is genetic diversity for the trait. So far, more than 2300 *L. culinaris* accessions collected from 50 countries maintained in global gene banks were evaluated for both races of anthracnose at Agriculture and Agri-Food Canada, Saskatoon. Consequently, promising *L. culinaris* landrace accessions with resistance to race 0 were identified. It was proposed that evaluations of these promising sources of resistance in relation to the resistance identified in *L. ervoides* could be required to use these accessions for further genetic studies and incorporate the resistance gene/s into breeding lines; thus, the first study of this thesis was initiated.

Disclosure

This chapter was published as part of a manuscript on February 05, 2020 in the journal "Plant Genetic Resources: Characterization and Utilization".

Gela, T. S., Banniza, S., and Vandenberg, A. (2020). Lack of Effective Resistance to the Virulent Race of *Colletotrichum lentis* in *Lens culinaris* Medikus subsp. *culinaris*. Plant Gen. Res. Char. Util. 18(2):81-87.

Author contributions

TSG and technical support under supervision of SB conducted experiments, TSG drafted the manuscript and analyzed the data. SB and AV conceived the study, participated in its design, and critically reviewed the manuscript.

CHAPTER 3

Lack of effective resistance to the virulent race of *Colletotrichum lentis* in cultivated lentil (*Lens culinaris*)

Abstract

Anthracnose caused by *Colletotrichum lentis* is an important fungal disease of lentil in western Canada. Two known pathogenic *C. lentis* races, race 0 and race 1 have been identified and current cultivars of lentil have little or no resistance to the virulent race 0. Seven *Lens culinaris* landrace accessions were previously reported to have resistance to *C. lentis* race 0. In this study, accession VIR-2633, with reported resistance to both races of *C. lentis*, and the seven *L. culinaris* accessions were assessed for race 0 resistance relative to LR-59-81, an interspecific line derived from a *L. culinaris* × *L. ervoides* cross. The results showed lack of effective resistance to race 0 among the *L. culinaris* accessions when compared to that of LR-59-81. A few sublines displayed modest improvements in resistance compared to the susceptible check cv. Eston but were significantly more susceptible than LR-59-81. Moreover, screening of the sublines of accession VIR-2633 identified 12 sublines with resistance to race 1, but all VIR-2633 sublines were susceptible to race 0. The study underlined the importance of wild lentil germplasm for broadening the genetic base of cultivated lentil and their usefulness in disease screening experiments as positive checks.

3.1. Introduction

Anthracnose caused by *Colletotrichum lentis* is an important fungal disease of lentil in western Canada. The disease was first reported in 1987 in the province of Manitoba (Morrall, 1988). A search for a source of resistance was initiated first by screening of a large number of accessions in cultivated lentil (*L. culinaris*) obtained from different gene banks worldwide (Buchwaldt et al., 2004; Shaikh et al., 2013). Based on these results, two pathogenic races of *C. lentis*, race 0 and race 1 were identified (Buchwaldt et al., 2004). Race 1 is a less virulent race to which partial resistance was found in *L. culinaris* accessions and race 0 is a more virulent race against which little or no resistance has been found in *L. culinaris* accessions.

Subsequently, a search for source of resistance in lentil wild relative species identified with high frequency to both races of *C. lentis* in accessions of *L. ervoides* (Tullu et al., 2006). Resistant

accession L-01-827A selected from the *L. ervoides* pool was crossed with susceptible *L. culinaris* cultivar Eston and an F_1 was developed using embryo rescue techniques (Fiala et al., 2009). The resulting F2 was advanced using single seed descent to develop an interspecific recombinant inbred line (RIL) population, named LR-59. The LR-59 population was evaluated for race 0 and race 1 resistance under controlled and field conditions (Fiala et al., 2009; Vail et al., 2012). The resistant line LR-59-81 with resistance to both races was selected and is being used as a resistant check for anthracnose disease screening for both races of *C. lentis* (Banniza et al., 2018).

Prior to this study, Shaikh et al. (2013) evaluated 579 *L. culinaris* accessions from 20 countries of central and eastern Europe by self-pollinating plants and then making single plant selections of the progeny. They reported seven, one and 15 landrace accessions with resistance to race 0, to both race 0 and race 1, and to race 1, respectively. Thus, the current study was initiated to evaluate the promising sources of resistance to *C. lentis* race 0 identified in the landrace accessions by Shaikh et al. (2013) in relation to the resistance in LR-59-81.

3.2. Materials and methods

3.2.1. Plant material and plant growth conditions

Seeds for eight *L. culinaris* accessions were obtained from Plant Gene Resources of Canada (PGRC), Saskatoon (Table 3.1). Seven were previously reported to be resistant to race 0, and accession, VIR-2633 was reported to have resistance to races 0 and 1 of *C. lentis* (Shaikh et al., 2013). For all 8 accessions, 35 arbitrarily selected seeds, which will be referred to as 'sublines' in this study, were planted individually in 4.5 L (15.5 cm diameter) pots in a growth chamber. The sublines were grown to generate seeds for a replicated pathogenicity test. Seeds were harvested from each plant separately and each subline was treated as an independent entry. For each of the eight accessions, 31 sublines were evaluated for disease reaction in individual experiments. *Lens culinaris* cultivars Eston (susceptible to both races) and CDC Robin (susceptible to race 0, partially resistant to race 1), as well as interspecific recombinant inbred line LR-59-81, derived from the cross *L. culinaris* Eston × *L. ervoides* L-01-827A (high levels of resistance to both races) (Fiala et al., 2009; Vail et al., 2012) were used as checks.

Thirty-one sublines of each accession in 10 replicates were used for each experiment. The experiments were conducted separately per accession. Two seeds of each subline were sown in

each cell of 38-cell cone trays (26.8 cm x 53.5 cm) filled with Sun Gro Horticulture Sunshine Mix LA4 (Sun Gro Horticulture, Bellevue, USA) and perlite (Specialty Vermiculite Canada, Winnipeg, MB) at 3:1 ratio. Ten replicate trays per accession were arranged in a randomized complete block design and the three checks were included in each tray. The experiments were conducted under controlled conditions in a growth chamber (Conviron, Model GR178; Winnipeg, MB) and in a greenhouse at the University of Saskatchewan. The day/night temperature of 21/18 °C and 23/22 °C, and photoperiod of 16 h and 17 h were maintained throughout the experiment using artificial light sources for growth chamber and greenhouse, respectively. After germination, the developing seedlings were thinned to one seedling/cell and a soluble mixture of N, P and K (20:20:20) at 2 g/l water was applied once per week.

Table 3.1. List of *Lens culinaris* accessions evaluated in the current study.

Accession[≠] (PGRC)	Original[¥] Name	Country of origin	Seed coat color	Cotyledon color	Reportedly resistant to*
CN 108287	VIR-2058	Czechoslovakia	Green	Yellow	Race 0
CN 108293	VIR-2068	Czechoslovakia	Green dotted	Yellow	Race 0
CN 108297	VIR-2076	Czechoslovakia	-	-	Race 0
CN 108301	VIR-2080	Hungary	Green	Yellow	Race 0
CN 108305	VIR-2086	Germany	Black	Red	Race 0
CN 108445	VIR-2826	Unknown	Green	Yellow	Race 0
CN 108446	VIR-2827	Czechoslovakia	Green	Yellow	Race 0
CN 108424	VIR-2633	Georgia	Tan	Red	Race 0 & 1

[≠]Plant Gene Resources of Canada (PGRC); [¥]Vavilov Institute of Plant Industry (VIR), Russia

* Adapted from Shaikh et al. 2013.

3.2.2. Fungal inoculum production, inoculation, and disease assessment

Colletotrichum lentis isolates CT-30 (race 0) and CT-21 (race 1) (Banniza et al., 2018) were used for inoculations in separate experiments. Conidia were revitalized on 50% oatmeal agar plates (30 g oatmeal [Quick Oats, Quaker Oats Co., Chicago, IL, USA], 8.8 g agar [Difco, BD®,

Sparks Glencoe, MD, USA], 1 L H2O) and incubated for 7-10 days at room temperature. Plates were then flooded with sterile deionized water and conidia were harvested by scraping the colonies with the edge of a sterile glass microscope slide. The suspension was collected and filtered through one layer of Mira-cloth into a clean Erlenmeyer flask. The concentration of the conidial suspension was adjusted to 5×10^4 conidia mL^{-1} using a hemocytometer. The surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) was added at the rate of 1 to 2 drops per 1000 mL of suspension and the suspension was shaken well before inoculation.

Four weeks after seeding, plants were inoculated with the spore suspension at 3 mL per plant using an airbrush. The inoculation for one accession (VIR-2633) was conducted in a growth chamber and for seven accessions (VIR-2058, VIR-2068, VIR-2076, VIR-2080, VIR-2086, VIR-2826, and VIR-2827) in the greenhouse. For VIR-2633, sublines were inoculated with *C. lentis* CT-30 and CT-21 in separate experiments in a growth chamber. Twelve sublines of accession VIR-2633 that showed race 1 resistance after growth chamber inoculation were inoculated with both races for further confirmation in the greenhouse. Immediately after inoculation, plants were incubated at 90-100% relative humidity for 48 h in incubation chambers in the growth chamber, and for 24 h in incubation chambers in the greenhouse. They were subsequently covered with clear plastic bags or sleeves, before being moved to regular growth chamber or greenhouse benches. In the growth chamber experiments, leaf wetness was maintained by misting water inside the bag until the final scoring. In the greenhouse, benches were equipped to mist for 30 seconds every 90 minutes. Individual plants were scored for *C. lentis* disease severity at 8-10 days post-inoculation (dpi), using a 0 to 10 rating scale with 10% increments in anthracnose severity. Data were converted to percentage/proportion disease severity using the class midpoints for data analysis.

3.2.3. Data analysis

Statistical analyses were conducted using SAS software (SAS 9.4, SAS Institute, Cary, North Carolina). Disease scores of each accession (31 sublines entry in 10 replicates) were analyzed separately. Normality and variance homogeneity of the residuals were tested using Shapiro-Wilk normality test and Levene's test for homogeneity, respectively. The data did not conform to the assumptions of a Gaussian distribution. As a result, a generalized linear mixed model with a beta distribution function was fitted to the data using PROC GLIMMIX with the LOGIT link function (SAS 9.4). The genotype was treated as a fixed factor and replicate as a

random factor. Means of the disease reactions were compared post hoc using Tukey's Honestly Significant Difference at $\alpha = 0.05$.

3.3. Results

3.3.1. Reaction of lentil landrace accessions to race 0 inoculation

Seven *L. culinaris* landrace accessions identified previously as promising sources of resistance to *C. lentis* race 0 (Shaikh et al., 2013) were evaluated to determine their reaction (Table 3.1). The susceptible checks Eston and CDC Robin had similar mean disease severity ranging from 93 - 95% in all experiments (Figure 3.1). The race 0 resistant check LR-59-81 had a disease severity of 14 - 36%, which was significantly lower than that of susceptible checks Eston and CDC Robin in all experiments ($p < 0.05$). Among the 217 sublines derived from VIR-2058, VIR-2068, VIR-2076, VIR-2080, VIR-2086, VIR-2826 and VIR-2827 (31 sublines per accession), all sublines were significantly more susceptible to *C. lentis* race 0 isolate CT-30 than the resistant check LR-59-81 (Figure 3.1).

Disease severity for the majority of the sublines were similar to those of the susceptible checks Eston and CDC Robin ($p > 0.05$). The overall mean disease severity of the accessions VIR-2058, VIR-2068, VIR-2076, VIR-2080, VIR-2086, VIR-2826 and VIR-2827 ranged from 88 - 93% and no disease severity scores of less than 80% were observed for any of the sublines of those accessions (Appendix A). Accession VIR-2826 had an overall mean disease severity of 90%, but two of its sublines had mean disease severity scores of 65% and 77%, which was significantly lower than that of the susceptible checks Eston and CDC Robin.

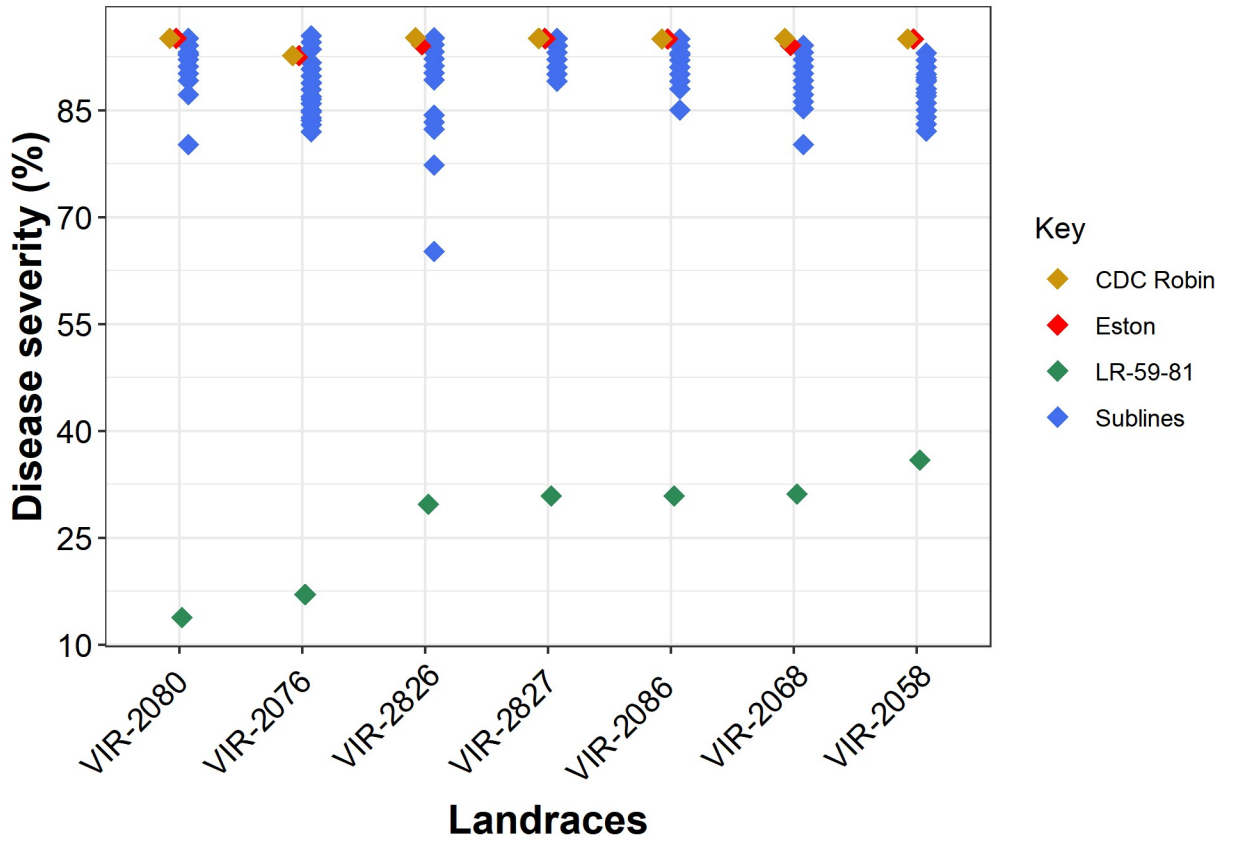

Figure 3.1. Anthracnose severity for seven *Lens culinaris* landrace accessions and checks evaluated under greenhouse conditions in response to infection with *Colletotrichum lentis* isolate CT-30 (race 0). Purple data points on the panel represent mean anthracnose severity values of 31 sublines evaluated for each landrace accession in comparison to susceptible checks CDC Robin and Eston, and resistant check LR-59-81. Each data point is the estimate based on 10 replications per subline and per check. Anthracnose severity was rated using a 0-10 scale with 10% increments in disease severity.

3.3.2. Reaction of lentil landrace accession VIR-2633 to both races of *C. lentis*

Race 0 inoculations of accession VIR-2633, previously identified as a potential source of resistance to both races of *C. lentis* in growth chamber experiments, revealed levels of anthracnose severity ranging from 58 - 84%, with an overall mean of 72%. The susceptible checks Eston and CDC Robin had mean disease severity of 94% and 88%, respectively (Figure 3.2). The resistant check LR-59-81 had a mean anthracnose severity of 29%, which was significantly lower than that

of all sublines of VIR-2633 ($p<0.05$). One subline had the lowest mean anthracnose severity (58%), significantly lower than that of the susceptible checks Eston and CDC Robin ($p<0.05$) in the growth chamber, but in greenhouse conditions, where scores were higher overall, anthracnose severity was similar to that of the susceptible checks Eston and CDC Robin (data not shown).

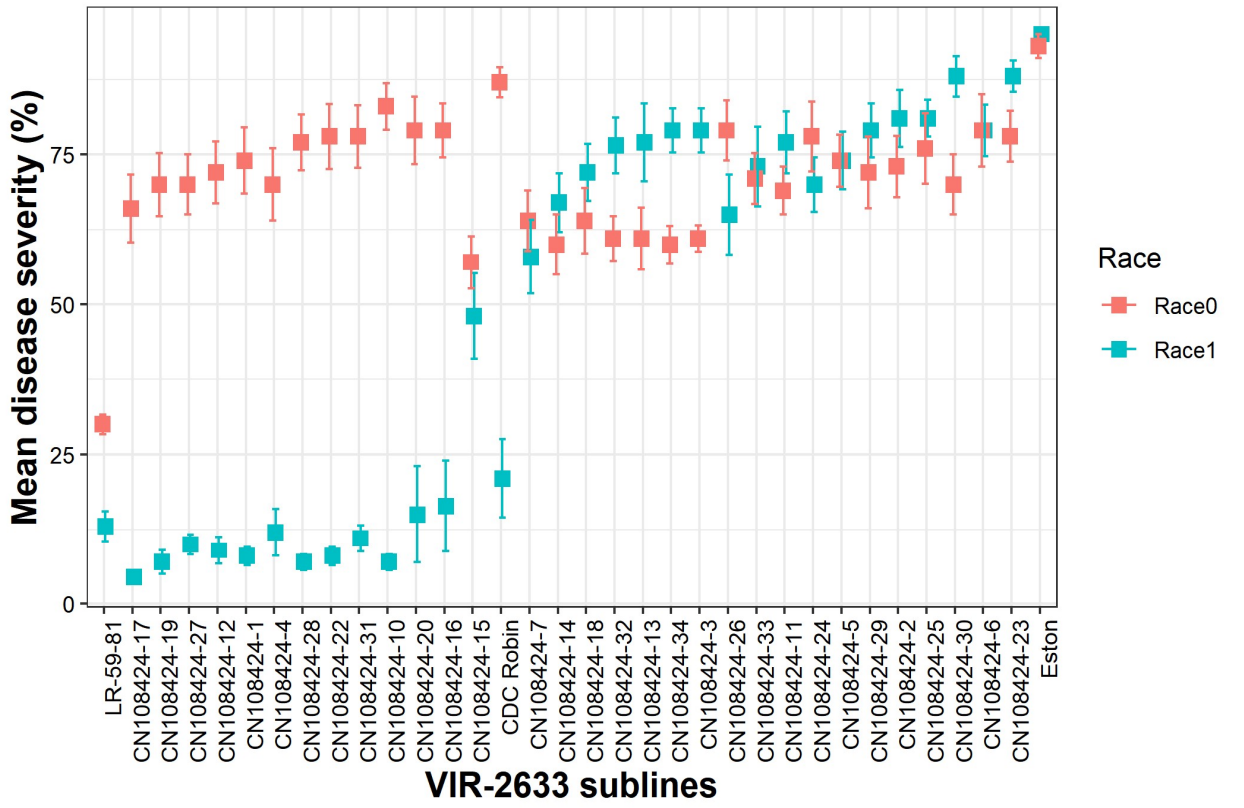

Figure 3.2. Anthracnose severity (%) of 31 sublines of *Lens culinaris* landrace accession VIR-2633 evaluated under growth chamber conditions for disease reaction to race 0 and race 1. Error bars indicate standard error of the mean. Anthracnose severity was rated using a 0-10 scale with 10% increments in disease severity.

Screening of VIR-2633 with the race 1 isolate CT-21 in the growth chamber revealed varying levels of resistance, with disease scores ranging from 5% (small lesions at stem base) to 95% (dead plant) and an overall mean of 49%. The resistant checks LR-59-81 and CDC Robin had mean scores of 13% and 21%, respectively. Of the 31 VIR-2633 sublines tested, 12 had scores equal to, or lower than the two resistant checks and were considered resistant to this race (Figure 3.2). When re-tested in the greenhouse with race 1 inoculation, these 12 sublines had mean disease

severity scores ranging from 11% to 33%, which was not significantly different from the resistant checks LR-59-81 (28%) and CDC Robin (23%), and much lower than the 95% score for the susceptible check Eston (Figure 3.3). When re-tested with race 0 in the greenhouse they had a minimum average disease severity score of 72%, which was not different from the susceptible checks CDC Robin and Eston and was significantly higher than that of the resistant check LR-59-81 which had a mean of 38%. These results indicate there was no resistance to race 0 in accession VIR-2633, but 12 sublines of the accession had scores equal to, or lower than the two resistant checks and were considered resistant to race 1.

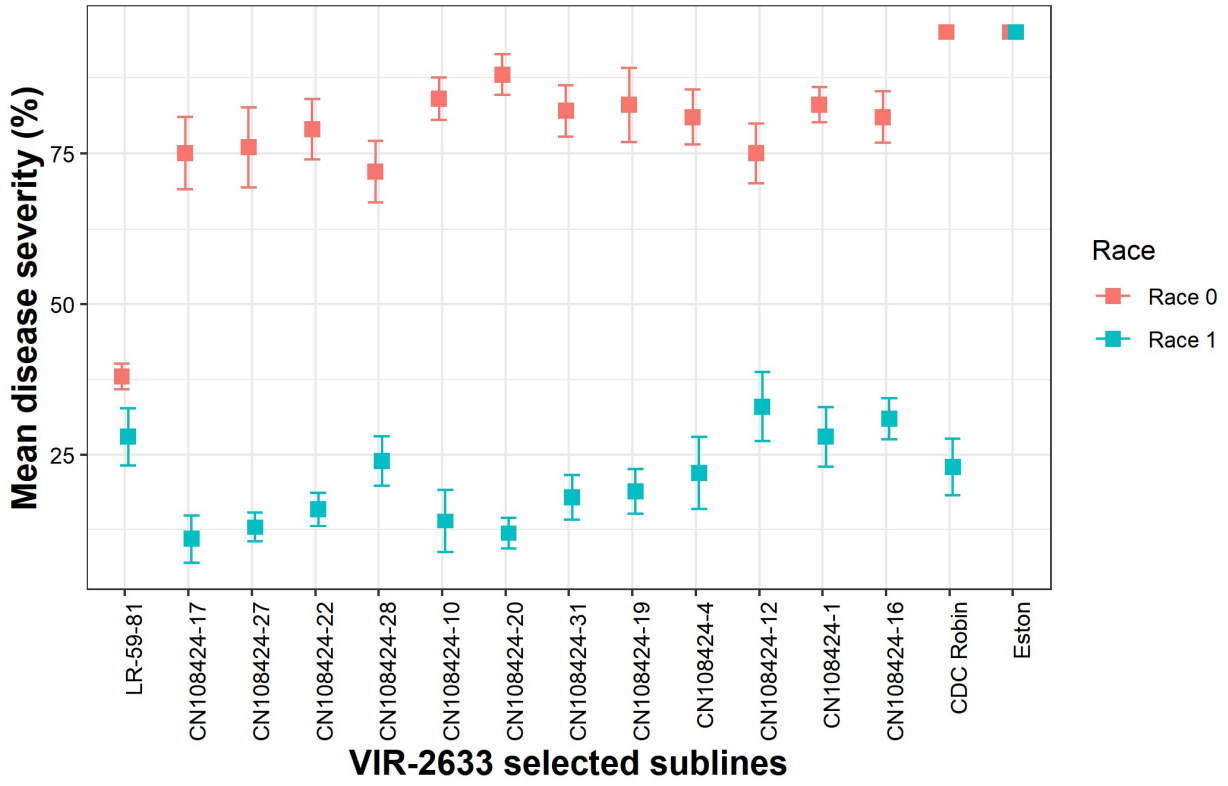

Figure 3.3. Anthracnose severity (%) of 12 sublines of *Lens culinaris* landrace accession VIR2633 resistant to race 1 evaluated under greenhouse conditions for both race 0 and race 1 reactions for further confirmation. The 12 sublines were selected after growth chamber inoculation with race 1. Error bars indicate standard error of the mean. Disease was rated using a 0-10 scale with 10% incremental increases in disease severity.

3.4. Discussion

Identification of new sources of resistance from landraces and subsequent introduction into an elite cultivated background can be efficient and is easily implemented in breeding programs with the goal of developing a variety with desirable genes/alleles. For *C. lentis* race 0, the sources of resistance in the cultivated species and its primary genepool remain limited (Buchwaldt et al., 2004; Tullu et al., 2006). In the genus *Lens*, the most effective resistance to both races were identified in *L. ervoides* and *L. lamottei* Czefr. (Tullu et al., 2006). However, interspecific hybridization with species in genetically distant gene pools is complicated by fertilization barriers, such as embryo abortion, chromosomal aberrations, and reduced pollen fertility (Abbo and Ladizinsky, 1991, 1994; Gupta and Sharma, 2007). Fiala et al. (2009) and Tullu et al. (2013) used ovule and embryo rescue techniques to start to transfer the resistance from *L. ervoides* accessions into *L. culinaris* germplasm. The resultant interspecific hybrid RIL lines had variable levels of fertility in subsequent segregating populations.

The interspecific RIL, LR-59-81(Fiala et al., 2009), has become a commonly used resistant check in all anthracnose disease screening nurseries and indoor assays at the Crop Development Centre (CDC), University of Saskatchewan. This RIL has shown a consistently high level of resistance to both races in greenhouse and field evaluations, even under high disease pressure (Fiala et al*.,* 2009; Vail et al., 2012), and the resistance is not dependent on plant age (Vail, 2010). Moreover, the response of LR-59-81 to the inoculation of 144 ascospore-derived *C. lentis* populations (race 0 × race 1) revealed lower levels of stem lesions and shoot die-back to all isolates of that population (Banniza et al., 2018).

Evaluations of anthracnose severity under controlled conditions in the current study confirmed the lack of resistance to race 0 in *L. culinaris* accessions in comparison to the resistance of LR-59-81. We found a few sublines that had improved resistance when compared to the susceptible check Eston. This partially agrees with the findings of Shaikh et al. (2013). They reported that, after a cycle of selfing and single plant selection, all the lentil accessions evaluated in the study had resistance to race 0 in comparison to the susceptible check Eston. However, the level of resistance in those accessions was less than the resistance of the interspecific RIL LR-59-81. A possible explanation for the discrepancy could be that lentil landrace accessions display heterogeneity due to either segregation at resistance loci or due to genotypic mixture (Buchwaldt et al., 2018). The

success of finding the desired level of resistance in such situations mainly relies on the frequency of targeted alleles in the accession. In VIR-2633, identified as a potential source of resistance to both races, 38.7% of the sublines were resistant to race 1, and none showed resistance to race 0. Another possible reason could be differences in race 0 isolates used for the two studies. It is possible that the race 0 isolate used in the current study was more virulent, potentially indicating a higher aggressiveness on *L. culinaris* accessions in comparison to the resistant check. Similar results were reported by Vail (2010), who evaluated the resistance to both races for accession VIR421 under field conditions, which had previously been reported resistant to race 0 (Buchwaldt and Diederichsen, 2004). Banniza et al. (2018) also found only modest improvement in resistance of VIR421 compared to Eston, and that resistance was significantly lower than LR-59-81 when tested against an ascospore-derived population of *C. lentis* from a cross of CT-30 (race 0) × CT-21 (race 1).

Based on these results, it was confirmed that sources of resistance to race 0 of *C. lentis* appear to be restricted to wild *Lens* species, especially accessions of *L. ervoides* as reported by Tullu et al. (2006). Exploiting the resistance in the tertiary gene pool species can be confounded by linkage drag (Tanksley and Nelson, 1996). Use of marker assisted selection (MAS) (Collard and Mackill, 2008) may improve resistance breeding strategies for transferring race 0 resistance genes from *L. ervoides* without the associated linkage drag. This may require deeper knowledge of genomic information considering that *L. culinaris* and *L. ervoides* have a chromosomal translocation between chromosome 1 and 5 (Gujaria-Verma et al., 2014; Bhadauria et al., 2017). Transfer of desired genes/alleles between the two species is possible only if the genes/alleles that control the resistance are not near the translocation breakpoint.

3.5. Conclusion

Seven *L. culinaris* landrace accessions reported to be resistant to *C. lentis* race 0 and accession VIR-2633, with reported resistance to both races of *C. lentis* were assessed for race 0 resistance relative to LR-59-81. No resistance to race 0 was detected among the *L. culinaris* accessions. A few sublines displayed slight improvements in resistance compared to the susceptible check Eston, but were significantly more susceptible than LR-59-81. Moreover, screening of the sublines of accession VIR-2633 identified 12 sublines with resistance to race 1, but all were susceptible to race 0. The study underlined the importance of the resistance genes introgressed from *L. ervoides*

in broadening the genetic base of cultivated lentil and their usefulness in disease screening experiments as checks.

Prologue to Chapter 4

Results from Chapter 3 supported existing reports that resistance to anthracnose race 0 is rare within the *L. culinaris* gene pool. However, a number of lentil genotypes with resistance to race 1 have been identified in cultivated lentil. Race 1 resistance from selected resistant germplasm were also successfully introgressed into elite breeding materials. The transfer and utilization of the resistance from different sources could be accelerated through marker assisted selection (MAS). MAS requires identification of molecular markers associated with quantitative trait loci (QTL) underlying disease resistance. Mapping of disease resistance QTLs in plants are commonly done via biparental linkage analysis and/or genome-wide association studies (GWAS) approaches. Thus, applications of the two mapping approaches for anthracnose race 1 resistance will be discussed in the next chapter.

Disclosure

The content of this chapter is currently a manuscript in preparation: Identification of anthracnose (*Colletotrichum lentis*) race 1 resistance loci in lentil by integrating linkage mapping and a genome-wide association study.

Gela, T. S., Ramsay, L., Haile, T. A., Vandenberg, A., and Bett, K. E. (2020). IN PREPARATION.

Author contributions

TSG conducted the experiments, drafted the manuscript, and analyzed the data; TAH constructed the genetic linkage map (LR-01);TSG and technical support under supervision of KEB preformed DNA extraction; technical support under supervision of KEB prepared exome capture libraries; LR performed bioinformatic analyses; AV and KEB participated in its design, and critically reviewed the manuscript.

CHAPTER 4

Identification of anthracnose (*Colletotrichum lentis*) race 1 resistance loci in lentil by integrating linkage mapping and a genome-wide association study

Abstract

Anthracnose caused by *Colletotrichum lentis* is a devastating disease of lentil in western Canada. Growing resistant lentil cultivars is the most cost-effective and environmentally friendly approach to prevent seed yield losses that can exceed 70%. To identify loci conferring resistance to anthracnose race 1 in lentil, biparental quantitative trait loci (QTL) mapping of two recombinant inbred line (RIL) populations was integrated with a genome-wide association study (GWAS) using 200 diverse lentil accessions from a lentil diversity panel (LDP). A major-effect QTL (*qAnt*1.*Lc*-3) conferring resistance to race 1 was mapped to lentil chromosome 3 and co-located on the lentil physical map for both RIL populations. Clusters of candidate nucleotide binding-leucine-rich repeats (NB-LRR) and other defence-related genes were uncovered within the QTL region. A GWAS detected 14 significant SNP markers associated with race 1 resistance on chromosomes 3, 4, 5, and 6. The most significant GWAS SNPs on chromosome 3 supported *qAnt*1.*Lc*-3 and delineated a region of 1.6 Mb containing candidate resistance genes. The identified SNP markers can be directly applied in marker-assisted selection to accelerate the introgression of race 1 resistance in lentil breeding.

4.1. Introduction

Lentil (*Lens culinaris* Medik., 2n=2x=14) is an annual self-pollinating pulse crop with genome size of ~4Gb (Arumuganathan and Earle, 1991). Lentil production in the northern Great Plains of North America, particularly western Canada, is challenged by anthracnose caused by the fungus *Colletotrichum lentis* (Damm) (Damm et al., 2014). Yield loss of more than 70% can occur when susceptible cultivars experience high disease pressure (Chongo et al., 1999; Morrall and Pedersen, 1990). Recommended integrated control strategies include 3-4-year crop rotations, fungicides, and genetic resistance (Buchwaldt et al., 2018). Breeding and deployment of resistant cultivars is the most cost-effective and environmentally friendly approach to prevent yield loss caused by

anthracnose. Successful deployment requires continuous incorporation of new sources of resistance into elite breeding materials.

Lentil accessions with resistance to anthracnose race 1 were identified in *L. culinaris* germplasm (Buchwaldt et al., 2004). Resistance to race 1 was transferred into elite breeding lines and resulted in the release of cultivars such as CDC Robin and CDC Redberry (Vandenberg et al., 2002, 2006). Since then, several cultivars with partial resistance to race 1 have been released and deployed in Saskatchewan lentil production (Government of Saskatchewan, 2019).

Successful incorporation of race 1 resistance into improved lentil cultivars is possible through classical breeding but would be greatly improved if molecular breeding strategies could be employed. This requires use of molecular markers to identify the genes that control the quantitative traits; however, little is known about the causal genomic regions controlling race 1 resistance in lentil. Tullu et al. (2003) mapped race 1 resistance in lentil accession PI 320937 using RAPD markers and identified a major dominant gene and several minor genes. Segregation analysis of race 1 resistance in PI 320952 and PI 345629 revealed control by recessive and dominant genes (Buchwaldt et al., 2013).

More precise knowledge of accurate localization of QTL/genes and identification of linked molecular markers is an important step in development of effective MAS in lentil breeding. It also facilitates pyramiding of the resistance genes into lentil cultivars to achieve high levels of resistance against both races of anthracnose. Integration of QTL mapping in biparental populations and GWAS provides the technology to identify trait loci associated with resistance while refining the genomic regions with high resolution (Zhu et al., 2008). Both mapping strategies have been successfully used to identify QTL for multiple traits in lentil (biparental: Fedoruk et al., 2013, Subedi et al., 2018; Sari et al., 2018; and GWAS: Khazaei et al., 2018; Kumar et al., 2018).

Exome capture genotyping, which targets the genic regions of the genome, has been demonstrated to be an efficient method of high-throughput SNP discovery in lentil (Ogutcen et al., 2018). Exome capture sequencing has been used on a diversity panel of lentil accessions (Haile et al., 2020) and a lentil biparental RIL population (LR-01; Haile et al. in prep.). The SNP markers targeting the functional region of a genome may be of great importance to breeders because they are attributable to traits of interest under artificial selection through MAS.

In this study, QTL mapping of anthracnose race 1 resistance was performed using two lentil biparental populations and a lentil diversity panel. The objectives were: (i) to identify QTLs for

anthracnose race 1 resistance in two lentil RIL populations, (ii) to conduct an association mapping study using genome-wide SNP markers to identify chromosomal regions associated with race 1 resistance, and (iii) to compare the QTL regions detected in both mapping strategies to identify candidate genes involved in disease resistance.

4.2. Material and methods

4.2.1. Plant material

Two biparental-derived lentil RIL populations were used for QTL mapping: LR-01, derived from the cross ILL 1704 × CDC Robin; and LR-18, developed from the cross CDC Robin × 964a-46 (Tar'an et al., 2003). Both RIL populations were advanced to F_7 by single seed descent before bulking and comprised a set of 102 and 139 RILs for LR-01 and LR-18, respectively. CDC Robin is a cultivar partially resistant to race 1 of *C. lentis* and resistant to ascochyta blight (Vandenberg et al., 2002). Parents ILL 1704 and 964a-46 are susceptible to anthracnose race 1. Breeding line 964a-46 was developed from ILL 5588, an ascochyta blight resistant landrace released as the cultivar Northfield in Australia (Ali, 1995). ILL 1704 is a landrace from Ethiopia with moderate resistance to ascochyta blight (Tullu et al., 2010).

For the GWAS panel, a subset of 200 lentil genotypes selected from the Lentil Diversity Panel (LDP; N=324) was used (Haile et al., 2020; http://knowpulse.usask.ca/Lentil-Diversity-Panel). The LDP consists of 324 accessions assembled from the gene banks of Plant Gene Resources of Canada (PGRC), the USDA, the International Center for Agricultural Research in the Dry Areas (ICARDA), and included cultivars developed at the Crop Development Centre (CDC), University of Saskatchewan (U of S).

4.2.2. Fungal inoculum production, inoculation, and disease assessment

Colletotrichum lentis isolate CT-21 representing race 1 (Banniza et al., 2018) was used to inoculate the RIL populations and GWAS panel. Fungal inoculum production and inoculation were done as described in section 3.2.2.

The RIL populations and parents were evaluated in a growth chamber environment at the U of S College of Agriculture and Bioresources phytotron facility. The GWAS panel was evaluated in a growth chamber and in an outdoor polyhouse. In growth chambers, plants of each

accession/RIL were grown in 38-cell cone trays (26.8 cm x 53.5 cm) filled with Sun Gro Horticulture Sunshine Mix LA4 (Sun Gro Horticulture, Bellevue, USA) and perlite (Specialty Vermiculite Canada, Winnipeg, MB) at 3:1 ratio. The susceptible control Eston and the RIL parental genotypes for RILs were included in each tray. Experiments were conducted separately for each population. Four weeks after seeding, plants were inoculated with the spore suspension at 3 mL per plant using an airbrush. The experiments were arranged in randomized complete block design with five and seven replications for the RIL populations and the GWAS panel, respectively, which were blocked over time. For the RIL populations, two plants of each RIL were included in each of five sequential experimental runs and the final disease score from each plant was the average per replicate (run). For the GWAS panel, one plant of each accession was evaluated as an individual and repeated seven times. For GWAS accessions exhibiting segregation for disease reaction, an additional three runs were conducted to obtain representative disease scores. For all experiments the plants were scored for race 1 disease severity at 8-10 days post-inoculation (dpi).

The polyhouse experiment was conducted at the Department of Plant Sciences Field Laboratory at the U of S. Four seeds of each accession and two seeds of Eston (susceptible control) were sown in 4.5 L pots (15.5 cm diameter) containing Sunshine Mix No. 4 (Sun Grow Horticulture® Ltd., Vancouver, BC, Canada). The plants were grown under open field ambient conditions for 6 weeks (early flowering stage). Then a polyhouse tunnel covered with translucent thin plastic sheeting suspended 1.5 m above the ground was installed to cover the pots immediately before inoculation. The tunnel area was equipped with a misting irrigation system. Each pot was sprayed with approximately 36 ml (6 ml plant $^{-1}$) of aqueous conidial suspension (5×10^4 spores mL^{-1}) of isolate CT-21 (race 1) 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 three 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.

4.2.3. Statistical analysis of phenotypic data

Analysis of variance (ANOVA) for the phenotypic data were performed using the PROC GLIMMIX procedure of SAS v.9.4 (SAS Institute, Cary, USA). Broad-sense heritability (h_B^2) of single and combined environments disease severity scores of the GWAS panel were calculated with the lme4 package (Bates et al., 2015) in R software (R Core Team, 2020) using the equation:

$$h_B^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_e^2}{r}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.1)$$

$$h_B^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{GE}^2}{E} + \frac{\sigma_e^2}{Er}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4.2)$$

where σ^2_G is the genotypic variance, σ^2_{GE} is variance of the genotype × environment interactions , $\sigma^2 e$ is the error variance, r is the number of replications in each environment and E is the number of environments (Knapp et al., 1985). Lsmeans of disease severity scores were calculated for each environment and for combined environments and subjected to square root transformation to improve the normality of the skewed distribution to perform GWAS analysis, as suggested by Li et al. (2019). Spearman's rank correlation of disease severity between test environments were performed using the procedure CORR in SAS. For RIL populations, mean disease severity data calculated from the replicates were used for QTL mapping.

4.2.4. DNA extraction and genotyping

The GWAS panel and LR-01 populations, including the parents, were genotyped with a custom exome capture assay using protocols previously described by Ogutcen et al. (2018) (Haile et al., 2020; Haile et al. in prep.). In brief, total genomic DNA was isolated from fresh leaves of 2-3-week-old seedlings. DNA quality and quantity for each sample was checked using gel-electrophoresis and PicoGreen. For library preparation, 200 ng high quality DNA was fragmented, ligated to end-repair and A-tailing adaptors. Dual-size selection and PCR application was performed following the steps of the HyperPrep protocol options of the SeqCap EZ HyperCap (Roche, Basel, Switzerland). Before post-capture hybridization the concentration, size distribution, and quality of individual libraries were checked on an Agilent Bioanalyzer using DNA 1000 chips (Agilent, Santa Clara, California, USA). Individual libraries were pooled based on the specific

index combinations recommended by the supplier (Illumina, San Diego, California, USA) for low-plex pooling. The libraries were sequenced on an Illumina HiSeq 2500 instrument at the Génome Québec Innovation Centre, McGill University, Montréal, Canada.

The raw reads were de-multiplexed into individual sample files and subsequently processed for quality control and trimming using FastQC (Andrews, 2010). The per-processed reads of all lines were aligned to the lentil genome assembly V2.0 (http: http://knowpulse.usask.ca) with Bowtie 2 (Langmead and Salzberg, 2012), mixed and discordant alignments were discarded. SAMtools 1.3.1 (Li et al., 2009) was used to convert the mapping results to a bam format and to further sort the reads. The reads caused by PCR duplication were removed by the SAMtools rmdup function. Genome coverage was assessed using BedTools (Quinlan and Hall, 2010) and visualized using IGV 2.3.90 (Thorvaldsdóttir et al., 2013). The resulting variant call format (VCF) file was further filtered using VCFtools (Danecek et al., 2011) to retain SNPs with less than 10% missing, allele calls (minimum read depth=5) and SNPs with minor allele frequency greater than 5%.

4.2.5. Linkage map construction and QTL mapping

LR-01 population. A draft genetic linkage map consisting of 21,634 SNPs grouped into seven linkage groups (Haile et al., *unpublished data*), corresponding to the seven haploid lentil chromosomes was retrieved from the KnowPulse database, U of S (http://knowpulse.usask.ca/portal/chado/genotype/Lens). The linkage map was generated using the MSTMap software (Wu et al., 2008). The high-density genetic map was subjected to bin grouping of the redundant SNP markers with a correlation coefficient of 1.0 using BIN functionality employed in QTL ICIMapping 4.1 software (Meng et al., 2015). A marker representing each bin was retained on the map, and the map distances in centimorgan (cM) between markers were calculated using the Kosambi function (Kosambi, 1944).

LR-18 population. The genetic linkage map developed earlier by Fedoruk et al., (2013) using the SNPs generated by a 1536-SNP Illumina Golden Gate array (Illumina, San Diego, CA) was used for QTL mapping. This map consisted of 550 SNP markers, seven SSR markers, and four morphological markers and total map distance of 697 cM with an average marker distance of 1.2 cM. All genotyping information of LR-18 genetic linkage map can be found through the KnowPulse database accessible at: http://knowpulse.usask.ca/portal/chado/genotype/Lens (accessed 20th March 2020).

The QTL analyses were performed using R/qtl software (http://www.rqtl.org/; Broman et al., 2003). The QTL genotype probabilities were calculated along the chromosome at 1 cM intervals assuming a genotyping error rate of $1.0e^{-4}$ and using the Kosambi map function (Kosambi 1944). Multiple QTL mapping was completed with *stepwiseqtl* function (Broman et al., 2003) using Haley-Knott regression (Haley and Knott, 1992). 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 using *stepwiseqtl* function. 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. The confidence intervals for each QTL were estimated using the "*lodint*" function that calculated the 1.5 LOD support intervals. The percentage of the phenotypic variance explained (PVE) and effects of QTLs were obtained by fitting a mixed linear model using the "*fitqtl*" function.

4.2.6. Association analysis

The population structure of the association mapping panel was assessed using a pruned subset of 6,516 unlinked SNP markers generated after removing SNPs with minor allele frequency of <10% and linkage disequilibrium (r^2 <0.2) at a sliding window of 1 Mb using SNPRelate package (Zheng et al., 2012). The Bayesian model-based clustering implemented in STRUCTURE V2.3.4 (Pritchard et al., 2000) was used to estimate the number of subpopulations (K). The number of K sets from K=2 to K=10, with 10 times independent runs for each K, 50,000 burn-in iterations, and 100,000 Markov chain Monte Carlo sampling replicates were conducted. The optimal K-values were determined using STRUCTURE HARVESTER (Earl & vonHoldt, 2012), and visualized by STRUCTURE PLOT (Ramasamy et al., 2014). Genotypes with membership probabilities <60% were considered admixtures (Falush et al., 2003). Principal component analysis (PCA) (Patterson et al., 2006) and the genetic kinship matrix were conducted using the Genomic Association and Prediction Integrated Tool (GAPIT) (Lipka et al., 2012).

Marker-trait associations (MTA) were tested using a compressed mixed linear model (CMLM) (Zhang et al., 2010a) including the population structure (Q) and kinship (K). Association tests were run using the software GAPIT implemented in R software (Lipka et al., 2012). The quantile-quantile (Q-Q) plot's fattiness was inspected to compare the results from Q + K (population structure and kinship), and PC + K (principal component and kinship) analysis.

However, because the Q-Q plots generated using the two approaches were similar, only the results of the PC+K analysis are presented. SNPs with $-\text{Log}_{10}(\text{p-value}) \geq 5.2$ were considered to have significant associations based on a Bonferroni threshold (1/n) correction at $p = 6.6\times10^{-6}$. Manhattan plots were generated with the R package qqman (Turner, 2014).

4.2.7. Candidate gene analysis

The physical map of the QTL intervals identified was used against the lentil reference genome (CDC Redberry genome assemble *v2*.0; https://knowpulse.usask.ca/genome-assembly/Lcu.2RBY) for the identification of candidate genes associated with disease resistance. The annotated genes identified were used in BLAST analysis of GenBank (NCBI) database to confirm their functions in other plant species. All reported disease resistance (R-) or defense-related genes in plants were considered for selection of candidate genes.

4.3. Results

4.3.1. Phenotypic variation of RIL populations

In both RIL populations, the resistant parent CDC Robin had a resistant reaction with an average disease severity of 23%. The susceptible parents, ILL 1704 and 964a-46 exhibited a susceptible reaction with the average disease score of 95% and 93%, respectively. Most of the RIL lines exhibited disease severity between the range of the resistant and susceptible parental lines, but some exhibited lower disease severity than CDC Robin (Figure 4.1). The disease reactions to anthracnose race 1 of the two RIL populations ranged from 5 to 95% (Figure 4.1), with an overall average mean of 53.6% and 54.2% for LR-01 and LR-18, respectively. The RILs in both populations had bimodal frequency distributions which fitted a 1 resistant: 1 susceptible segregation ratio, indicating monogenic segregation for resistance to *C. lentis* race 1 (Table 4.1).

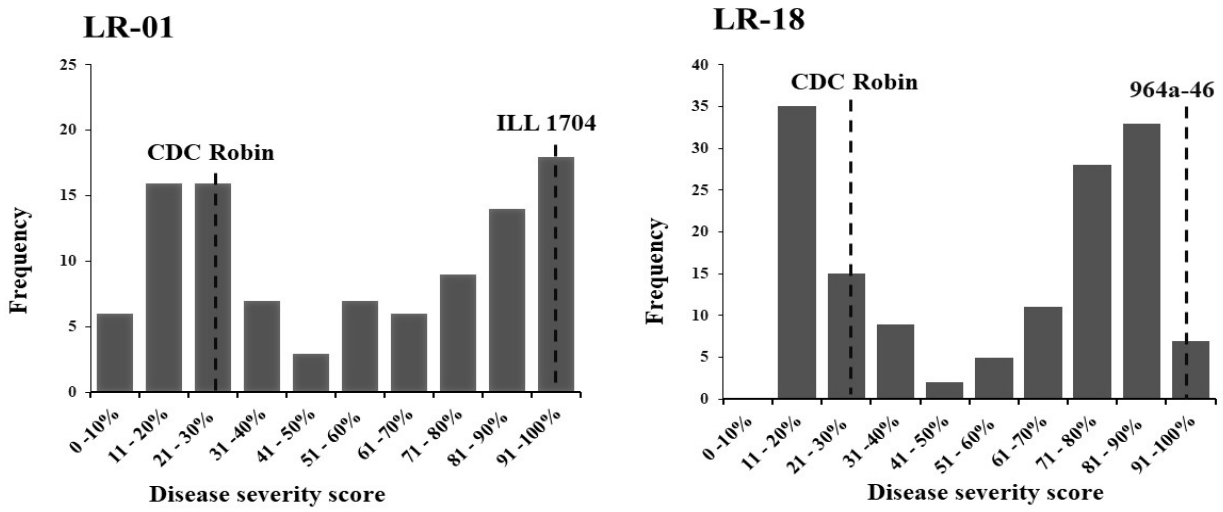

Figure 4.1. Frequency distribution of anthracnose race 1 severity in 102 RILs of LR-01 (ILL 1740 × CDC Robin) and severity in 139 RILs of LR-18 (CDC Robin × 964a-46) in growth chamber conditions. The vertical lines indicate the average disease severity of the parents. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Table 4.1. Segregation of anthracnose race 1 in LR-01 and LR-18 populations, χ 2 test for 1:1 Mendelian ratio and corresponding probability.

Population	Resistant	Susceptible	Total[#]	χ^2 1:1	P
LR-01	47	55	102	0.63	0.428
LR-18	58	80	138	3.51	0.061

[#]one RIL line showed a heterozygous reaction was not included in LR-18 population

4.3.2. Phenotypic variation of the GWAS panel

For the association mapping study, two hundred lentil genotypes were evaluated for reactions to race 1 under growth chamber (phytotron) and polyhouse conditions. Disease severity distribution for the panel was skewed towards susceptibility in both testing environments (Figure 4.2). Under growth chamber conditions, 6.5, 8.5, and 85%; and for polyhouse conditions, 9, 25.5 and 65.5% of the genotypes had resistant, intermediate, and susceptible reactions, respectively.

The results suggest the presence of limited sources of resistance among most of the genotypes tested against race 1, even though it was less aggressive than *C. lentis* race 0. The differences in disease severity scores among the genotypes were highly significant ($p<0.0001$) in both environments. Significant genotype by environment interaction was also observed ($p<0.001$), indicating the influence of experimental conditions on disease development. The estimated broad-sense heritability was high, 0.96 for growth chamber, 0.88 for polyhouse and 0.92 for combined analysis of both environments (Table 4.2), demonstrating that race 1 resistance was controlled by genetic factors and that the data could be used for accurate mapping of race 1 resistance genes.

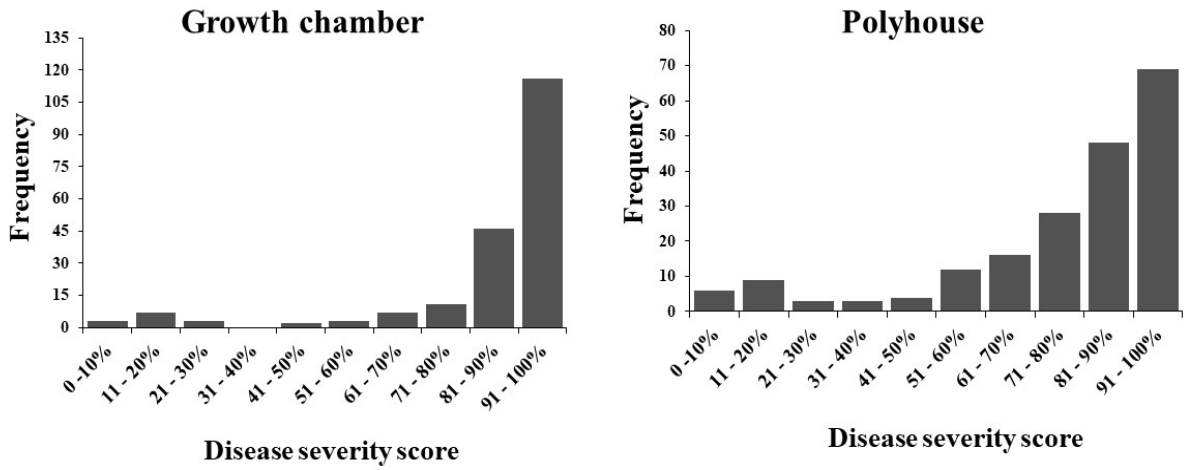

Figure 4.2. Frequency distribution of anthracnose race 1 severity of the 200 lentil genotypes in the GWAS panel evaluated under growth chamber and polyhouse conditions. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Although disease progression was continuous (more intermediates) at the polyhouse compared to the growth chamber (Figure 4.2), a highly significant positive correlation (Spearman $r = 0.61$, $p < 0.001$) was observed between growth chamber and polyhouse data for race 1 response of genotypes. Resistant genotypes such as PI 320952 (Indianhead), CDC Robin (Vandenberg et al., 2002), and PI 320937 (Tullu et al., 2003) showed resistant reactions in both environments, and the other resistant genotypes identified may be additional sources of resistance if they are non-allelic.

Table 4.2. Analysis of variance components for anthracnose race 1 severity of 200 lentil genotypes evaluated under growth chamber and polyhouse conditions.

Environment	Mean	Range	h_B^2	σ^2_G	σ^2_{GE}	σ^2_e
Growth chamber	84.2	6.4 - 95	0.96	405.1***	-	104.8
Polyhouse	76.0	5.0 - 95	0.88	454.5***	-	180.5
Combined	80.1	5.7 - 95	0.92	382.8***	45.55***	133.2

*** p<0.0001, σ^2_G, genotypic variance; σ^2_{GE}, genotype × environment variance; σ^2e, error variance; h_B^2, heritability.

4.3.3. Linkage map construction for the LR-01 population

A total of 21,634 SNPs were available for construction of the genetic map of the LR-01 population (http://knowpulse.usask.ca/portal/chado/genotype/Lens). Seven linkage groups (LG) corresponding to the haploid number of chromosomes of the lentil genome were resolved. The detailed information for the LR-01 linkage map is provided in Table 4.3. The number of SNPs assigned to each linkage group (LG) ranged from 1710 SNP markers (LG 7) to 5120 (LG 2). The SNPs were further refined and SNPs with redundant information were binned. The 21,634 SNPs mapped were grouped into 921 recombination bins and 1807 single markers. In total, 2728 informative SNP markers were distributed along the seven LGs and were retained on the initial LR-01 linkage map. The map covered a total length of 1643.8 cM, with an average distance between the neighboring SNP markers of 0.6 cM. The length of each LG varied from 164.6 cM for LG 7 to 299.5 cM for LG 3. Linkage groups were assigned to their respective chromosomes based on where markers lie in the reference genome.

Table 4.3. Summary statistics of the lentil LR-01 (ILL 1704 × CDC Robin) population genetic linkage map.

Linkage groups	Number of SNP markers	Number of independent marker loci[¥]	Numbers of BIN markers	Number of singleton markers	Map length (cM)	Average marker interval (cM)	Maximum gap (cM)
LG1	2806	337	104	233	200.6	0.6	4.7
LG2	5120	392	124	268	250.1	0.6	4
LG3	4540	442	146	296	299.5	0.7	6.5
LG4	3563	484	158	326	271.2	0.6	4.8
LG5	2004	333	115	218	197.4	0.6	4.3
LG6	1891	445	169	276	260.5	0.6	2.9
LG7	1710	295	105	190	164.6	0.6	3
Total	21634	2728	921	1807	1643.9	0.6	4.3

[¥] Number of independent marker loci includes the number of BIN markers and number of singletons

4.3.4. QTL mapping of anthracnose race 1 resistance

A single significant QTL conferring resistance to race 1 was identified on chromosome 3 using both LR-01 and LR-18 RIL populations and designated as *qAnt*1.*Lc*-3 (Figure 4.3). On the LR-18 linkage map the QTL was flanked by SNP markers LcC03673p249/LcC03441p105 and LcC09426p518, with an interval ranging from 43.2 to 51.5 cM. The SNP markers were mapped within a 7.6 Mb (30704841 to 38275723 bp) physical interval on the CDC Redberry genome assembly v.2.0 (Lcu.2RBY, https://knowpulse.usask.ca/genome-assembly/Lcu.2RBY).

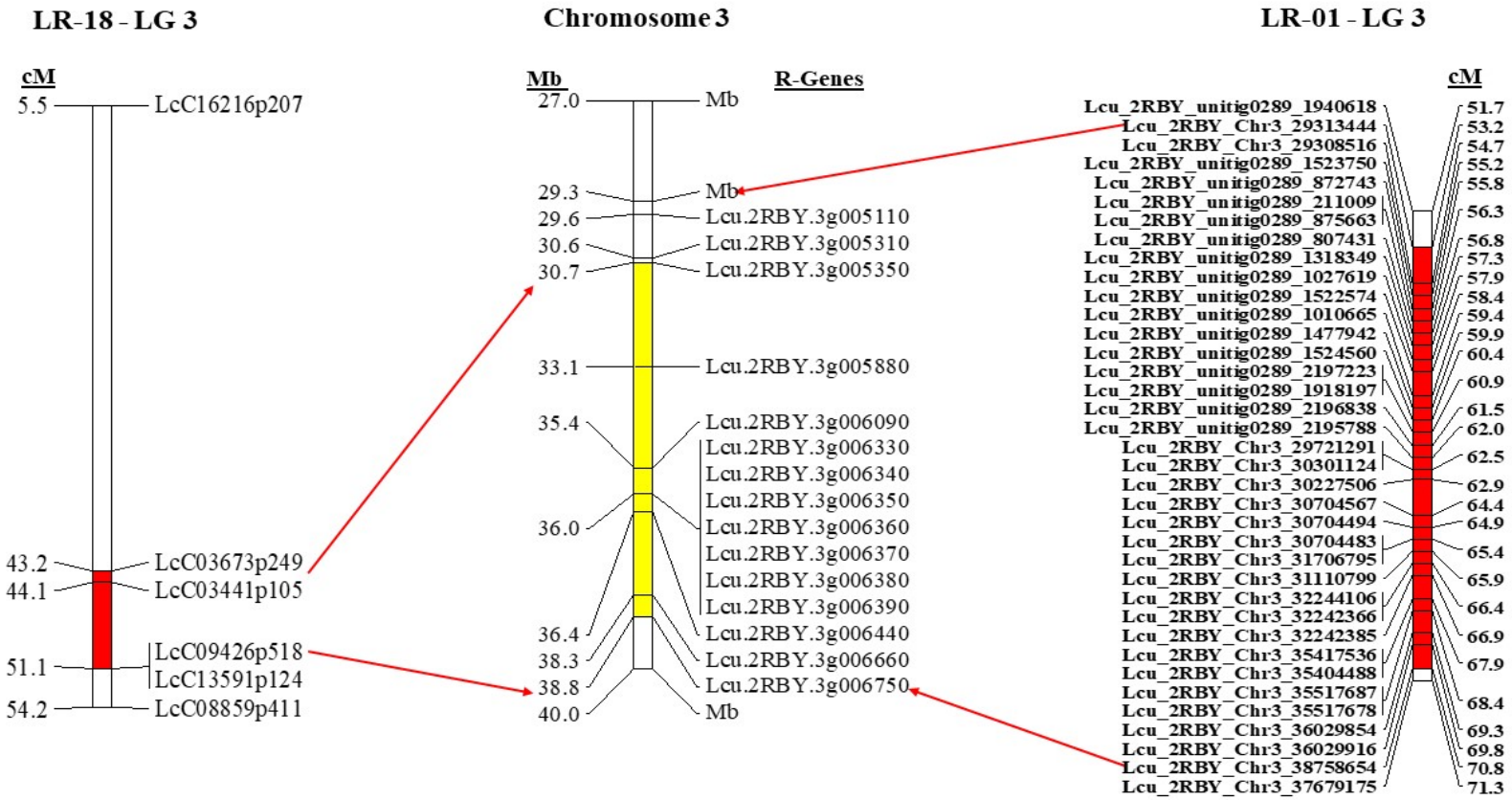

Figure 4.3. Position of anthracnose race 1 resistance QTL on linkage group (LG) 3 of the recombinant inbred line (RIL) populations LR-18 (CDC Robin × 964a-46; left) and LR-01 (ILL 1704 × CDC Robin; right) evaluated under growth chamber conditions. The red regions on the bar highlights the QTL interval on LGs; and the yellow region depicts the interval overlapped for both LGs on lentil chromosome 3 (*Ref. genome v*2.0) and the predicted candidate disease resistance genes (R-genes) is on the right. The positions are in centimorgan (cM) and mega base pairs (Mb) as indicated on the top of the bars.

For the LR-01 population genetic map, *qAnt*1.*Lc*-3 was mapped to an interval of 53.2 cM to 70.8 cM corresponding to a physical location of 29313444 to 38758654 bp (9.4 Mb region) on chromosome 3. Importantly, the high-density genetic map of the LR-01 population contains a number of SNP markers associated with race 1 resistance in the interval of the QTL region. Among these, a cluster of significantly associated SNP markers were from unitig0289 (*Ref. genome v.*2.0), suggesting that they would possibly correspond to chromosome 3. The percentage of the variation in race 1 resistance explained by *qAnt*1.*Lc*-3 varied from 66.6 to 69.8%, with a LOD score value ranging from 24.3 to 37.1 (Table 4.4). As expected, CDC Robin (the resistant parent) contributed the resistance allele for *qAnt*1.*Lc*-3, with additive effects of -25.9 and -26.5 for LR-01 and LR-18 populations, respectively.

Table 4.4. QTL mapping of anthracnose race 1 resistance detected by multiple QTL models of R/qtl in two biparental RIL populations: LR-01 (ILL 1704 × CDC Robin) and LR-18 (CDC Robin × 964a-46).

Population	QTL[€]	LG[#]	Peak LOD	Position (cM)	1.5 LOD interval		PVE[¥] (%)	Add[$]
					Left (cM)	Right (cM)		
LR-01	*qAnt*1.*Lc*-3	3	24.3	60.9	53.2	70.8	66.6	-25.9
LR-18	*qAnt*1.*Lc*-3	3	35.1	44.1	43.2	51.5	69.8	-26.6

[#]LG - linkage group (chromosome), [¥]PVE - Phenotypic variation explained, [$]Add - additive effect, [€]QTL nomenclature: *qAnt*1.*Lc*-3 (*q*=QTL, *Ant*=anthracnose, 1=race 1, *Lc*=resistance derived from *Lens culinaris*, 3=chromosome)

4.3.5. Genome-wide association study of anthracnose race 1 resistance

A total of 152,011 SNP markers were used for marker-trait association analysis. The number of SNP markers per chromosome varied from 16,848 SNPs on chromosome 7 to 26,349 SNPs on chromosome 2 (Figure 4.4). The average distance between two markers used in this study was approximately 26 kb, across a genome size of ~4 Gb with a mean of 21,716 SNP markers per chromosome. The SNP markers were evenly distributed and adequately covered the genome for the purpose of GWAS analysis.

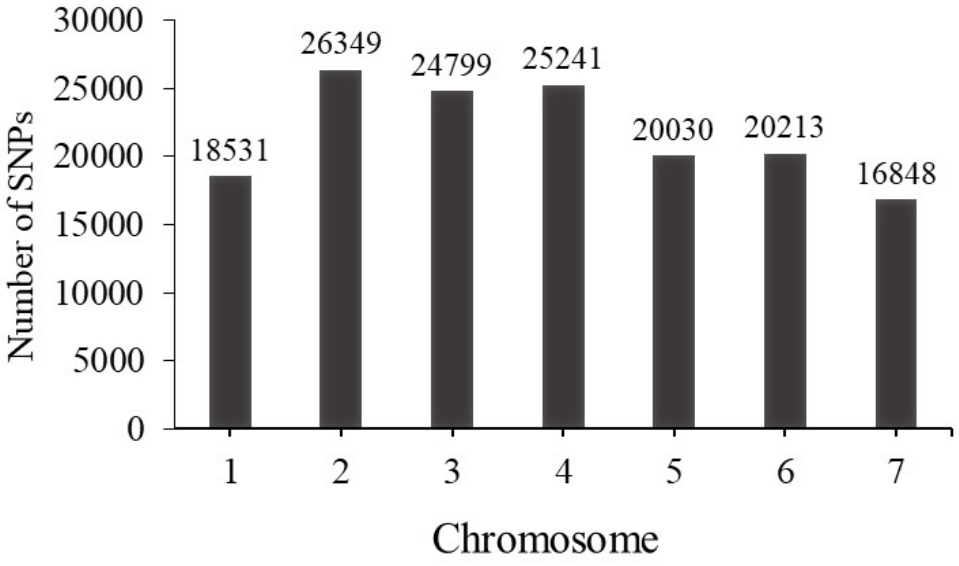

Figure 4.4. Summary of SNP markers per chromosome used for GWAS analysis

The model-based population structure analysis revealed that the 200 lentil genotypes could be grouped into three major subpopulations, and that finer hierarchical structures were evident in the diversity panel (Figure 4.5a). Using k = 3, 90.5% of the accessions were assigned to three groups, and only 9.5% of the accessions were assigned to mixed populations (Figure 4.5b). The principal component analysis (PCA) showed that the variance explained by the eigenvalue of each principal component (PC) dropped rapidly after the first three PCs, which explained approximately 35% of the total genetic variances for the diversity panel (Figure 4.5c). The results were consistent between STRUCTURE and PCA cluster analyses (Fig. 4.5d) and were also confirmed by visual inspection of Q-Q plots (appendix I). Consequently, the first three PCAs were used as a covariate in the mixed linear model in the GWAS analysis.

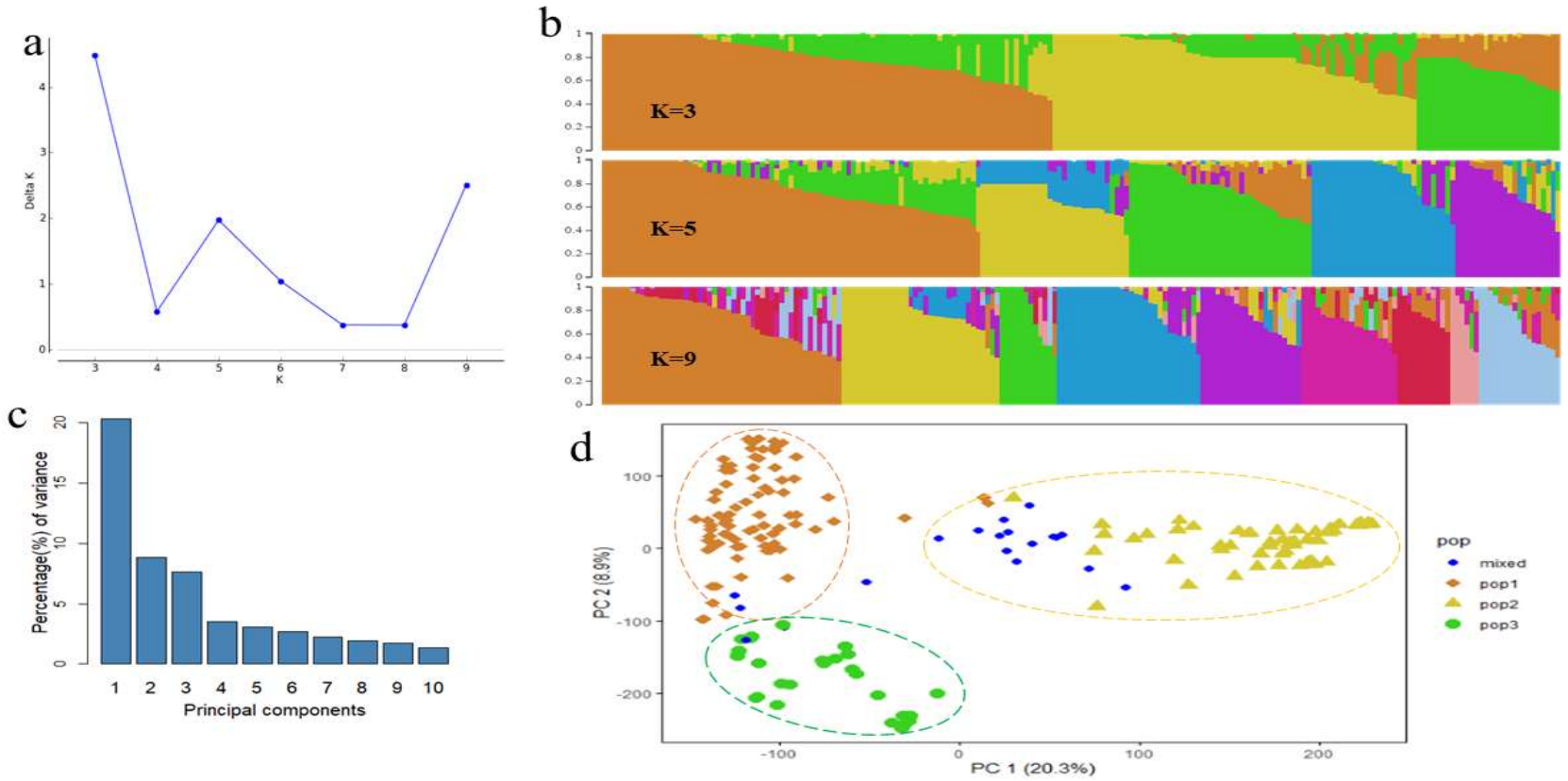

Figure 4.5. The population structure of 200 lentil accessions was identified by the STRUCTURE admixture model and principal component analysis (PCA), which were then used for GWAS analysis. (**a**) delta K values, (**b**) population structure for models with K = 3, K=5 and K = 9, each genotype is represented by a vertical line, (**c**) percent of the variation explained by the first ten principal components, (**d**) scattered plot of the first and second principal components. The PCA plot is colored based on subpopulations (K=3) from the admixture model, whereas the blue dots represent genotypes with estimated membership fraction <60% and assigned as a mixed population.

GWAS analysis using the combined disease severity data detected 14 SNPs that were significantly associated with race 1 resistance in the lentil genome ($-Log_{10}^{(p)} \geq 5.2$) (Figure 4.6). Detailed information of the SNPs is provided in Table 4.5. The GWAS analysis for single environments identified 26 and 11 SNPs, respectively, that were associated with race 1 resistance (Figure 4.6 and Appendix G) under growth chamber and polyhouse conditions. Most of the loci detected were common between both environments with varying p-values of SNP surrounding the loci. The phenotypic variation (R^2) explained by an individual significant SNP marker ranged from 58 to 69%.

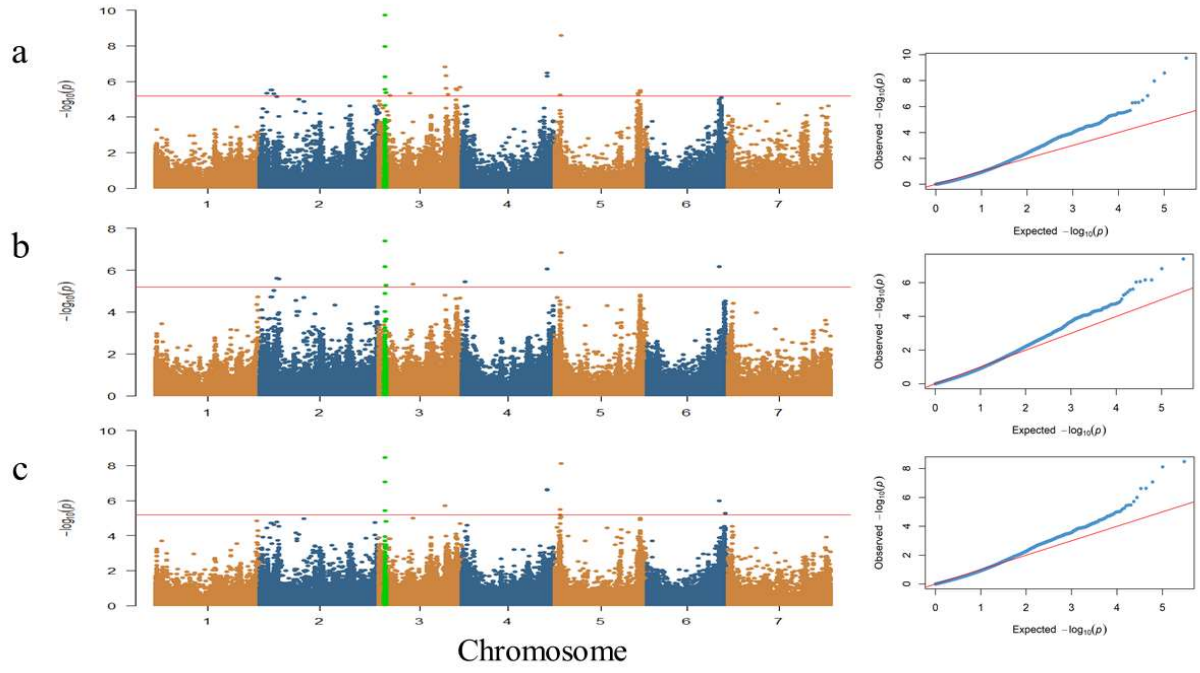

Figure 4.6. Manhattan and Quantile-quantile (Q-Q) plots of genome wide association study (GWAS) for anthracnose race 1 resistance in 200 lentil accessions evaluated in (**a**) the growth chamber, (**b**) the polyhouse and (**c**) the combined lsmean of disease severity scores from both environments. Each color indicates a different chromosome, the Y-axis indicates $-\log_{10}$ of p-values with significant association at 5.2 (red line). The green dots on chromosome 3 represent the SNP marker in the QTL (*qAnt*1.*Lc*-3) interval from biparental populations.

The SNPs surrounding the *qAnt*1.*Lc*-3 regions on chromosome 3 were the most significant with $-\log_{10}(p) = 9.6$ and $R^2 = 69\%$ (Figure 4.6 and Table 4.5) and were detected in all analyses. This region was tagged by four significant SNP markers (Lcu.2RBY.Chr3.33827173, Lcu.2RBY.Chr3.33827185, Lcu.2RBY.Chr3.34117023 and Lcu.2RBY.Chr3.35384298) and spanned 1.6 Mb. Two other genomic regions displayed a peak SNP marker at 341.3 Mb (R^2 = 66%) and 417.9 Mb (R^2 = 65% were identified on chromosome 3 for combined and growth chamber analysis (Table 4.5). Two SNP markers associated with race 1 resistance were detected on chromosome 4 within 4 bp (442702129 bp to 442702133 bp) in all analyses. Two significant regions were identified for combined and growth chamber data on chromosome 5 at interval positions of 28.4 - 33.7 Mb for all analysis and at intervals ranging from 427.5 - 437.9 Mb. One SNP marker located at 374.3 Mb was detected on chromosome 6 for the combined and the polyhouse experiments (Table 4.5).

Table 4.5. SNP markers associated with anthracnose race 1 resistance using combined lsmean data of disease severity from growth chamber and polyhouse for a set of 200 lentil accessions.

SNP Marker	Chr	Position (Mb)[#]	*P*.value	MAF	$R^{2\$}$
Lcu.2RBY.Chr3.33827173	3	33827173	1.38E-06	0.16	0.65
Lcu.2RBY.Chr3.33827185	3	33827185	2.43E-08	0.15	0.67
Lcu.2RBY.Chr3.34117023	3	34117023	2.47E-10	0.14	0.69
Lcu.2RBY.Chr3.35384298	3	35384298	3.24E-06	0.14	0.65
Lcu.2RBY.Chr3.341261994	3	341261994	4.30E-07	0.23	0.66
Lcu.2RBY.Chr3.417940994	3	417940994	6.13E-06	0.06	0.64
Lcu.2RBY.Chr4.442702129	4	442702129	8.32E-08	0.11	0.66
Lcu.2RBY.Chr4.442702133	4	442702133	8.48E-08	0.11	0.66
Lcu.2RBY.Chr5.28582530	5	28582530	3.74E-06	0.12	0.65
Lcu.2RBY.Chr5.28637458	5	28637458	3.74E-06	0.12	0.65
Lcu.2RBY.Chr5.33721990	5	33721990	1.82E-09	0.21	0.68
Lcu.2RBY.Chr5.437910070	5	437910070	3.23E-06	0.13	0.65
Lcu.2RBY.Chr5.437944230	5	437944230	3.95E-06	0.13	0.65
Lcu.2RBY.Chr6.374326758	6	374326758	8.88E-07	0.11	0.65

[#]Physical positions, [$]Explained phenotypic variance per marker

4.3.6. Candidate gene prediction

We explored the candidate genes associated with anthracnose race 1 resistance in the region of *qAnt*1.*Lc*-3 physical genomic intervals that overlapped for both genetic maps. The interval covered 8 Mb from 30.0 - 38.0 Mb on chromosome 3 and a total of 119 annotated genes were identified. Among these, 11 genes encode for typical resistance (R) genes, which are nucleotide-binding-leucine-rich repeat (NB-LRR) domain disease resistance proteins (Table 4.6), and 46 are known to be involved in defense response reactions to pathogens and other stresses (Appendix H). Moreover, two NB-LRR domain genes (Lcu.2RBY.L001220 and Lcu.2RBY.L001240) were tagged by SNPs from unitig Lcu.2RBY.unitig0289 mapped in the QTL region of the LR-01 genetic map, providing further evidence that they possibly correspond to chromosome 3 NB-LRR domain clusters. Within the *qAnt*1.*Lc*-3 region, the most significant GWAS SNPs, located at 103 kb upstream of the gene encoding for an anthranilate N-benzoyltransferase protein (Lcu.2RBY.3g005860), two significant SNPs reside within genes involved with cellulose synthase (Lcu.2RBY.3g005880), and one significant SNP is located within a gene encoding for a disease resistance protein TIR-NBS-LRR domain (Lcu.2RBY.3g006090). Almost all the significant GWAS SNPs identified are located within or close to the annotated gene (Appendix H).

Table 4.6. A subset of candidate resistance genes associated with anthracnose race 1 resistance identified in the interval of QTL and GWAS regions according to gene annotation.

Chr[$]	Gene ID	Physical position (bp)[#]		Annotation
Chr3	Lcu.2RBY.3g005310	30641401	30646173	NB-ARC domain disease resistance
Chr3	Lcu.2RBY.3g005350	30735946	30736590	Wall-associated receptor kinase protein
Chr3	Lcu.2RBY.3g005880[β]	33819256	33828131	Cellulose synthase
Chr3	Lcu.2RBY.3g005910[β]	34117126	34118497	Anthranilate N-benzoyltransferase
Chr3	Lcu.2RBY.3g006090[β]	35383081	35387584	TIR-NBS-LRR domain disease resistance
Chr3	Lcu.2RBY.3g006330	35972988	35973704	LRR & NB-ARC domain disease resistance
Chr3	Lcu.2RBY.3g006340	35974000	35976197	LRR & NB-ARC domain disease resistance
Chr3	Lcu.2RBY.3g006350	35976249	35981698	LRR & NB-ARC domain disease resistance
Chr3	Lcu.2RBY.3g006360	35981709	35982218	NB-ARC domain disease resistance protein
Chr3	Lcu.2RBY.3g006370	35982494	35982865	CC-NBS-LRR resistance protein
Chr3	Lcu.2RBY.3g006380	36028314	36032907	LRR & NB-ARC domain disease resistance
Chr3	Lcu.2RBY.3g006390	36037105	36044777	LRR & NB-ARC domain disease resistance
Unitig0289	Lcu.2RBY.L001220	1917198	1921007	LRR & NB-ARC domain disease resistance
Unitig0289	Lcu.2RBY.L001240	1939874	1942161	NB-ARC domain disease resistance protein

[#] Position in bp according to CDC Redberry genome ref. v.2, [β]Genes identified with GWAS SNPs, [$]Chromosome

4.4. Discussion

Developing host resistance is the most preferable, economical, and sustainable strategy for managing anthracnose in lentil production. However, the process requires sufficient information about genetic sources of resistance and identification of resistance loci associated with races of anthracnose to use marker assisted breeding strategy for gene pyramiding. We used a combination of traditional QTL mapping and GWAS to increase our understanding of anthracnose race 1 resistance in lentil. To our knowledge, this study is the first on anthracnose race 1 resistance in lentil to employ biparental QTL mapping and GWAS using a large number of physical SNP marker positions.

Analysis of the differential responses of the RIL populations and GWAS accessions to race 1 inoculation provided frequency distributions and heritability estimates for the inheritance of

disease resistance (Figure 4.1, 4.2 and Table 4.2). The disease reactions of the RILs of both populations displayed a bimodal distribution that fit a 1:1 ratio indicating a Mendelian one gene model, indicative of a major QTL identified in the populations. Analysis of variance for the GWAS panel revealed significant variation among genotypes. However, disease reaction frequencies of the lines showed a skewed distribution to greater susceptibility. The narrow genetic base for anthracnose resistance is evident in current collections of lentil accessions from different gene banks. For example, previous evaluations of resistance to anthracnose race 1 of lentil accessions from 50 countries identified 16 (0.9%) of 1771 (Buchwaldt et al., 2004), and 15 (2.6%) of 579 (Shaik et al., 2013). This may be attributable to genetic bottlenecks that were created at the time of domestication (Sonnante et al., 2009) due to the absence of the disease in its centre of origin/domestication. Until recently, the disease was considered minor or had not been reported in other parts of the world (Banniza et al., 2018), including its center of origin and/or diversity where lentil have been grown for centuries. The rapid expansion of the lentil crop in the prairie ecosystem coincides with the incidence of anthracnose (Morrell, 1997). Tanksley and McCouch (1997) argued expansion of a few improved cultivars into a modern agricultural system can result in emergence of new disease threats that can occur due to adaptation of plant pathogens (Silva et al., 2012).

Significant correlation between field inoculations and anthracnose screening under controlled conditions has been reported for resistant *L. culinaris* germplasm and interspecific lines (Vail et al., 2012; Chongo and Bernier, 1999). We also found high correlation between growth chamber and polyhouse environments, confirming high heritability estimates, indicating a repeatable and reliable dataset for GWAS analysis.

Breeding for partial resistance to anthracnose race 1 in lentil started with release of the cultivar CDC Robin (Vandenberg et al., 2002), which was derived from PI 320952 (Indianhead). The resistance in PI 320952 was shown to be governed by a recessive and a closely linked dominant gene (Buchwaldt et al., 2013). In this study, we mapped a major QTL (*qAnt*1.*Lc*-3) associated with race 1 resistance from CDC Robin on chromosome 3 in both populations. The QTL *qAnt*1.*Lc*-3 accounts for 63 -72% of the variance in resistance to race 1. Bhadauria et al. (2017) reported race 1 resistance QTL on chromosomes 2, 3, and 5 of the *L. ervoides* genome. We do not have sufficient information to determine if the QTL on chromosome 3 of *L. ervoides* is the same as that in the cultivated lentil. Large portions of the physical interval of *qAnt*1.*Lc*-3 were co-

localized for the LR-01 and LR-18 population genetic maps, indicating a strong association of the genomic region with race 1 resistance.

The association mapping approach is suited for the detection of high-resolution QTLs, as it captures a larger portion of the recombination events that have accumulated inside an association panel (Zhu et al., 2008). Exploration of high throughput marker data makes GWAS more efficient and provides a rapid method to identify significant genomic regions associated with traits of interest for candidate gene prediction (Yano et al., 2016). In the current study, marker-trait associations identified 14 SNPs associated with race 1 resistance on chromosomes 3, 4, 5 and 6 (Figure 4.6 and Table 4.5). The major locus on chromosome 3 identified by biparental populations was confirmed by GWAS. The most significant SNPs associated with race 1 resistance were located within the region of *qAnt*1.*Lc*-3 (Figure 4.6). The GWAS SNPs fine mapped the *qAnt*1.*Lc*-3 region to 1.6 Mb containing candidate resistance genes (Table 4.6).

A total of 57 candidate genes involved in plant defense against biotic and abiotic stress are predicted within the region of *qAnt*1.*Lc*-3 in the biparental mapping (Table 4.6 and Appendix H). Among these, 13 are NB-LRR class R genes, and 14 are transmembrane protein (TM) genes known as 'other' R genes (Sekhwal et al., 2015). In plant genomes, the NB-LRR class R genes are abundant and often clustered on specific chromosomes due to tandem and segmental duplications (Leister, 2004). Congruently, most of the annotated NBS-LRR genes in the lentil genome are located on chromosome 3 (Koh, C. personal communication, University of Saskatchewan, Saskatoon, Canada). Thus, the candidate R gene clusters identified within the region of *qAnt*1.*Lc*-3 could possibly account for improved resistance to race 1 in lentil. Transmembrane (TM) proteins are part of a plant cell complex membrane-associated receptors that mediate signal transduction between the extra- and intracellular environments in defense system and are mainly involved in conferring a broader resistance spectrum in plants, including the known *Mlo* gene (Büschges et al., 1997; Xiao et al., 2001; Brandwagt et al., 2002; Ma et al.,2014). The most significant SNP markers from GWAS analysis also identified genes involved with cellulose synthase and anthranilate N-benzoyltransferase protein in *qAnt*1.*Lc*-3. Cellulose synthases play an important role in mediating cell wall changes in the epidermal layers in response to defense against pathogens (Douchkov et al., 2016). Anthranilate N-benzoyltransferase genes are important in phytoalexins biosynthesis, which provide enhanced protections against pathogens (Grayer and Kokubun, 2001).

4.5. Conclusion

In this study, we combined the use of biparental QTL mapping and GWAS to identify QTL and candidate genes for anthracnose race 1 resistance in lentil. The major effect QTL (*qAnt*1.*Lc*-3) identified on chromosome 3, explained 66.6 to 69.8% of the phenotypic variance and was confirmed via GWAS analysis. Across the genome, GWAS identified 14 SNPs associated with race 1 resistance. The SNP markers identified that were associated with the candidate genes can be used for MAS to advance molecular breeding approaches for improved anthracnose resistance in lentil.

Prologue to chapter 5

In the previous chapters, *C. lentis* race 0 and race 1 resistance in cultivated lentil was examined and genomic regions conferring resistance to race 1 in cultivated lentil germplasm was mapped. The results from the study described in chapter 3 and existing reports indicate that *C. lentis* race 0 resistance in the primary gene pool is limited. As described in Chapter 2, sources of resistance to race 0 have been identified in *L. ervoides* accessions. Subsequently, *L. ervoides* accessions IG 72815, with superior resistance to both races of anthracnose was crossed with a *L. culinaris* cultivar, and an interspecific RIL populations were developed at the Crop Development Centre, University of Saskatchewan. We conducted linkage analysis of this interspecific RIL population to identify the QTL associated with anthracnose resistance from *L. ervoides* and characterize the gene that controls the trait. Thus, the experiments described in the next chapter of this thesis were initiated.

Disclosure

The content of this chapter was submitted as part of a manuscript: Gela, T. S., Koh, C. S., Chen, L., Caron, C., Vandenberg, A., and Bett, K. E. (2020). QTL mapping of lentil anthracnose (*Colletotrichum lentis)* resistance from *Lens ervoides* accession IG 72815 in an interspecific RIL population. ***Euphytica*** (*in review*).

Author contributions

TSG conducted the experiments, drafted the manuscript, and analyzed the data; CSK and LC provided the genetic linkage map; CC performed bioinformatic analyses; LC and technical support under supervision of KEB preformed DNA extraction and GBS libraries; AV and KEB conceived the study, participated in its design, and critically reviewed the manuscript.

CHAPTER 5

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

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 resistance in the growth chamber and polyhouse. A QTL analysis identified major resistance loci on chromosomes 3 and 7, accounting together for 50.2 to 73.3% of total phenotypic variance. 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.

5.1. Introduction

Lentil (*Lens culinaris* M.) is an economically important pulse crop on a global scale that is consumed for its high levels of dietary fiber, micronutrients, vitamins, and protein (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 2013-2017 (FAOSTAT, 2017). Lentil productivity in western Canada is challenged by diseases such as anthracnose, ascochyta blight, stemphylium blight and aphanomyces root rot. Anthracnose, caused by the fungal ascomycete pathogen *Colletotrichum lentis* (Damm) (Damm et al., 2014), 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 from the province of Manitoba in 1987 (Morrall, 1988), and since then it has become widespread in western Canada.

Low genetic diversity and a narrow genetic base for anthracnose resistance in current collections of lentil accessions in the primary gene pool has been reported (Buchwaldt et al., 2004;

Gela et al., 2020, Chapter 3). This pronounced loss of genetic diversity can be reintroduced by going back to the crop wild relatives (reviewed by Coyne., 2020; Dempewolf et al., 2017). Wild relatives of crop species are natural genetic reservoirs that retain much of the genetic diversity lost during the process of domestication and/or deliberate selections for cultivar development (Tanksley and McCouch, 1997). *Lens* species in the tertiary gene pool, *L. ervoides,* show resistance to many lentil diseases, including resistance to both races of anthracnose (Tullu et al., 2006), ascochyta blight (Tullu et al., 2010), and stemphylium blight (Podder et al., 2012). Therefore, resistance breeding for the highly virulent race 0 of *C. lentis* is especially dependent on the use of resistant germplasm from *L. ervoides*. Anthracnose resistance from *L. ervoides* accessions L-01-827A and IG 72815 was successfully transferred to a *L. culinaris* cultivar through interspecific hybridization using embryo rescue techniques (Fiala et al., 2009; Tullu et al., 2013) to develop two interspecific RIL populations (LR-59: *L. culinaris* Eston × *L. ervoides* L-01-827A; LR-26: *L. culinaris* Eston × *L. ervoides* IG 72815).

The genetic inheritance of resistance to race 0 in accession L-01-827A was determined to be governed by two recessive genes/alleles (Fiala et al., 2009). Use of genetic sources of host resistance is one of the integral parts of the IPM practices, but little is known about the genomic regions and molecular markers linked to the anthracnose resistance gene(s). The identification of QTL conferring resistance to race 0 and race 1 on chromosomes 2, 3, 5, and 7 of the *L. ervoides* genome (Bhadauria et al., 2017) was a first step forward. However, the fate of these QTL in the interspecific population has yet to be reported.

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 regions of the *L. ervoides* genome associated with anthracnose resistance in the interspecific RIL population developed from a cross between *L. ervoides* accession IG 72815 and the susceptible cultivar Eston.

5.2. Material and methods

5.2.1. Plant material and fungal isolates

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 conditions resistance to both races of anthracnose (Tullu et al., 2006). Cultivar Eston, a small seeded, yellow cotyledon, green seed coat, early maturing line released in Canada in 1980 (Slinkard,1981). Eston is susceptible to both races of anthracnose.

5.2.2. 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 plants in a growth chamber at the University of Saskatchewan (U of S) College of Agriculture and Bioresources phytotron facility, and in an outdoor polyhouse. Fungal inoculum production was done as described in section 3.2.2.

The parents and LR-26 RILs were evaluated for resistance to *C. lentis* in growth chambers (inoculated with race 0 or race 1, separately) and under polyhouse (inoculated with race 0) conditions. In the growth chamber experiments, plants were grown in 38-cell cone trays (26.8 x 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. Growth chamber inoculations were conducted as described in section 3.2.2; they were maintained at 21/18ºC day/night temperature under a 16 h photoperiod. Briefly, the disease severity data were collected per individual plant (an experimental unit). The RILs of the LR-26 population and the parents were randomized in a set of trays per replicate. The experiment was repeated at least 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 in the summer of 2017 at the Department of Plant Sciences field laboratory at the U of S as described in section 4.2.2. Briefly, 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 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.

5.2.3. Statistical analysis

Disease scores data were subject to analysis of variance using SAS v.9.4 (SAS Institute, Cary, USA) and analysed as described in section 3.2.3. The mean disease scores were transformed using log transformation for QTL analysis to reduce skewness. Spearman's rank correlation of disease severity between races and test environments was performed using procedure CORR in SAS.

5.2.4. Genotyping and linkage mapping

Total genomic DNA (gDNA) from bulk leaf tissue of parents and LR-26 RILs was extracted using the DNeasy® 96 Plant Kit (Qiagen, Germany) (Chen, 2018). 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. Briefly, 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 a GBS pipeline written in Perl and developed by the Pulse Bioinformatics group in the Department of Plant Sciences, U of S, Canada (https://knowpulse.usask.ca/software/GBS-Pipeline; Wong et al., 2015). The pipeline demultiplexes raw reads and removes barcode sequences prior to trimming using Trimmomatic-0.38 (Bolger et al., 2014). The pipeline then aligns the trimmed reads to the *L. culinaris* cultivar CDC Redberry genome v2.0 using Bowtie2-2.2.9 (Langmead and Salzberg, 2012) and allows reads to multimap up to three times, then filters for the best hit. The final step in the pipeline is variant-calling and SNP calling of the combined samples using SAMtools-1.9 and BCFtools-1.6 (Li et al., 2009), 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. Then raw SNPs 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 et al., 2017) with the parameters: segregation ratio=75:25 and p value = 10^{-5}; and treating heterozygous calls as missing values.

5.2.5. Segregation distortion (SD) analysis

Deviation from the expected 1:1 Mendelian segregation ratio for each SNP marker was determined by a chi-square test at a p-value of 0.05 with 1 degree of freedom. Since lentil is a diploid with seven pairs of chromosomes, at least 14 independent genomic regions are expected. A Bonferroni adjustment threshold of at least $0.05/14 \approx 0.00357$ would be required to obtain a genome wide error rate of $\alpha = 0.05$. Segregation distortion regions (SDR) were considered when at least three closely linked SNPs exhibited significant distortion. Patterns and distribution of SDs along the chromosome (LG) were visualized using the r/qtl package (Broman et al., 2003). SNPs showing SD were integrated into the map.

5.2.6. QTL analysis

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, which were employed 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). The multiple QTL model was performed as described in section 4.2.5. For composite interval mapping (CIM), five markers were selected as cofactors by forward selection to control genomic background effects. The percentage of the phenotypic variance explained (PVE) and effects of QTL were obtained by fitting a mixed linear model using the "*fitqtl*" function. Thresholds for declaring QTL were determined by 1000 permutations at a significance of $\alpha = 0.05$ for both methods. The confidence intervals for each QTL were estimated using the "*lodint*" function that calculates the 1.5 LOD support intervals.

5.3. Results

5.3.1. 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 (Figure 5.1A and B). With disease severity scores ranged from 8.33 to 95% and 5 to 95%, and a mean of 79.4% and 76.0% for race 0 and race 1, respectively. From the polyhouse ratings, the distribution of the disease reactions revealed relatively continuous variation with a skew toward the higher level of disease severity and ranged from 12.5 to 95%, with a mean of 65.7% (Figure 5.1C). Correlation analysis indicated a significant positive relationship between the two races and test environments (Table 5.1), suggesting the resistance derived from IG 72815 to both races may be controlled by the same gene or by tightly linked genes.

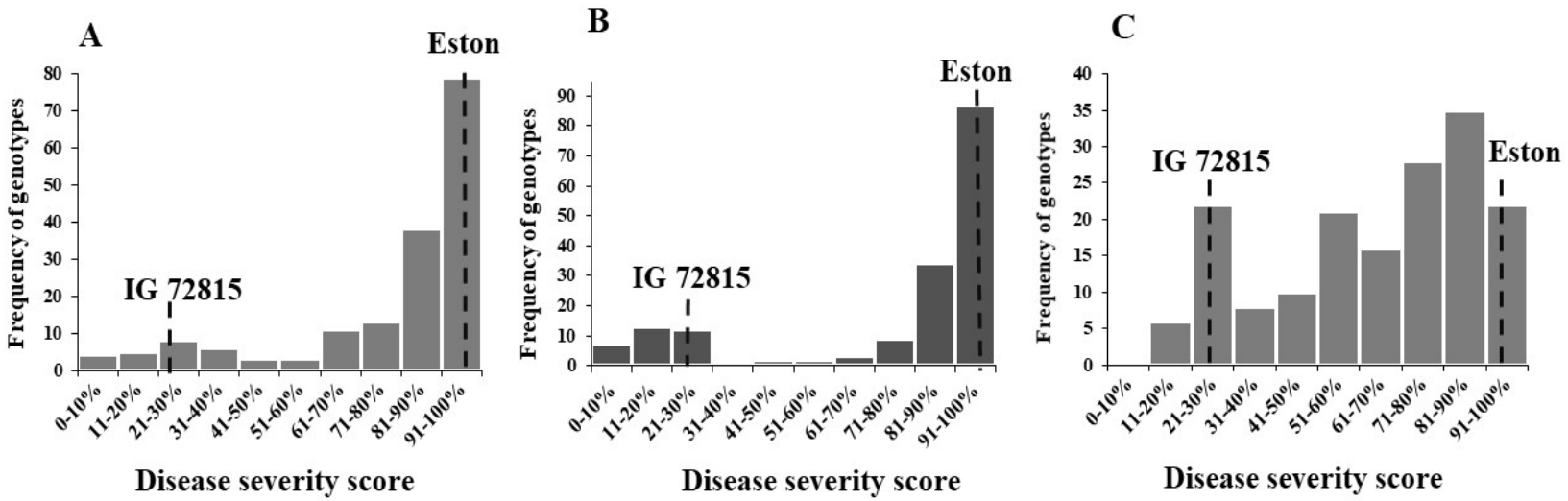

Figure 5.1. Frequency distributions of percent anthracnose severity for 168 members of the interspecific RIL population LR-26 derived from the interspecific cross *Lens culinaris* Eston × *L. ervoides* IG 72815 following inoculation with: a) race 0, and b) race 1 of *C. lentis* under growth chamber (phytotron) 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.

Table 5.1. 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[≠]	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***

[≠]degrees of freedom, *** Significant at the 0.001 probability level

5.3.2. Linkage map and segregation distortion (SD)

A genetic map was constructed from 5491 SNP markers that mapped to eight linkage groups representing the seven chromosomes of lentil (Table 5.2). The linkage groups were numbered to match the respective haploid number of chromosomes of the lentil reference genome (v2.0; https://knowpulse.usask.ca/genome-assembly/Lc.2RBY). SNPs on chromosome 5 mapped to two separate linkage groups: LG 5 and LG 5.1. The linkage map spanned a total genetic distance of 3449.6 cM with an average marker interval at 0.6 cM (Table 5.2). The two linkage groups that make up chromosome 5 contained the smallest number of SNPs: 36 for LG 5.1 and 342 for LG 5. Whereas LG 2 and LG 4 contained the highest number of SNPs (Table 5.2).

Table 5.2. Summary statistics of genetic linkage map and percentage of markers displaying segregation distortion (SD) in the LR-26 interspecific lentil population.

Linkage groups	Number of markers	Map length (cM)	Average marker interval (cM)	Maximum gap (cM)	SD%
LG1	667	603.3	0.9	40.8	94.5
LG2	1002	443.3	0.4	19.9	37.1
LG3	783	363.5	0.5	21.2	50.2
LG4	1002	387.6	0.4	11.9	14.2
LG5	342	627.4	1.8	21.3	87.1
LG5.1	36	196.8	5.5	42.9	91.7
LG6	790	399.9	0.5	14.3	54.1
LG7	869	428.0	0.5	11.2	26.9
Total	5491	3449.6	0.6		

SD%, percent of markers exhibiting segregation distortion

Among the 5491 SNPs, 2529 (46%) showed significant SD ($\alpha = 0.00357$). The distortions were detected in all chromosomes, with all favoring the cultivated parent allele, except for SNPs on linkage group 5.1, which were biased towards the wild parent (Figure 5.2). Notably, the SNPs on linkage groups 1 (94.5%), 5 (87.1%) and 5.1 (91.7%) displayed high levels of SD, most likely attributable to a reciprocal translocation between chromosome 1 of *L. culinaris* and chromosome 5 of *L. ervoides* (Bhadauria et al., 2017; Gujaria-Verma et al., 2014), due to non-homologous pairing during meiosis (Ladizinsky et al., 1985). Non-randomly distributed segregation distortion regions were detected on other LGs with varying genetic distance intervals, and SD ranged from 14.2% (LG 4) to 54% (LG 6) (Table 5.2 and appendix K).

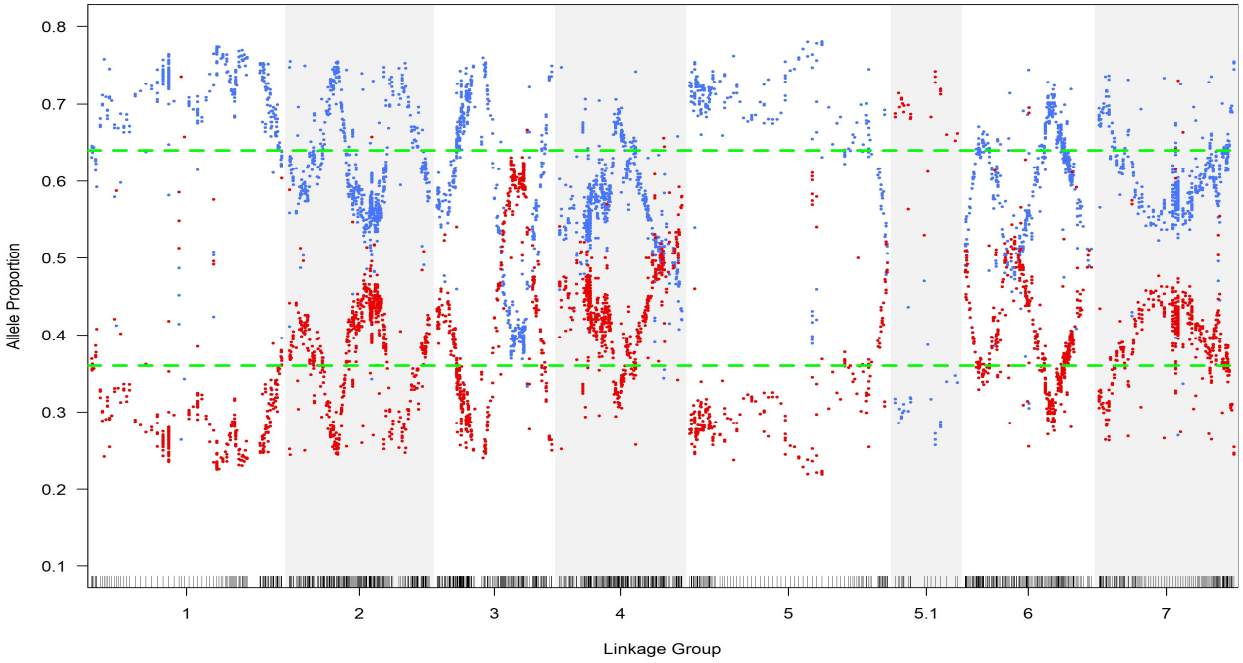

Figure 5.2. Distribution of SNP segregation ratios of alleles along the genetic linkage map. Blue and red dots represent alleles of Eston and IG 72815, respectively. SNPs with distorted segregation occur outside of the green doted lines of the confidence interval for the chi-square test. The threshold was declared at $\alpha = 0.05$ with Bonferroni correction for genome wide error (0.05/14 = 0.00357), considering at least 14 independent genomic regions (seven pair of chromosomes) in lentil.

5.3.3. QTL for anthracnose resistance

We detected one large effect QTL on chromosome 3, and another on chromosome 7, associated with anthracnose resistance (Figure 5.3). A genome-wide view of the QTL detected with individual data sets across the chromosomes (LGs) and the details of each QTL identified is presented in Table 5.3 (and appendix L). 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 the position of the QTL 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 (r^2 = 0.8 - 0.9) between race 0 and race 1 phenotypes in the LR-26 population.

Table 5.3. 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	QTL	LG	Peak LOD	Position (cM)	1.5 LOD interval (cM)		[¥]PVE (%)	Add[≠]	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	

[¥] Percentage of phenotypic variance explained by QTL, [≠]Additive effect

[$] Inoculation with race 0 and race 1 of *C. lentis*

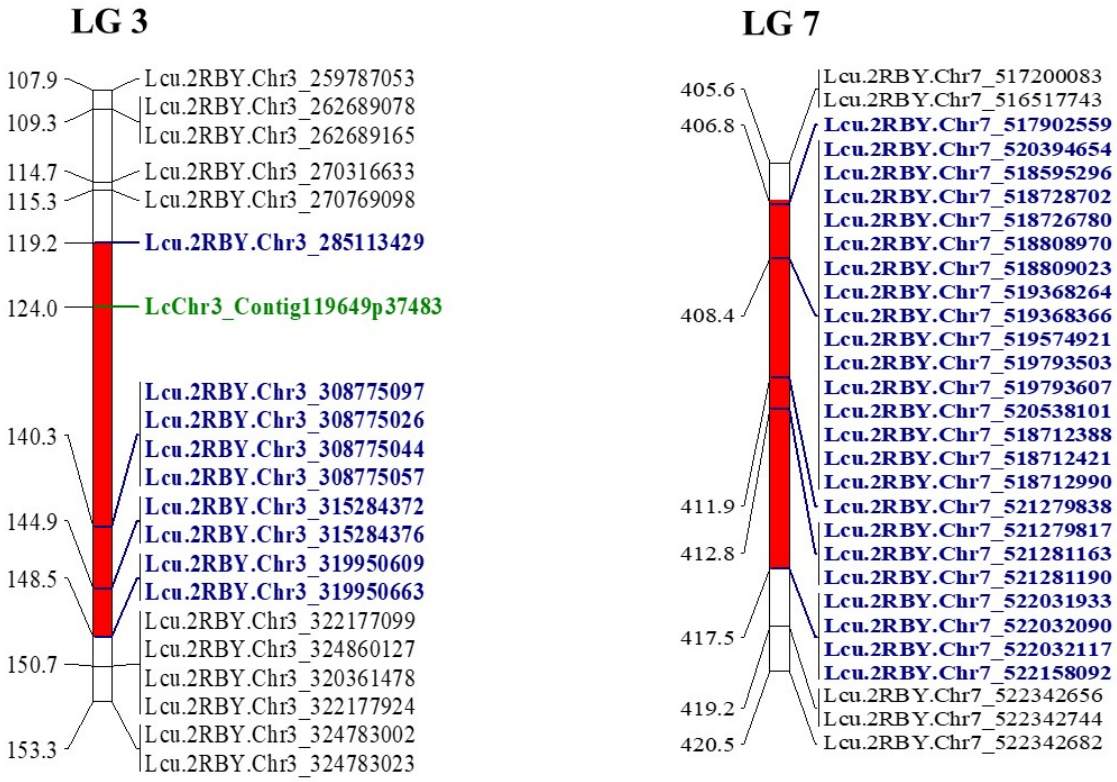

Figure 5.3. Location of anthracnose resistance QTL in IG 72815 on linkage groups (LG) 3 and 7; the linkage map was constructed from an interspecific LR-26 recombinant inbred line (RIL) population derived from a cross between *L. culinaris* Eston and *L. ervoides* accession IG 72815. The QTL positions 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.

5.3.4. Identification of candidate genes underlying anthracnose QTL

Scanning the annotated reference lentil genome (v2.0; https://knowpulse.usask.ca/genome-assembly/Lc.2RBY) revealed more than 290 and 140 genes of known function, and hypothetical and uncharacterized proteins, located within a 1.5-LOD interval of the anthracnose resistance QTL found on chromosomes 3 and 7, respectively. 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, based on the current lentil genome assembly (Lcu.2RBY, www.knowpulse.usask.ca/genome-assembly/Lc.2RBY). Genes within these QTL

intervals that may condition anthracnose resistance are genes encoding NB-ARC domain disease resistance genes, LRR receptor-like kinase (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 5.4).

Table 5.4. Candidate resistance and/or defense-related genes associated with QTL for anthracnose resistance based on gene annotations in v2.0 of the *L. culinaris* CDC Redberry genome, listed based on their similarity of annotated functions within the chromosome.

Chr[¥]	QTL	Start	End	Gene ID	Descriptions
Chr3	qANT.3	312119382	312129996	Lcu.2RBY.3g049830	Cellulose synthase-interactive protein
Chr3	qANT.3	313600052	313601816	Lcu.2RBY.3g050180	NB-ARC domain disease resistance
Chr3	qANT.3	307335035	307335337	Lcu.2RBY.3g048920	LRR receptor-like kinase
Chr3	qANT.3	307335338	307336435	Lcu.2RBY.3g048930	LRR receptor-like kinase
Chr3	qANT.3	308956617	308961348	Lcu.2RBY.3g049220	LRR receptor-like kinase
Chr3	qANT.3	319619573	319622321	Lcu.2RBY.3g051370	Wall-associated receptor kinase protein
Chr3	qANT.3	307893203	307893451	Lcu.2RBY.3g049040	Transmembrane protein
Chr3	qANT.3	308773941	308775644	Lcu.2RBY.3g049180	Transmembrane protein
Chr3	qANT.3	310039708	310042005	Lcu.2RBY.3g049580	Transmembrane protein
Chr3	qANT.3	317226830	317227192	Lcu.2RBY.3g050840	Transmembrane protein
Chr3	qANT.3	320992828	320993826	Lcu.2RBY.3g051640	Transmembrane protein
Chr3	qANT.3	308616445	308619238	Lcu.2RBY.3g049150	Serine/Threonine-kinase SAPK1 protein
Chr3	qANT.3	314179028	314205441	Lcu.2RBY.3g050290	Peroxidase
Chr3	qANT.3	320293338	320300539	Lcu.2RBY.3g051480	PPR containing plant-like protein
Chr3	qANT.3	319601916	319605267	Lcu.2RBY.3g051360	ABC transporter-like family-protein
Chr3	qANT.3	309702980	309718949	Lcu.2RBY.3g049520	Peroxisomal ABC transporter
Chr3	qANT.3	307861899	307869214	Lcu.2RBY.3g049030	zinc finger protein
Chr3	qANT.3	320023409	320023951	Lcu.2RBY.3g051430	FAR1 Zinc finger, SWIM-type
Chr3	qANT.3	310931369	310932626	Lcu.2RBY.3g049660	PLAT-plant-stress protein
Chr3	qANT.3	314869623	314876125	Lcu.2RBY.3g050390	F-box only protein
Chr3	qANT.3	320614247	320614699	Lcu.2RBY.3g051520	F-box protein interaction domain
Chr3	qANT.3	320869182	320873334	Lcu.2RBY.3g051560	F-box SKP2A-like protein
Chr7	qANT.7	521985326	521989849	Lcu.2RBY.7g074710	NB-ARC domain disease resistance
Chr7	qANT.7	519458266	519460043	Lcu.2RBY.7g073650	Threonine synthase-like protein
Chr7	qANT.7	520397546	520401681	Lcu.2RBY.7g074090	Receptor-like Serine/Threonine-kinase

Chr7	qANT.7	520796219	520801130	Lcu.2RBY.7g074280	Serine/Threonine kinase family protein
Chr7	qANT.7	520796219	520801130	Lcu.2RBY.7g074280	Serine/Threonine kinase family protein
Chr7	qANT.7	520106647	520121478	Lcu.2RBY.7g073920	Receptor-like kinase
Chr7	qANT.7	520636322	520638414	Lcu.2RBY.7g074200	Receptor-like kinase
Chr7	qANT.7	520636322	520638414	Lcu.2RBY.7g074200	Receptor-like kinase
Chr7	qANT.7	520536608	520539771	Lcu.2RBY.7g074150	Transmembrane protein
Chr7	qANT.7	520703372	520707345	Lcu.2RBY.7g074250	Transmembrane protein
Chr7	qANT.7	520722011	520724447	Lcu.2RBY.7g074260	Transmembrane-like protein
Chr7	qANT.7	520536608	520539771	Lcu.2RBY.7g074150	Transmembrane protein
Chr7	qANT.7	520703372	520707345	Lcu.2RBY.7g074250	Transmembrane protein
Chr7	qANT.7	520722011	520724447	Lcu.2RBY.7g074260	Transmembrane-like protein
Chr7	qANT.7	522029604	522032476	Lcu.2RBY.7g074760	Transmembrane protein
Chr7	qANT.7	520103100	520105166	Lcu.2RBY.7g073900	PPR containing plant protein
Chr7	qANT.7	520106625	520110842	Lcu.2RBY.7g073910	PPR containing plant protein
Chr7	qANT.7	520110896	520111520	Lcu.2RBY.7g073930	PPR containing plant protein
Chr7	qANT.7	520111537	520112148	Lcu.2RBY.7g073940	PPR containing plant protein
Chr7	qANT.7	520122026	520123948	Lcu.2RBY.7g073960	PPR containing plant protein
Chr7	qANT.7	519824641	519826943	Lcu.2RBY.7g073770	Peroxidase
Chr7	qANT.7	522017047	522020857	Lcu.2RBY.7g074740	Peroxidase
Chr7	qANT.7	521276880	521281728	Lcu.2RBY.7g074430	Transporter ABC domain protein
Chr7	qANT.7	521276880	521281728	Lcu.2RBY.7g074430	Transporter ABC domain protein
Chr7	qANT.7	520496423	520498601	Lcu.2RBY.7g074140	GATA type zinc finger transcription factor
Chr7	qANT.7	520496423	520498601	Lcu.2RBY.7g074140	GATA type zinc finger transcription factor

¥ Chromosome

5.4. Discussion

The absence of allelic diversity in the cultivated lentil gene pool for anthracnose race 0 resistance necessitated the introduction of the resistance allele from a wild relative, *L. ervoides*. Interspecific introgresssion of anthracnose resistance into elite cultivars could be facilitated using marker-assisted selection. 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 of 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 *L. ervoides* intraspecific RIL population (Bhadauria et al., 2017).

The GBS-based linkage map was generated for interspecific RIL populations covering the seven chromosomes of lentil with 5491 SNP markers. The genetic linkage map spanned 3449.6 cM with an average marker density of 0.6 cM. Stange et al. (2013) reported an increase in marker density from 5 to 1 cM could increase the power sufficiently to precisely localize and resolve closely linked QTL. Marker segregation distortions that were widespread throughout the lentil genome were observed in the current study. In lentil, SD has been reported in both an intraspecific and interspecific cross (Galasso, 2003; Eujayl et al., 1997; Zamir and Tadmor, 1986). In all cases, the transmission favored the alleles from the cultivated parent, except in the distorted region on LG 5.1 where the alleles are switched (Figure 5.2). Evidence from recent linkage map studies indicate that distorted markers, if handled properly, have little or no effect on the accuracy of linkage maps and, can potentially improve the accuracy of grouping the markers and QTL detection (Xu, 2008; Zhang et al., 2010b; Bartholome et al., 2015, Zuo et al., 2019). Moreover, exclusion of the SD markers could possibly reduce genome coverage (Luo et al., 2005) and form a marker-gap when the SDs are in clusters as detected in the present study. Thus, the LR-26 genetic map developed in this study has enough 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 on the distal end of linkage group 7. Importantly, both QTLs were detected inside the segregation distortion regions.

A 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 (Figure 5.3), and it is quite likely the same QTL. Another QTL, with moderate effect, was detected on chromosome 7 (qANT-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 derived from race 0 and race 1 resistant *L. ervoides* parents (L-01-827A × IG 72815). However, the qANT-7 identified in IG 72815 in this study conferred resistance to both races. A plausible explanation for the discrepancy could be that accessions L-01-827A and IG 72815 carry the same race 1 resistance in

this region of chromosome 7, or perhaps this is a different locus. Similarly, Murube et al. (2019) reported a co-occurring QTL conferring anthracnose resistance in common bean (*Phaseolus vulgaris* L.) to multiple races of *C. lindemuthianum*, where multiple race-specific genes (co-genes) are found in clusters on chromosomes. Thus, the race 0 and race 1 resistance in accession IG 72815 are possible 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, with an interval of 25.7 cM. 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 the current study we found a wealth of candidate genes that may play a role in disease resistance and plant defense-related genes in the 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). In this study, 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 of 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 transcript regulations such as RNA editing, splicing and translation modification (Schmitz-Linneweber, 2008) and are involved in plant disease resistance (Garcia-Andrade et al., 2013). Other candidate plant defense-related genes found in the QTL regions (Tables 5.4), 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.

5.5. 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.

Prologue to Chapter 6

In chapter 5 we mapped genomic regions conferring resistance to *C. lentis* race 0 in an *L. ervoides* accession using an interspecific RIL population. Standard interspecific RIL populations have many introgression regions throughout the genome and for this reason they are not regularly used to uncover the novel genes/alleles from the wild species. Mainly due to the inheritance of deleterious alleles of the wild parent along with the alleles for the traits of interest. Backcross derived populations are commonly used to dissect the genetic mechanism of traits introgressed from the wild parent. Thus, an advanced backcross-QTL mapping strategy is proposed to provide an opportunity for the efficient use of the desired traits of interest by minimizing the presence of the unwanted traits in individual introgression lines (Tanksley and Nelson 1996). Therefore, the experiments described in the next chapter of this thesis were initiated to implement the advanced backcross-QTL analysis approach in lentil.

Disclosure

The content of this chapter was submitted as part of a manuscript: Gela, T. S., Adobor, S., Khazaei, H., and Vandenberg, A. (2021). An advanced lentil backcross population developed from a cross between *Lens culinaris* × *L. ervoides* for future disease resistance and genomic studies. ***Plant Genetic Resources***, (*in review*).

The content of this chapter is also currently a manuscript in preparation: Mapping of anthracnose (*Colletotrichum lentis*) race 0 resistance in an interspecific advanced backcross population of lentil.

Gela, T. S., Adobor, S., Ramsay, L., Bett, K. E., and Vandenberg, A. (2021). IN PREPARATION.

Author contributions

TSG conducted the experiments, drafted the manuscript, and analyzed the data; LR performed bioinformatic analyses; TSG, SA and technical support under supervision of KEB preformed DNA extraction and exome capture libraries; AV and KEB conceived the study, participated in its design, and critically reviewed the manuscript.

CHAPTER 6

Mapping of anthracnose (*Colletotrichum lentis*) race 0 resistance in an interspecific advanced backcross population of lentil

Abstract

Resistant cultivars are one strategy to reduce yield losses due to lentil anthracnose (caused by *C. lentis*). Genetically accessible resistance to the highly virulent race 0 of the pathogen is limited to *L. ervoides* in the tertiary gene pool. We developed a lentil advanced backcross (LABC-01) population in cultivar CDC Redberry background, based on *L. ervoides* genes/alleles derived from an interspecific RIL LR-59-81. A total of 217 LABC-01 ($BC_2F_{3:4}$) population lines were evaluated for their response to anthracnose race 0 in the growth chamber to dissect the genomic region conferring resistance to race 0. A marker-trait association analysis identified a resistance locus (*qAnt*0.*Le*-3) on chromosome 3. We identified eight SNP markers associated with *qAnt*0.*Le*-3 that accounted for 12.5 to 20.7% of the phenotypic variation conferring resistance to race 0. The genomic interval of the locus harbors genes encoding disease resistance and other plant defense related protein domains. The results suggested that developing lentil cultivars with improved race 0 resistance is possible by introgressing the resistance identified in *L. ervoides* accessions, and the identified SNPs can be used for marker assisted selection (MAS) to facilitate the introgression.

6.1. Introduction

Global lentil production has increased dramatically in the last five decades compared to the other major pulse crops (Khazaei et al., 2019), mainly due to the introduction of the crop into new regions like western Canada (FAO, 2013-2017). The increased production of lentil in the Canadian prairies overlapped with the appearance of anthracnose caused by *Colletotricum lentis,* Damm. (Morrall, 1988). The disease is now one of the most important foliar diseases of lentil in western Canada and can cause 70% yield loss on susceptible cultivars (Chongo et al., 1999).

Although growing resistant lentil cultivars is the most cost-effective and environmentally friendly approach to control anthracnose, sources of resistance, especially to the highly virulent race 0 of the pathogen, are limited within the *L. culinaris* primary gene pool. Therefore, breeding for resistance to race 0 is highly dependent on the wild species in the *L. ervoides* tertiary gene pool

(Tullu et al., 2006) in which a high frequency of resistant accessions to both races have been identified among *Lens* species.

Incorporation of resistance genes from the tertiary gene pool into elite cultivar of lentil requires breeding strategies that do not compromise the favorable yield, quality and agronomic traits that have improved the crop over the long course of continuous breeding. Although wild species carry useful allelic variations for traits of interest, they also carry unadapted deleterious alleles of traits that were removed from the cultivated species through domestication and breeding. These alleles/genes are often transferred along with favourable exotic alleles into interspecific hybrid progeny, which make it difficult to use this germplasm directly. As an example of this in lentil, Tullu et al. (2013) and Chen (2018) observed the segregation of wild lentil traits such as pod dehiscence in *L. ervoides* interspecific lines.

The advanced backcross-quantitative trait loci (AB-QTL) strategy was proposed to reduce the confounding effect of these unwanted wild alleles (Tanksley and Nelson, 1996). This approach combines the QTL discovery and transfer of valuable traits from a wild donor parent to an adapted recurrent parent. The AB populations are developed through multiple backcrossing (BC_2 or BC_3) followed by multiple rounds of selfing. The AB lines may contain single or multiple, fixed or non-fixed segments of the introgressed genome of the wild species in the genetic background of an adapted parent (Fulton et al., 1997). The AB populations are also useful material for developing chromosome segment substitution lines (CSSL) or near isogenic lines (NILs), which consist of fixed lines that carry homozygous chromosomal segments of the wild donor parent in an adapted background. These can be directly incorporated into breeding programs (Eshed and Zamir, 1994; Zamir, 2001; Eduardo et al., 2005, Tian et al., 2006). The AB-QTL strategy has been used in many crop species to identify introgression QTL for traits of interest, including disease resistance (Yun et al., 2006; Schmalenbach et al., 2008; Taguchi-Shiobara et al., 2013).

In this study, we developed a lentil advanced backcross (LABC-01) population with cultivar CDC Redberry as the recurrent parent and an interspecific RIL LR-59-81 as a donor: (i) to determine the genomic regions conferring anthracnose race 0 resistance, and (ii) to evaluate the genetic characteristics of the LABC-01 population.

6.2. Materials and methods

6.2.1. Plant material

The LABC-01 population was developed from the interspecific recombinant inbred line (RIL) LR-59-81. A donor line selected from the LR-59 interspecific RIL population (Fiala et al., 2009), which was developed from a cross between *L. culinaris* Eston × *L. ervoides* accession L-01-827A (Figure 1). Embryo rescue techniques were used to obtain the F_1 hybrid (Fiala et al., 2009). Line LR-59-81 has been evaluated for resistance to anthracnose, ascochyta blight and stemphylium blight (Table 1) and has been commonly used in lentil breeding as a resistant check for both races of anthracnose at CDC, U of S (Banniza et al., 2018). The recurrent parent for the LABC-01 population was the cultivar CDC Redberry, a red lentil cultivar released by the CDC for its high yield and partial resistance to anthracnose race 1 (Vandenberg et al., 2006). Recently, cultivar CDC Redberry was sequenced to develop the lentil reference genome (reference assembly; Bett et al., 2016).

6.2.2. LABC-01 population development

The LABC-01 population was developed by crossing a single LR-59-81 plant to CDC Redberry to obtain the F_1 generation (Figure 1). The hybridity of the F_1 plants were confirmed by flower color and the hybrid was fertile. CDC Redberry has the typical *L. culinaris* flower phenotype, mostly white with light blue veins. It was used as the female parent in all crosses, and flower color was used as a marker to trace the hybridity of the wild species genome, where purple (typical *L. ervoides)* flower is dominant over white with blue veins (Singh et al., 2014). Two F_1 plants from this cross were backcrossed to CDC Redberry to create BC_1F_1 seeds. To avoid genetic drift, all efforts were made to achieve the maximum number of cross combinations. A total of 111 and 73 BC_1F_1 seeds were harvested from the two F_1 plants, respectively. A second backcross was made independently with all 184 BC_1F_1 plants to generate the BC_2 population, and one or two BC_2F_1 seeds were advanced to BC_2F_2 for each successful BC_1F_1 backcross. Then, one seed of each member of the BC_2F_2 population was arbitrarily selected and selfed by single-seed descent to generate the BC_2F_3 generation and onward. To minimize the effect of an unintended environmental selection, the population was developed under controlled conditions.

Table 6.1. Differences in plant and yield related characteristics between parents of the LABC-01 population.

		Characteristics	CDC Redberry	LR-59-81
1		Disease reaction		
	1.1.	Anthracnose race 0	Susceptible	Resistant
	1.2.	Anthracnose race 1	Partially Resistant	Resistant
	1.3.	Ascochyta blight	Resistant	Resistant
	1.4.	Stemphylium blight	Susceptible	Resistant
2		Days to flower	Late	Early
3		Days to maturity	Late	Early
4		Seed cotyledon	Red	Red
5		Seed coat color/pattern	Gray/unpatterned	Brown/black marble
6		Grain yield	High	Low
7		Seed weight	High	Less
8		Flower color	White with light blue veins	Purple
9		Plant height	Tall	Short/weak stem
10		Other	Ref. genome of lentil	

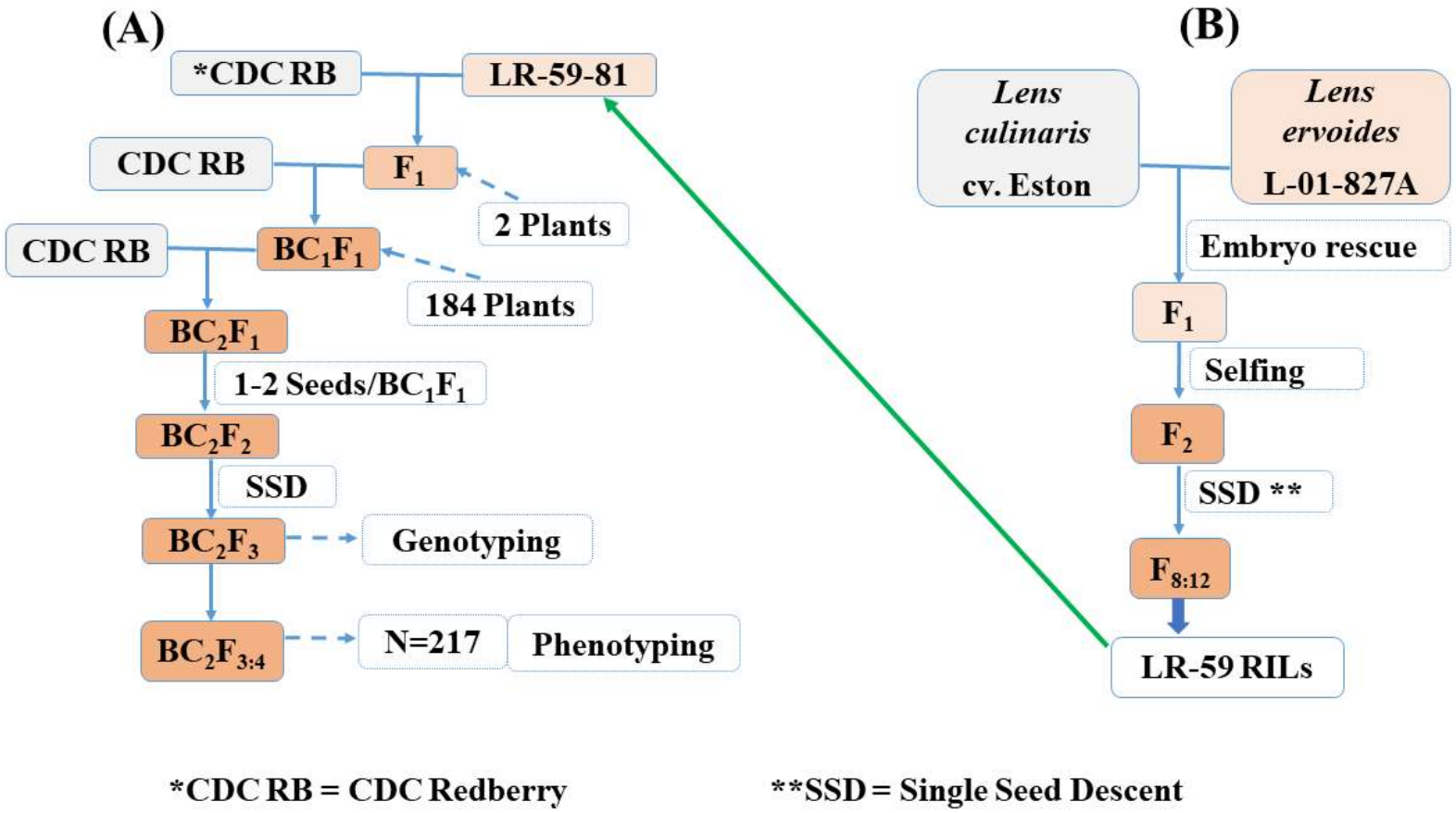

Figure 6.1. (A) Schematic diagram of lentil advanced backcross (LABC-01) mapping population development, (B) Donor parent RIL RL59-81 development (Fiala et al., 2009).

6.2.3. Disease phenotyping

A total of 217 $BC_2F_{3:4}$ individuals of the LABC-01 population and parents were evaluated for anthracnose race 0 resistance in a growth chamber. Fungal inoculum production, inoculation and plant growth condition were as described in section 3.2.2. Briefly, the experiment was arranged in a randomized complete block design with seven replications blocked over time. Two plants of each line were included in each of seven sequential experimental runs and the final disease score from each plant was the mean score per replicate (run). Two plants of the susceptible control Eston and the parental genotypes were included in each tray. Four-week-old seedlings were inoculated with a spore suspension of *C. lentis* race 0 isolate CT-30 (Banniza et al*.,* 2018) at 3 mL per plant. Disease severity data were collected 8-10 d after inoculation using a 0 to 10 rating scale with 10% increments. Data were converted to percent disease severity using the class midpoints for statistical analysis and analyzed as described in section 3.2.3.

6.2.4. DNA extraction and genotyping

DNA of the LABC-01 population and parents were isolated from fresh leaves of 2-3-week-old seedlings using a Qiagen MiniPrep Kit (Qiagen). The quality and quantity of each sample was checked using a gel-electrophoresis and a Qubit 2.0 fluorometer (Thermo Fisher Scientific Inc., Canada). Sequence library construction protocols of the custom exome capture assay, sequence read mapping and single-nucleotide polymorphism (SNP) identification were done as previously described by Ogutcen et al. (2018) and in section 4.2.4. A total of 190 lines of the LABC-01 population and both parental lines were sequenced at a depth of approximately 2 ×. The resulting variant call format (VCF) file was filtered using VCFtools (Danecek et al., 2011) to retain SNPs called in at least 95 lines, minimum quality score 30 and SNPs with minor allele frequency greater than 5%. The VCF file was subsequently further filtered to retain only markers polymorphic between the parental lines. The missing genotypes were imputed by BEAGLE 5.0 software (Browning et al., 2018) for QTL mapping. The imputation was conducted based on the parameter selection adopted for crop data imputation (Pook et al., 2020) using ne = 1000, err = 0.00005, window = 200, imp-segment = 50, imp-step = 0.05, cluster = 0.00005 and without a reference panel.

6.2.5. Genotype analysis and linkage mapping

For linkage map construction a total of 4073 SNP markers segregating between the parents were available. However, our attempts to generate a linkage map of the LABC-01 population were not successful due to severe segregation distortions, which may be attributed to the nature of the LABC-01 population development. As described in the section 6.2.2. only one to two seeds of BC_2F_1 generation were selected and advanced to BC_2F_2 for each successful BC_1F_1 backcross (Figure 6.1). Therefore, the LABC-01 population could possibly violate the basic analytical assumptions of linkage mapping packages that are typically designed for balanced populations. Consequently, the QTL analysis was conducted using a QTL mapping mixed model approach (Malosetti et al., 2011) as described in the next section.

To analyse the genomic composition of the LABC-01 lines, the SNPs were further filtered for missing data >25% and a segregation distortion cut-off of $p<1\times10^{-10}$. The genomic composition of the lines was calculated using the CSSL finder program (Lorieux, 2005) and displayed graphically using R/qtl software (Broman et al., 2003).

6.2.6. QTL mapping for anthracnose resistance

The QTL mapping mixed model for the designed crosses (Malosetti et al., 2011) has been successfully applied to advanced backcross populations and recombinant chromosome-segment substitution lines (Saxena et al., 2020; Mora et al., 2016). The method was proposed for populations that violates the basic assumptions of QTL mapping procedures (details in the discussion section). Analytically, it accounts for the non-homogeneous genetic covariance (genetic relatedness) among lines in the population caused by the uneven sharing of genetic background (Malosetti et al., 2011). The marker-trait association was completed using a mixed model implemented in TASSEL 5.0 (Bradbury et al., 2007). To minimize the rate of false QTL detection and to optimize the appropriate test, kinship relationships among the samples were calculated based on the genotyping data and embedded in the model as genetic covariance (Malosetti et al., 2011). Associations were declared at a Bonferroni correction threshold of $P<0.05$.

6.3. Results

6.3.1. Development of backcross populations

A total of 181 BC_1 plants were obtained by crossing two F_1 hybrid plants of CDC Redberry × LR-59-81 back to CDC Redberry (Figure 6.1). The flower color phenotype of the 181 BC_1 plants was used as check for hybridity, where purple (typical to *L. ervoides*) is dominant over white with light blue veins. The flower color segregation of the BC_1F_1 population fit a 1:1 ratio (88 white: 93 purple, $\chi^2_{(1:1)} = 0.14$, $P_{(0.05)} = 0.71$), indicating unbiased segregation of the BC_1F_1. The second backcross resulted in 145 BC_2 progeny obtained from each successful cross of 145 of 181 BC_1F_1 plants to the recurrent parent. The resulting population involved 1-2 BC_2F_1 from each BC_2 cross for a total of 217 lines that were advanced by selfing to BC_2F_4-derived lines. The preliminary observation during seed increases of the $BC_2F_{3:4}$ generation showed that the LABC-01 population was segregating for days to flowering, flower color (ratio of 191 white: 26 purple), seed coat color (190 gray: 27 tan) and seed coat pattern (185 absent: 32 marbled) (Appendix M).

6.3.2. Reactions of LABC-01 to *C. lentis* race 0

The parents and the 217 LABC lines were screened for reaction to anthracnose race 0. The donor parent LR-59-81 had a resistant reaction with a mean disease severity of 36% and the recurrent parent, CDC Redberry 85% (susceptible). The frequency graph for race 0 resistance of the LABC lines showed a slightly skewed distribution toward the susceptible parent, with disease severity ranging from 17 - 95% and a mean of 70.2%. Transgressive variation for race 0 resistance relative to that of the resistant LR-59-81 was observed (Figure 6.2). Anthracnose race 0 resistance levels among the LABC-01 lines differed ($F=3.98$; $p=0.001$).

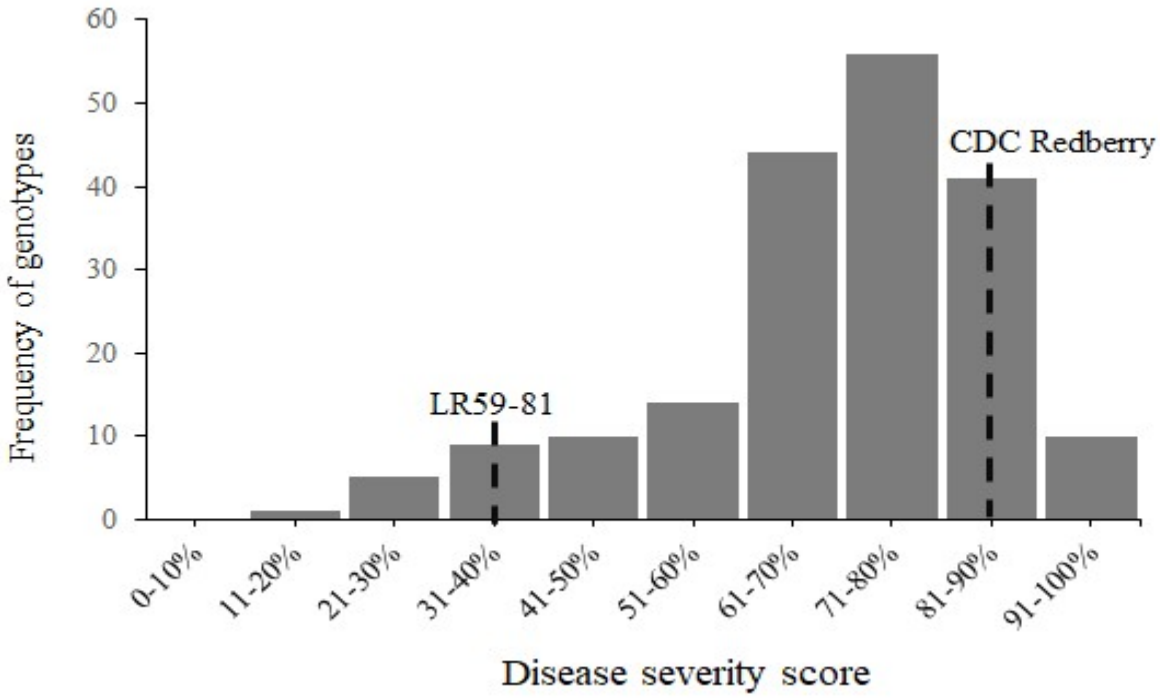

Figure 6.2. Frequency distribution of anthracnose race 0 severity for LABC-01 population derived from cultivar CDC Redberry × an interspecific cross with RIL LR-59-81 under growth chamber condition. The vertical lines indicate the average disease severity of the parents. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments. Data were converted to percent disease severity using the class midpoints for data analysis.

6.3.3. Genotypic characterization of LABC-01 lines

The genomic region of the LR-59-81 (donor parent) introgression in the CDC Redberry background was evaluated with 829 SNP markers (segregation distortion cut-off $p<1\times10^{-10}$); however, for the SNPs on chromosome 7, which showed severe segregation distortion, less stringent criteria were applied to retain some SNPs. Since the LABC-01 population was genotyped at BC_2F_3, the expected marker segregation was: 84.4% homozygous recurrent parent allele, 6.3% heterozygous allele, and 9.4% homozygous donor allele. Whereas the total expected donor allele frequency for a BC_2 derived individual is 12.5%. The details of the genomic composition of the LABC-01 population were summarized in Table 6.2, Figure 6.3 and Appendix M.

Based on the SNP markers segregation, the percent of the LR-59-81 donor-genome introgression per chromosome (Chr) ranged from 6.7% (Chr7) to 16.0% (Chr 1 and 2), with an overall average of 12.2%. Individual lines contain 1.5 to 33.9% of the donor parent introgression, average 13.5% wild genome introgression per line. The mean number of donor segments introgressed per line was 16.8 (Appendix M), and the average length of donor chromosome segments was 26.8, 20.3, 11.4, 23.9, 33.2, 12.7 and 48.2 Mb for chromosome 1 to 7, respectively, with an average of 25.2 Mb. For the recurrent parent, the average genome coverage per chromosome by the lines ranged from 73.0% (Chr1) to 85.2% (Chr7), average 80.2%. The LABC-01 lines also carried 5.8% (Chr4) to 11.0% (Chr1) heterozygous markers with an average of 7.6% per chromosome (Table 6.2).

Table 6.2. Genome composition of LABC-01 populations based on SNP markers.

Chr[≠]	**% Donor**			**% Recurrent**			**% Heterozygous**		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
chr1	5.6	33.9	15.9	40.5	86.3	73.0	2.8	34.8	11.0
chr2	4.3	33.7	16.0	50.2	93.2	77.0	0.0	16.9	7.0
chr3	2.2	25.6	13.1	66.6	91.4	79.4	0.0	22.5	7.5
chr4	7.5	19.8	12.9	70.6	90.8	81.4	0.3	19.8	5.8
chr5	7.5	19.8	10.4	76.5	87.6	81.7	0.1	14.1	7.9
chr6	3.8	14.4	10.4	77.1	87.1	83.7	0.1	15.5	5.9
chr7	1.5	12.3	6.7	73.9	92.6	85.2	0.7	24.7	8.1

[≠] Chromosome, [%] Percent of markers with donor, recurrent and heterozygous alleles

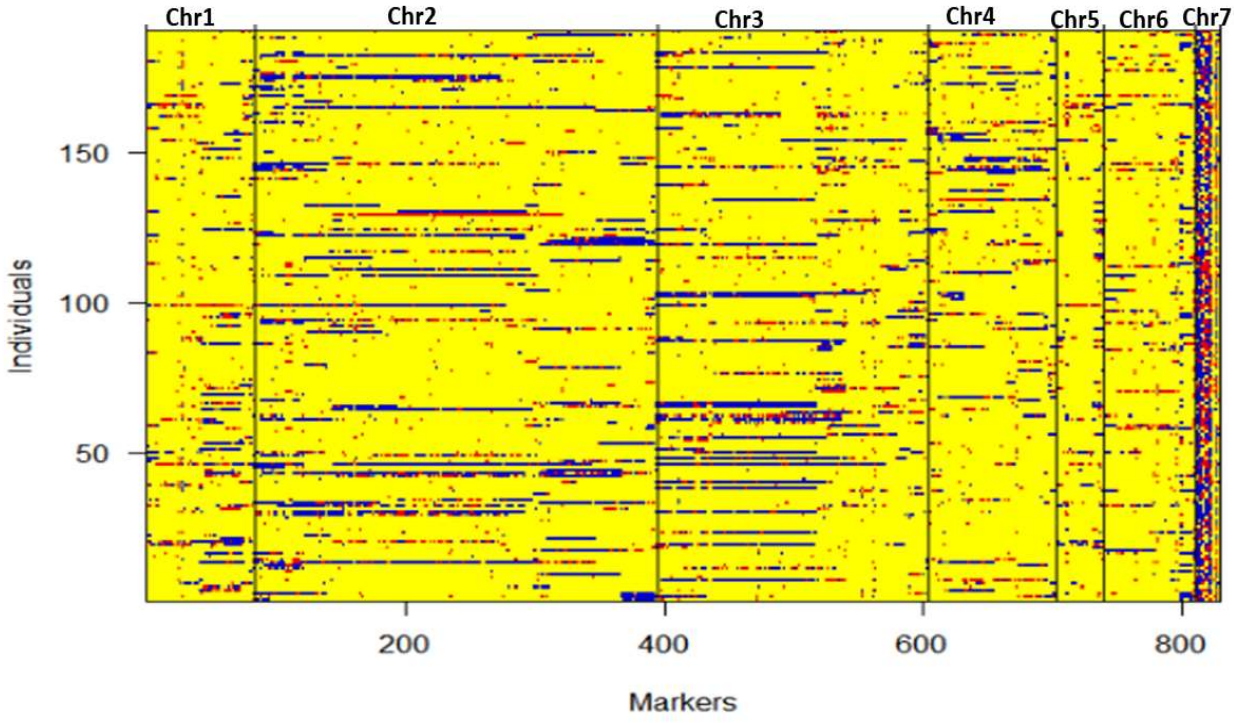

Figure 6.3. Genotypic composition of the LABC-01 population; yellow and blue correspond to CDC Redberry and LR-59-81 parental genomes, respectively and red represents the heterozygous regions. Lines are arranged in ascending order of their membership in LABC-01 population.

6.3.4. QTL mapping of anthracnose race 0

The anthracnose race 0 disease severity data together with a total of 4073 SNP markers distributed along the lentil chromosomes were used for marker-trait association analysis using a mixed linear model approach. A total of eight marker-trait associations were detected for anthracnose race 0 resistance at a significant threshold of $-\log_{10}$ (p-value) ≥ 5 (Figure 6.4 and Table 6.3). The SNPs were identified at a QTL on chromosome 3 with a p-value ranging from $1.24e^{-5}$ and $3.74e^{-10}$. Each of these SNPs explained approximately 11.4 to 20.0% of the total phenotypic variation (Table 6.4). This locus is designated *qAnt*0.*Le*-3, indicating anthracnose race 0 (Ant0) introgressed from *L. ervoides* (*Le*) into chromosome 3. The interval delimited by the SNPs extended from 305.7 to 312.3 Mb and the three most significant SNPs were located within a span of 34 kbp. However, one SNP marker was located at a physical position of 369.3Mb (Table 6.4). In addition to the marker-trait associations declared significant at p=0.05, we also observed two

genomic regions associated with race 0 resistance, one on chromosome 3 and another on chromosome 5 at a Bonferroni threshold (1/n) -$\log_{10}$ (p-value) ≥ 3.6. The locus identified on chromosome 3 was located at a physical position of 58.7 Mb and explained 9.0% of the phenotypic variance. Whereas the genomic region detected on chromosome 5 explained approximately 9.0 to 10.0% of the total phenotypic variance and was located at between positions of 12.2 to13.4 Mb.

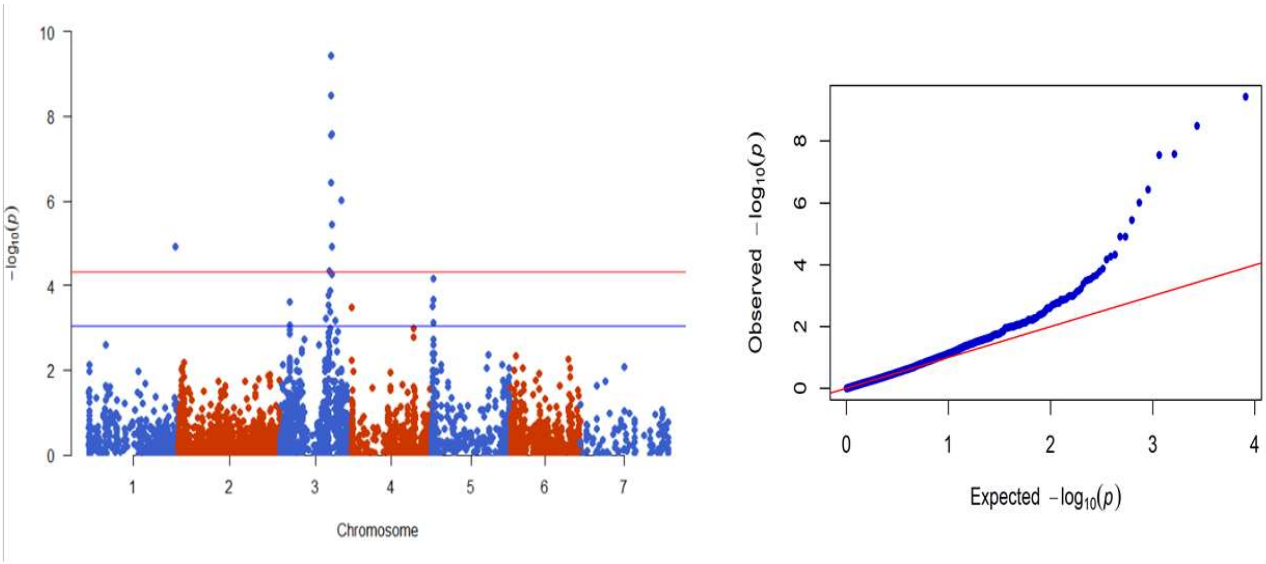

Figure 6.4. Manhattan and Q-Q plots of marker-trait association for anthracnose race 0 resistance in the 190 LABC-01 population evaluated under growth chamber conditions. Each color indicates a different chromosome, the Y-axis indicates -$\log_{10}$ of p-values with a significant association at 5.0 (red line) and the blue line indicates associations at 3.6.

Table 6.3. SNP markers associated with anthracnose race 0 resistance using mixed model QTL analysis in the LABC-01 population.

SNP Marker	Chr	Position (Mb)[#]	p-value	$R^{2\$}$	$-\log_{10}(p)$
Lcu.2RBY.Chr3p306217907	3	306217907	3.74E-10	20.7	9.4
Lcu.2RBY.Chr3p305730188	3	305730188	3.18E-09	18.9	8.5
Lcu.2RBY.Chr3p312282110	3	312282110	2.60E-08	17.0	7.6
Lcu.2RBY.Chr3p306217963	3	306217963	2.80E-08	17.0	7.6
Lcu.2RBY.Chr3p305745854	3	305745854	3.66E-07	14.7	6.4
Lcu.2RBY.Chr3p369331975	3	369331975	9.74E-07	13.8	6.0
Lcu.2RBY.Chr3p310041296	3	310041296	3.66E-06	12.5	5.4
Lcu.2RBY.Chr3p310041217	3	310041217	1.24E-05	11.4	4.9
Lcu.2RBY.Chr3p296783786	3[G]	296783786	4.63E-05	10.1	4.3
Lcu.2RBY.Chr3p309584404	3	309584404	5.50E-05	10.0	4.3
Lcu.2RBY.Chr3p302743637	3	302743637	1.33E-04	9.1	3.9
Lcu.2RBY.Chr3p290394179	3	290394179	1.66E-04	8.9	3.8
Lcu.2RBY.Chr3p58655542	3	58655542	2.41E-04	8.5	3.6
Lcu.2RBY.Chr5p13390420	5	13390420	6.77E-05	9.8	4.2
Lcu.2RBY.Chr5p12201902	5	12201902	2.14E-04	8.6	3.7

[#]Physical positions, [$]Explained phenotypic variance per marker, [G]Highlighted, significant SNPs at $-\log_{10}(p) > 3.6$

6.4. Discussion

In this study we developed a lentil advanced backcross population (LABC-01) to explore the valuable genetic variation introgressed from wild lentil (*L. ervoides*) into adapted cultivar CDC Redberry. CDC Redberry, the source of the lentil reference genome, has a complex pedigree that contains many agronomically important traits including disease resistance (Vandenberg et al., 2006). *Lens ervoides* accession L-01-827A, the donor parent to the interspecific RIL LR-59-81 used as the donor parent to LABC-01, has favorable variation for abiotic stress tolerance (Gorim and Vandenberg, 2017) and biotic stress resistance against pathogens such as ascochyta blight

(Tullu et al., 2010), stemphylium blight (Podder et al., 2012), anthracnose (Vial et al., 2012) and broomrape (*Orobanche crenata*) (Bucak et al., 2014). The line LR-59-81 was selected from the LR-59 population (Fiala et al., 2009) for its high level of anthracnose resistance (Vial et al., 2012) and pilot studies indicated that the line has other desirable traits from its wild parent accession L-01-2872-A, which could provide an avenue to explore the genetic variation for the traits of interest in the line (Vail, 2010 and Chen, 2018).

The LABC-01 population could possibly combine important key traits from wild parents for lentil genetic improvement as a pre-breeding genetic source and as a valuable resource on which to conduct further genetic studies. The LABC-01 lines have shown variation for days to flowering, flower color, seed coat patterns, seed coat color, and for disease resistance such as stemphylium blight (S. Adobor, personal communication) and anthracnose race 0, as observed during population advancement. Understanding the genetic architecture of the favorable traits from the unadapted germplasm provides breeders information that can aid in the introgression of the traits while avoiding linkage drag of deleterious characteristics of the wild species (Tanksley and Nelson 1996). Thus, the LABC-01 population is available as an initial resource for QTL analysis and genetic characterization of agronomic and disease resistance traits that have been introgressed into CDC Redberry. Additionally, some targeted lines could be selected from this population to further develop chromosomal segment substitution lines (CSSL) and/or near-isogenic lines (NILs) after a few rounds of backcrossing and/or selfing for QTL fine mapping and utilization of the favourable alleles that might contribute to lentil breeding programs.

In the present study, 829 filtered SNPs distributed along the lentil chromosomes were used to estimate the proportion and size of the donor parent introgression. However, the SNPs on chromosome 7 showed severe segregation distortion and most of them were removed. The LABC-01 lines had an average of 16% donor-parent (LR-59-81) genome introgression into the recurrent parent, CDC Redberry, which did not differ from the expected amount [12.5% ($\chi^2_{(87.5:12.5)} = 1.12$, $p_{(0.05)} = 0.29$)] for a BC_2 population in the absence of selection (Stam and Zeven, 1981). The proportion of heterozygous alleles ranged from 5.8% (chromosome 4) to 11.0% (chromosome 1), whereas the expected segregation for heterozygous alleles at BC_2F_3 is 6.3%. The higher proportion of heterozygous alleles at chromosome 1 and 5 may be due to a reciprocal translocation between chromosomes 1 and 5 in *L. culinaris* relative to *L. ervoides* (Bhadauria et al., 2017; Gujaria-Verma et al., 2014).

The standard QTL mapping procedures have been developed mainly for balanced populations (e.g. RILs, F_2, BC, DH), with the assumption that there is no selection, no mutation, no migration and no genetic drift (Malosetti et al., 2011). Likewise, in the current study our attempts to generate linkage groups to develop a linkage map of the LABC-01 population was not successful due to irregular recombination fractions. The possible explanations could be that natural or artificial selection during population advancement might have resulted in severe allele frequency distortion (e.g., chromosome 7). However, as discussed in chapter 5, incorporation of distorted markers into an initial genetic map may be an advantage in QTL mapping. The other plausible explanation could be the low-coverage sequencing SNP data used in this study, which has large amounts of missing data and therefore potential genotyping errors (Swarts et al., 2014). Similarly, severe segregation distortion was observed in many of the backcross populations developed using wild species, for instance in pigeonpea (Saxena et al., 2020), cotton (Li et al., 2018), and barley (Mora et al., 2016). Thus, a mixed model QTL mapping strategy (analogous to association mapping studies) for crossing populations that violate the basic assumptions of the standard QTL mapping approaches has been proposed (Malosetti et al., 2011) and successfully implemented in advanced backcross populations (Saxena et al., 2020; Mora et al., 2016).

The QTL mapping conducted in the present study revealed an association of resistance to anthracnose race 0 on chromosome 3 within a physical interval of 305.7 to 312.3 Mb, and the three peak SNPs within 487.8 kb. The locus explained total phenotypic variation ranging from 12.5 to 20.7% conferring resistance to race 0. The locus is localized within the interval of the major QTL detected in *L. ervoides* accessions IG 72815 (Chapter 5). Similarly, Bhadauria et al. (2017) reported QTL conferring resistance to both races on chromosome 3 using the intraspecific RIL population derived from race 0 and 1 resistant *L. ervoides* parents (L-01-827A × IG 72815). They indicated that acquisition of alleles from both parents is required to trigger the greatest resistance response to the pathogens. As discussed in Chapter 5, a number of candidate disease resistance genes were identified in this genomic region including LRR receptor-like kinase, transmembrane protein and serine/threonine-kinase SAPK1-like proteins (Table 5.4). Therefore, race 0 resistance in accessions IG 72815 and L-01-827A could be controlled by the tightly linked genes demarcated on chromosome 3.

6.5. Conclusion

In this study, we developed a lentil advanced backcross population (LABC-01) with cultivar CDC Redberry as the recurrent parent and an interspecific RIL LR-59-81 as a donor to map the genomic regions conferring anthracnose race 0 resistance. A locus associated with anthracnose race 0 resistance was detected on chromosome 3. Eight SNP markers related to this locus were identified. These SNP markers can be used in marker-assisted selection (MAS) studies in breeding programs to select material resistant to this pathogen and facilitate its introgression.

CHAPTER 7

General discussion and future research directions

7.1. General discussion

7.1.1. Resistance to *C. lentis* race 0 was not detected in a global collection of cultivated lentil

Lentil is grown in more than 70 countries worldwide and lentil production in western Canada contributes about 46% of world lentil production (FAOSTAT, 2017; Canadian Grain Commission, 2018). The expansion of lentil production in western Canada is coupled with the appearance of anthracnose. Although it is considered a minor disease of lentil in other parts of the world, anthracnose has become the most important foliar fungal disease of lentil in western Canada since 1987 (Morrall, 1988). Local research was initiated to characterize the pathogen, determine the disease management options and to identify sources of resistance. The search for resistant germplasm was initially focused on screening cultivated lentil accessions obtained from global gene banks (Buchwaldt et al., 2004). Resistance to race 1 in cultivated germplasm was identified and transferred into lentil cultivars, and a number of cultivars with partial resistance to race 1 were released and deployed in Saskatchewan lentil production (Vandenberg et al., 2002, 2006; Government of Saskatchewan, 2019). This likely contributed to the decline in the proportion of race 1 isolates in the pathogen population, which is currently dominated by race 0 isolates (Durkin et al. 2015; Menat et al., 2016).

Efforts to identify effective sources of resistance to the highly virulent race 0 of the pathogen have only been successful in *L. ervoides.* Due to difficulty with hybridization with a tertiary gene pool species, and to reduce the problems related to linkage drag, breeders have often preferred introgression of new alleles/genes from the cultivated pool (Feuillet et al., 2008). Shaikh et al. (2013) evaluated 579 accessions from 20 countries of central and eastern Europe and identified eight promising *L. culinaris* landrace accessions with resistance to race 0.

In this context, the first study of this project was initiated to evaluate promising sources of resistance to race 0 identified in *L. culinaris* landrace accessions by Shaikh et al. (2013) relative to the resistance in LR-59-81, an interspecific RIL derived from a cross of *L. culinaris* cv. Eston × *L. ervoides* accession L-01-827A (Fiala et al., 2009). The results indicate lack of resistance to race 0 among the *L. culinaris* accessions in comparison to the resistant check LR-59-81. This

confirmed that a narrow genetic base exists for resistance to virulent race 0 in the cultivated lentil gene pool in agreement with the previous report by Buchwaldt et al. (2004).

7.1.2. Genetic dissection of anthracnose race 1 resistance QTL in cultivated lentil

Identifying molecular markers associated with QTL underlying disease resistance is the first step for implementing marker-assisted selection (MAS) in lentil breeding programs. The two most common methods for identifying quantitative traits in plants are linkage analysis and genome-wide association mapping. Both mapping approaches were performed to identify markers significantly associated with QTL controlling anthracnose race 1 resistance in cultivated lentil. The linkage mapping was conducted using two bi-parental RIL populations (N=102 and N=139) and the GWAS was performed using a lentil diversity panel consisting of 200 accessions (Chapter 4). Analysis of the differential responses of the RIL populations and GWAS accessions to race 1 inoculation revealed significant variation among the genotypes and high heritability estimates of the disease for the GWAS panel. The disease reactions of both RIL populations displayed a bimodal distribution that fit a Mendelian one gene model.

A major QTL conferring resistance to anthracnose race1 was detected on chromosome 3 in both populations, which was inherited from cultivar CDC Robin (Vandenberg et al., 2002). Cultivar CDC Robin has been used as one of the parents in many cycles of hybridization and its resistance was transferred into elite lines of the lentil breeding program of the CDC, University of Saskatchewan. The QTL physical genomic interval that overlapped for both genetic maps cover 8 Mb from 30.0 - 38.0 Mb on chromosome 3. This region contains 57 candidate genes involved in plant defense against biotic and abiotic stress. Among these, many are known R genes with NB-LRR class and transmembrane proteins (TM) also known as 'other' R genes (Sekhwal et al., 2015). The one or more of the members of the cluster of candidate R genes identified within the region of the QTL could possibly account for enhanced durability and resistance to race 1 in lentil.

The association mapping approach is suited for the detection of high-resolution QTL, as it captures a larger portion of the recombination events that have accumulated inside an association panel (Zhu et al., 2008). Marker-trait associations in this study identified 14 significant SNPs associated with race 1 resistance on chromosomes 3, 4, 5 and 6. However, the strongest marker-trait associations were associated with the SNPs on chromosome 3, and the most significant SNPs on this chromosome confirmed the QTL identified by the bi-parental populations. The results

delineated a physical region of the QTL to 1.6 Mb, containing candidate disease resistance genes, including R-genes with a TIR-NBS-LRR domain and cellulose synthases. Cellulose synthases play an important role in mediating cell wall changes in the epidermal layers in response to defense against pathogens (Douchkov et al., 2016). These SNP markers can be immediately applied in marker-assisted selection to accelerate the introgression of race 1 resistance in lentil breeding.

7.1.3. Mapping of anthracnose race 0 resistance QTL derived from *Lens ervoides* accessions

It was previously estimated that lentil has lost approximately 40% of its genetic diversity during the domestication process (Alo et al., 2011). The absence of genetic diversity for resistance to anthracnose race 0 is observed in the cultivated lentil gene pool, making it necessary to introduce resistance alleles from the *L. ervoides* gene pool through different breeding strategies. To that end, *L. ervoides* accessions L-01-827A and IG 72815, with superior resistance to both races of anthracnose, were identified. The anthracnose resistance of these accessions was introgressed to cultivated lentil through interspecific hybridization using embryo rescue techniques (Fiala et al., 2009; Tullu et al., 2013), and resulted in development of interspecific RIL populations.

In Chapter five, a GBS-based interspecific genetic linkage map was analyzed to dissect QTL controlling resistance to anthracnose race 0 and race 1 in the interspecific LR-26 RIL population consisting of 168 lines derived from a cross between *L. culinaris* cv. Eston × *L. ervoides* accession IG 72815 (Tullu et al., 2013). This population displayed significant variation in disease reaction that is conferred by resistance genes/alleles for both races. A highly positive correlation of disease reaction was found between race 0 and race 1, suggesting that closely linked genes may confer resistance to both races.

The QTL analysis detected two QTL, a major one on chromosome 3 and another on chromosome 7. The QTLs are co-localized for both races of the pathogen, which agreed with the observed high correlation of disease reaction between race 0 and race 1 in this study. The QTL accounted for 50.2 to 73.3% of the total phenotypic variance among the RILs. In the physical interval of the QTL, a wealth of candidate genes that encode plant disease resistance can be found (Table 5.4). This result will require consideration in planning of follow-up validation studies.

In Chapter 6 we described the introgression efforts made to develop lentil advanced backcross (LABC-01) populations (Tanksley and Nelson, 1996) that could be used to extract useful QTL/genes introgressed from *L. ervoides* accession L-01-827A. Most often, introgression of genes

of interest from a distant wild relative into elite cultivars results in disruption of the long-accumulated agronomic and quality traits in breeding programs due to linkage drag and/or epistatic interactions of deleterious genes (Young and Tanksley, 1989; Tanksley et al., 1989). Tanksley and McCouch (1997) described the need for molecular markers to facilitate and improve the efficiency of the introgression instead of phenotypic based evaluation. Advanced backcross-QTL analysis was suggested as a tool to minimize the undesirable segments of the wild genome through repeated backcrossing to the adapted cultivar and simultaneous mapping of QTL underlying the trait of interest (Tanksley and Nelson, 1996).

The lentil advanced backcross (LABC-01) population containing the full introgression genome of the wild genome was developed in the background of cultivar CDC Redberry. The introgression lines displayed variation in resistance reaction for the virulent race 0 of *C. lentis* and other traits observed during population advancement. Therefore, the LABC-01 lines could provide lentil breeders the ability to map many traits inherited from the donor parent, accession L-01-827A. Accession L-01-827A is a reservoir for many biotic and abiotic stress resistance/tolerance genes such as those for ascochyta blight (Tullu et al., 2010), stemphylium blight (Podder et al., 2012), broomrape (*Orobanche crenata*) (Bucak et al., 2014) and drought stress (Gorim and Vandenberg, 2017).

The QTL mapping conducted for resistance to anthracnose race 0 in the LABC-01 population in the present study identified a single locus associated with race 0 resistance on chromosome 3. The locus explained total phenotypic variation ranging from 12.5 to 20.7% for resistance to race 0. This locus was co-localized with QTL identified in the LR-26 interspecific population (Chapter 5) on the lentil physical genetic map. This suggests that the physical position of the genes conferring resistance to anthracnose derived from accessions L-01-827A and IG 72815 are found in closely clustered regions. Similar results were reported for anthracnose (caused by *C. lindemuthianum*) resistance in common bean (*Phaseolus vulgaris* L.), where multiple race-specific genes (co-genes) identified from differential lines form a resistance cluster on specific chromosome regions (Murube et al., 2019).

7.1.4. Conclusions

The main conclusions from the studies are:

i. Evaluation of promising sources of resistance to anthracnose race 0 identified in *L. culinaris* landrace accessions indicated a lack of resistance to race 0, while interspecific-derived resistance in RIL LR-59-81 provided an unprecedented level of resistance to anthracnose.
ii. The major QTL identified for race 1 resistance using bi-parental populations were validated via GWAS analysis on chromosome 3, and associated candidate disease resistance genes were detected. The SNP markers identified will be useful for MAS to improve anthracnose race 1 resistance in lentil.
iii. The genetic source of resistance in *L. ervoides* accession IG 72815 appeared to be derived from resistance loci on chromosomes 3 and 7, and the positions were co-localized for race 0 and race 1 at both loci.
iv. The anthracnose race 0 resistance locus derived from *L. ervoides* accession L-01-817A and found in RIL LR-59-81 was coincident with the QTL derived from *L. ervoides* accession IG 72815 on chromosome 3.
v. Regions of lentil chromosome 3 were demonstrated to be a hotspot for anthracnose resistance QTL/genes, which suggested multiple anthracnose resistance genes are co-located in a cluster on this chromosome.

7.2. Future Research Directions

The previous screening of the accession VIR-2633, reported to be resistant to both races of *C. lentis,* identified 12 resistant sublines with resistance to race 1 compared to the partial resistance of CDC Robin. The sublines demonstrated consistent race 1 reactions under growth chamber and greenhouse conditions. Lentil landrace accessions display heterogeneity due to either segregation at resistance loci or due to genotypic mixture. Future research should consider testing allelic variation to discard duplicate sublines and use of specific resistant lines for gene pyramiding in the breeding program. Results from the screening of *L. culinaris* accessions confirmed the lack of resistance to the more virulent race 0 in the *L. culinaris* germplasm pool. Thus, if additional resistance sources are required in the future, a targeted search for a new source of resistance should

focus on accessions in the *L. ervoides* gene pool. Expansion of this gene pool should be a priority for germplasm collection activity.

The QTL identified for race 1 resistance in the bi-parental populations was validated by GWAS SNPs on chromosome 3. Those SNP markers associated with race 1 resistance can be converted to SNP-based markers such as kompetitive allele-specific PCR (KASP) assays and used to evaluate F_2 populations and/or diverse germplasm segregating for race 1 resistance to select the best diagnostic marker(s) for use in marker-assisted selection in lentil breeding programs.

In the interval of the QTL derived from *L. ervoides* accessions on chromosome 3, several candidate genes were found. Future research should consider fine mapping of the locus using large intra- and inter-specific F_2 populations to further delineate the locus. In addition, gene expression studies of the candidate genes identified using qRT-PCR could also help to shorten the list of candidate genes and to identify the most differentially expressed genes. Validation of the SNP markers associated with the highly expressed gene will facilitate the transfer of the candidate genes into elite *L. culinaris* cultivars.

In Chapter 6 the LABC-01 population showed variation for some favorable traits observed during population advancement that might be used in lentil breeding programs. In addition, Vail (2010) and Chen (2018) reported that populations derived from accession L-01-827A segregate for many agronomic and plant related traits such as plant height, number of pods per node, seed size, pod dehiscence, seed coat background color, seed coat pattern, plant vigour, and seed iron concentration (Podder, 2018). Thus, the LABC-01 population is a resource available for further phenotyping that can be converted to near isogenic lines (NILs) and/or chromosome segment substitution lines (CSSLs) for use in fine mapping of the introgression of the beneficial segments or QTL conferring the traits of interest.

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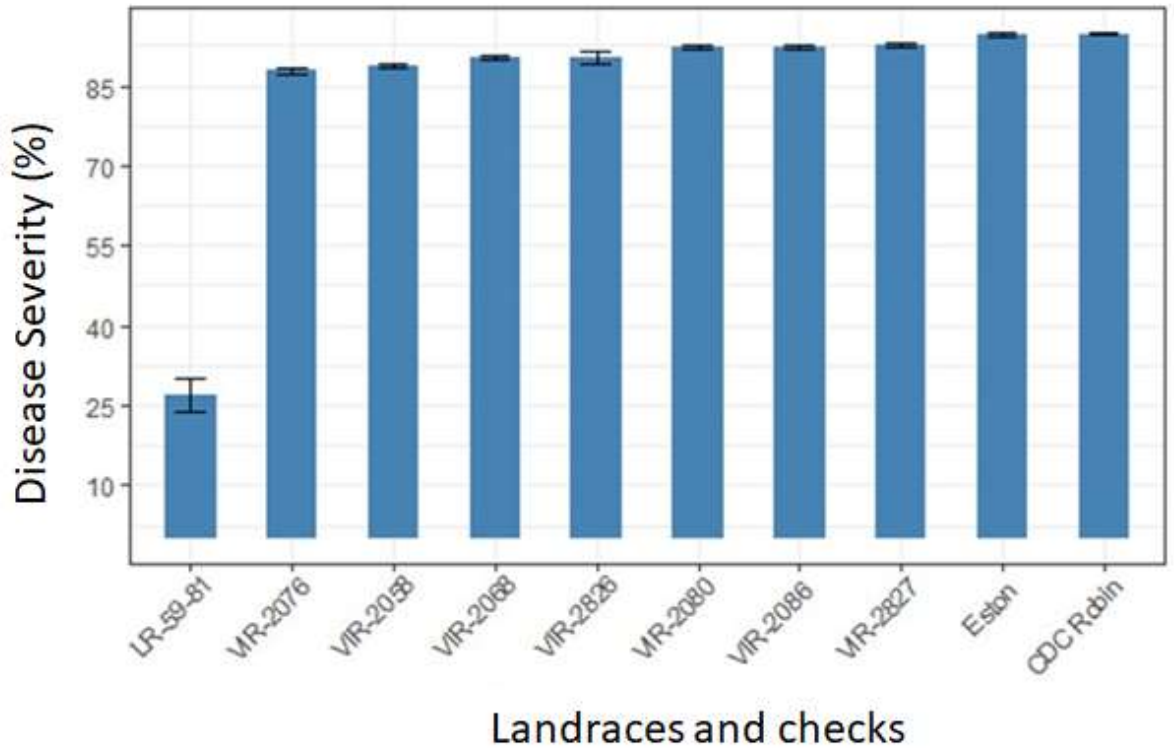
9. APPENDICES

Appendix A. Overall mean anthracnose severity score of seven Lens culinaris landrace accessions and checks infected with Colletotrichum lentis isolate CT-30 (race 0). The data are the means of 10 replications of 31 sublines evaluated for each landrace accession and for 10 replications for each, susceptible checks CDC Robin and Eston, and resistant check LR-59-81. Error bars indicate ± standard error of the mean. Anthracnose severity was rated using a 0-10 scale with 10% increments in disease severity.



Appendix B. Mean anthracnose severity for eight *Lens culinaris* accessions inoculated with *Colletotrichum lentis* race 0 under growth chamber and greenhouse conditions. The data were summarized from 10 replications of each subline per accession. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Note: For all eight accessions, 31 arbitrarily selected seeds (referred to as 'sublines') were grown individually and seeds were generated per subline before pathogenicity test.

*Original name	#Subline name	¥ DS%	£ std	Original name	Subline name	¥ DS%	£ Std
VIR-2058	CN108287-01	86	7	VIR-2068	CN108293-02	93	6
VIR-2058	CN108287-02	90	7	VIR-2068	CN108293-03	91	8
VIR-2058	CN108287-03	84	7	VIR-2068	CN108293-04	92	7
VIR-2058	CN108287-04	90	7	VIR-2068	CN108293-05	93	6
VIR-2058	CN108287-05	82	13	VIR-2068	CN108293-06	90	7
VIR-2058	CN108287-06	.	.	VIR-2068	CN108293-07	94	3
VIR-2058	CN108287-07	89	7	VIR-2068	CN108293-09	85	9
VIR-2058	CN108287-08	90	8	VIR-2068	CN108293-10	91	5
VIR-2058	CN108287-09	89	7	VIR-2068	CN108293-11	94	3
VIR-2058	CN108287-10	87	7	VIR-2068	CN108293-12	89	11
VIR-2058	CN108287-11	87	8	VIR-2068	CN108293-13	89	11
VIR-2058	CN108287-12	87	6	VIR-2068	CN108293-14	94	3
VIR-2058	CN108287-13	90	7	VIR-2068	CN108293-15	91	8
VIR-2058	CN108287-14	92	7	VIR-2068	CN108293-16	87	12
VIR-2058	CN108287-15	90	7	VIR-2068	CN108293-17	91	5
VIR-2058	CN108287-16	89	9	VIR-2068	CN108293-18	91	10
VIR-2058	CN108287-17	86	9	VIR-2068	CN108293-19	89	7
VIR-2058	CN108287-18	83	25	VIR-2068	CN108293-20	94	3
VIR-2058	CN108287-19	89	5	VIR-2068	CN108293-22	90	8
VIR-2058	CN108287-21	91	5	VIR-2068	CN108293-23	88	13
VIR-2058	CN108287-22	89	7	VIR-2068	CN108293-25	88	11
VIR-2058	CN108287-23	91	7	VIR-2068	CN108293-26	90	5

VIR-2058	CN108287-24	92	5	VIR-2068	CN108293-28	88	16
VIR-2058	CN108287-25	91	5	VIR-2068	CN108293-29	91	5
VIR-2058	CN108287-26	85	11	VIR-2068	CN108293-30	91	7
VIR-2058	CN108287-27	91	5	VIR-2068	CN108293-31	92	7
VIR-2058	CN108287-28	93	6	VIR-2068	CN108293-32	86	13
VIR-2058	CN108287-29	86	6	VIR-2068	CN108293-33	90	11
VIR-2058	CN108287-30	89	7	VIR-2068	CN108293-34	88	8
VIR-2058	CN108287-31	88	9	VIR-2068	CN108293-35	80	12
VIR-2058	CN108287-32	93	4	VIR-2068	CN108293-36	94	3
VIR-2076	CN108297-01	80	23	VIR-2080	CN108301-01	94	3
VIR-2076	CN108297-02	85	15	VIR-2080	CN108301-02	92	7
VIR-2076	CN108297-03	83	14	VIR-2080	CN108301-03	92	9
VIR-2076	CN108297-04	89	8	VIR-2080	CN108301-04	92	5
VIR-2076	CN108297-06	84	11	VIR-2080	CN108301-06	92	5
VIR-2076	CN108297-07	89	7	VIR-2080	CN108301-07	93	4
VIR-2076	CN108297-08	87	8	VIR-2080	CN108301-12	93	4
VIR-2076	CN108297-09	81	13	VIR-2080	CN108301-13	92	7
VIR-2076	CN108297-10	80	16	VIR-2080	CN108301-14	93	4
VIR-2076	CN108297-12	89	10	VIR-2080	CN108301-15	87	15
VIR-2076	CN108297-13	84	10	VIR-2080	CN108301-16	95	0
VIR-2076	CN108297-14	91	7	VIR-2080	CN108301-17	94	3
VIR-2076	CN108297-15	86	13	VIR-2080	CN108301-18	92	7
VIR-2076	CN108297-16	81	16	VIR-2080	CN108301-19	89	8
VIR-2076	CN108297-17	83	11	VIR-2080	CN108301-20	92	7
VIR-2076	CN108297-18	93	4	VIR-2080	CN108301-21	94	3
VIR-2076	CN108297-19	95	0	VIR-2080	CN108301-22	94	3
VIR-2076	CN108297-20	84	14	VIR-2080	CN108301-23	87	12
VIR-2076	CN108297-21	89	8	VIR-2080	CN108301-24	95	0
VIR-2076	CN108297-22	86	14	VIR-2080	CN108301-25	95	0
VIR-2076	CN108297-24	94	3	VIR-2080	CN108301-26	91	7
VIR-2076	CN108297-25	87	12	VIR-2080	CN108301-27	91	10

VIR-2076	CN108297-26	91	8	VIR-2080	CN108301-28	80	14
VIR-2076	CN108297-27	88	11	VIR-2080	CN108301-29	95	0
VIR-2076	CN108297-28	90	8	VIR-2080	CN108301-30	90	7
VIR-2076	CN108297-29	84	11	VIR-2080	CN108301-31	93	4
VIR-2076	CN108297-30	94	3	VIR-2080	CN108301-32	93	4
VIR-2076	CN108297-31	87	10	VIR-2080	CN108301-33	93	4
VIR-2076	CN108297-32	88	8	VIR-2080	CN108301-34	93	4
VIR-2076	CN108297-33	84	10	VIR-2080	CN108301-35	93	6
VIR-2076	CN108297-34	82	15	VIR-2080	CN108301-36	94	3
VIR-2086	CN108305-01	89	5	VIR-2826	CN108445-01	91	5
VIR-2086	CN108305-02	90	16	VIR-2826	CN108445-02	93	4
VIR-2086	CN108305-03	95	0	VIR-2826	CN108445-03	92	7
VIR-2086	CN108305-04	94	3	VIR-2826	CN108445-04	91	7
VIR-2086	CN108305-05	92	7	VIR-2826	CN108445-05	92	9
VIR-2086	CN108305-06	90	13	VIR-2826	CN108445-06	94	3
VIR-2086	CN108305-07	88	16	VIR-2826	CN108445-07	94	3
VIR-2086	CN108305-08	95	0	VIR-2826	CN108445-08	84	11
VIR-2086	CN108305-09	92	7	VIR-2826	CN108445-09	94	3
VIR-2086	CN108305-11	93	6	VIR-2826	CN108445-10	93	6
VIR-2086	CN108305-12	91	7	VIR-2826	CN108445-11	95	0
VIR-2086	CN108305-13	88	11	VIR-2826	CN108445-12	93	6
VIR-2086	CN108305-14	92	5	VIR-2826	CN108445-13	95	0
VIR-2086	CN108305-15	95	0	VIR-2826	CN108445-14	94	3
VIR-2086	CN108305-16	85	9	VIR-2826	CN108445-15	94	3
VIR-2086	CN108305-17	94	3	VIR-2826	CN108445-16	83	10
VIR-2086	CN108305-18	91	7	VIR-2826	CN108445-17	77	14
VIR-2086	CN108305-19	91	7	VIR-2826	CN108445-18	92	5
VIR-2086	CN108305-21	93	4	VIR-2826	CN108445-20	65	23
VIR-2086	CN108305-22	92	7	VIR-2826	CN108445-21	91	7
VIR-2086	CN108305-23	95	0	VIR-2826	CN108445-22	82	12
VIR-2086	CN108305-24	95	0	VIR-2826	CN108445-23	91	7

VIR-2086	CN108305-25	94	3	VIR-2826	CN108445-24	90	7
VIR-2086	CN108305-26	92	5	VIR-2826	CN108445-25	89	19
VIR-2086	CN108305-27	93	4	VIR-2826	CN108445-26	90	7
VIR-2086	CN108305-28	95	0	VIR-2826	CN108445-27	93	4
VIR-2086	CN108305-30	91	7	VIR-2826	CN108445-28	91	7
VIR-2086	CN108305-31	90	8	VIR-2826	CN108445-29	94	3
VIR-2086	CN108305-33	91	10	VIR-2826	CN108445-30	93	4
VIR-2086	CN108305-34	92	7	VIR-2826	CN108445-31	92	5
VIR-2086	CN108305-35	93	6	VIR-2826	CN108445-32	89	7
VIR-2827	CN108446-01	94	3	VIR-2633	CN108424-23	78(88)	13
VIR-2827	CN108446-02	90	7	VIR-2633	CN108424-30	70(88)	16
VIR-2827	CN108446-03	92	5	VIR-2633	CN108424-02	73(81)	16
VIR-2827	CN108446-04	92	7	VIR-2633	CN108424-25	76(81)	19
VIR-2827	CN108446-05	91	7	VIR-2633	CN108424-03	61(79)	7
VIR-2827	CN108446-06	92	5	VIR-2633	CN108424-06	79(79)	19
VIR-2827	CN108446-07	95	0	VIR-2633	CN108424-29	72(79)	19
VIR-2827	CN108446-08	95	0	VIR-2633	CN108424-34	60(79)	10
VIR-2827	CN108446-09	91	10	VIR-2633	CN108424-11	69(77)	13
VIR-2827	CN108446-10	94	3	VIR-2633	CN108424-13	61(77)	16
VIR-2827	CN108446-11	91	13	VIR-2633	CN108424-32	61(77)	12
VIR-2827	CN108446-13	94	3	VIR-2633	CN108424-05	74(74)	14
VIR-2827	CN108446-14	93	4	VIR-2633	CN108424-33	71(73)	13
VIR-2827	CN108446-15	92	5	VIR-2633	CN108424-18	64(72)	17
VIR-2827	CN108446-16	95	0	VIR-2633	CN108424-24	78(70)	18
VIR-2827	CN108446-17	93	6	VIR-2633	CN108424-14	60(67)	16
VIR-2827	CN108446-18	94	3	VIR-2633	CN108424-26	79(65)	16
VIR-2827	CN108446-19	91	7	VIR-2633	CN108424-07	64(58)	16
VIR-2827	CN108446-20	93	4	VIR-2633	CN108424-15	57(48)	14
VIR-2827	CN108446-21	93	4	VIR-2633	CN108424-16	79(24)	14
VIR-2827	CN108446-22	95	0	VIR-2633	CN108424-12	72(21)	16
VIR-2827	CN108446-23	93	4	VIR-2633	CN108424-01	74(18)	17

VIR-2827	CN108446-24	93	6	VIR-2633	CN108424-04	70(17)	19
VIR-2827	CN108446-25	95	0	VIR-2633	CN108424-28	77(28)	15
VIR-2827	CN108446-26	89	8	VIR-2633	CN108424-31	78(15)	16
VIR-2827	CN108446-27	91	7	VIR-2633	CN108424-20	79(14)	18
VIR-2827	CN108446-28	93	4	VIR-2633	CN108424-19	70(13)	16
VIR-2827	CN108446-29	93	6	VIR-2633	CN108424-22	78(12)	17
VIR-2827	CN108446-30	90	10	VIR-2633	CN108424-27	70(12)	16
VIR-2827	CN108446-31	94	3	VIR-2633	CN108424-10	83(11)	12
VIR-2827	CN108446-32	91	8	VIR-2633	CN108424-17	66(8)	18
Eston		Susceptible check for both races				94(95)	
CDC Robin		Susceptible check for race 0; resistant to race 1				94(23)	
LR-59-81		Resistant check for both races				28(21)	

[≠]Sublines named based on Plant Gene Resources of Canada (PGRC); [*]Vavilov Institute of Plant Industry (VIR), Russia; [¥]DS% - average disease severity percentage score; [£]std - standard deviations**; g**ray highlighted - accession VIR-2633 evaluated for both races, the scores for race 1 shown in brackets.

Appendix C. Mean anthracnose race 1 severity of LR-01 (ILL 1740 × 'CDC Robin') RILs evaluated under controlled conditions. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Genotype	¥ DS%	£std	Genotype	¥ DS%	£std	Genotype	¥ DS%	£std
LR-01-02	25.0	10.6	LR-01-131	90.0	8.7	LR-01-36	31.0	6.5
LR-01-03	88.0	8.4	LR-01-132	87.4	7.5	LR-01-37	18.0	8.4
LR-01-04	29.0	11.9	LR-01-133	43.0	17.9	LR-01-38	29.0	19.8
LR-01-05	91.2	6.5	LR-01-134	12.0	7.6	LR-01-39	58.8	2.2
LR-01-06	94.0	2.2	LR-01-135	68.0	10.4	LR-01-40	87.4	13.0
LR-01-07	77.0	24.1	LR-01-136	58.0	19.2	LR-01-41	32.0	19.2
LR-01-08	23.0	6.7	LR-01-137	22.0	7.6	LR-01-42	88.0	11.0
LR-01-09	9.0	4.2	LR-01-139	8.0	4.5	LR-01-43	15.0	7.9
LR-01-10	60.0	12.7	LR-01-14	94.0	2.2	LR-01-44	30.0	7.9
LR-01-103	80.0	21.2	LR-01-141	7.6	2.5	LR-01-45	55.0	18.4
LR-01-104	63.0	12.0	LR-01-142	73.8	20.1	LR-01-46	56.0	23.3
LR-01-105	48.0	18.6	LR-01-143	68.0	21.4	LR-01-48	95.0	0.0
LR-01-106	13.0	5.7	LR-01-145	38.0	16.0	LR-01-49	80.0	20.6
LR-01-107	91.0	8.9	LR-01-15	28.0	20.2	LR-01-50	78.0	17.2
LR-01-11	95.0	0.0	LR-01-16	33.0	15.2	LR-01-51	87.4	4.3
LR-01-110	73.0	21.7	LR-01-17	80.0	14.1	LR-01-52	27.4	5.6
LR-01-111	5.0	0.0	LR-01-18	95.0	0.0	LR-01-53	93.0	4.5
LR-01-113	10.0	8.7	LR-01-19	21.2	6.5	LR-01-54	55.0	21.5
LR-01-114	18.8	8.9	LR-01-20	88.0	6.7	LR-01-55	95.0	0.0
LR-01-115	73.8	36.8	LR-01-21	23.0	5.7	LR-01-56	43.0	24.6
LR-01-116	82.0	17.9	LR-01-22	13.0	11.0	LR-01-57	29.0	12.9
LR-01-117	15.0	10.0	LR-01-23	19.0	4.2	LR-01-58	6.2	2.2
LR-01-118	18.0	9.1	LR-01-24	85.0	9.4	LR-01-59	13.0	9.1
LR-01-119	91.0	8.9	LR-01-25	68.0	31.5	LR-01-60	33.0	22.0
LR-01-12	21.0	5.5	LR-01-26	57.0	7.6	LR-01-61	93.0	4.5
LR-01-120	95.0	0.0	LR-01-27	25.0	3.5	LR-01-62	16.0	8.2

LR-01-121	88.0	11.0	LR-01-28	73.0	7.6	LR-01-63	17.0	9.1
LR-01-122	95.0	0.0	LR-01-29	95.0	0.0	LR-01-69	86.2	8.9
LR-01-123	32.4	21.9	LR-01-30	67.0	27.5	LR-01-71	17.0	12.5
LR-01-124	28.0	6.7	LR-01-31	29.0	21.9	LR-01-72	95.0	0.0
LR-01-126	16.0	8.2	LR-01-32	14.0	8.2	LR-01-73	92.0	4.5
LR-01-128	33.0	13.0	LR-01-33	89.0	13.4	LR-01-76	83.0	16.4
LR-01-129	95.0	0.0	LR-01-34	83.0	11.5	LR-01-79	12.0	9.7
LR-01-13	95.0	0.0	LR-01-35	70.0	30.6	LR-01-84	22.0	9.7

[¥] DS% - average disease severity percentage score; [£]std - standard deviations

Appendix D. Mean anthracnose race 1 severity of LR-18 ('CDC Robin' × 964a-46) RILs evaluated under controlled conditions. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

[#]Genotype	[¥]DS%	[£]std	Genotype	[¥]DS%	[£]std	[#]Genotype	[¥]DS%	[£]std
LR - 18 -2	53.0	12.5	LR - 18-70	86.0	10.2	LR - 18-142	79.0	18.2
LR - 18 -3	60.5	33.0	LR - 18-72	86.0	12.4	LR - 18-143	83.0	8.4
LR - 18 -5	89.0	13.4	LR - 18-73	20.5	4.5	LR - 18-145	22.5	13.5
LR - 18 -8	85.0	12.2	LR - 18-74	89.0	6.5	LR - 18-146	16.0	10.2
LR - 18-9	23.5	2.2	LR - 18-75	27.5	17.1	LR - 18-149	10.0	7.1
LR - 18-11	84.0	13.4	LR - 18-76	58.0	19.9	LR - 18-150	70.0	10.0
LR - 18-12	15.0	11.7	LR - 18-78	94.0	2.2	LR - 18-151	73.5	13.6
LR - 18-13	89.0	5.5	LR - 18-79	83.5	21.6	LR - 18-152	81.5	8.9
LR - 18-14	22.0	8.4	LR - 18-80	91.0	5.5	LR - 18-153	26.5	10.8
LR - 18-15	19.5	11.0	LR - 18-81	15.0	7.9	LR - 18-154	74.0	18.8
LR - 18-16	21.5	5.5	LR - 18-84	16.0	11.4	LR - 18-155	14.5	7.2
LR - 18-18	13.5	7.4	LR - 18-85	87.0	13.0	LR - 18-156	86.0	8.9
LR - 18-19	11.0	6.5	LR - 18-86	17.5	5.0	LR - 18-157	16.0	5.5
LR - 18-20	83.0	16.8	LR - 18-87	52.5	31.1	LR - 18-158	77.0	13.0
LR - 18-21	48.0	43.2	LR - 18-88	15.5	3.7	LR - 18-160	79.5	9.1
LR - 18-22	85.5	11.0	LR - 18-89	19.5	9.4	LR - 18-161	87.0	8.4
LR - 18-26	89.0	5.5	LR - 18-90	16.0	8.9	LR - 18-162	75.0	6.1
LR - 18-28	89.0	8.9	LR - 18-92	83.5	16.4	LR - 18-163	74.0	15.2
LR - 18-30	77.0	16.4	LR - 18-95	90.0	8.7	LR - 18-164	86.5	11.9
LR - 18-31	14.5	5.7	LR - 18-96	85.0	11.7	LR - 18-165	78.0	14.8
LR - 18-32	64.5	18.6	LR - 18-98	25.0	9.4	LR - 18-166	89.0	5.5
LR - 18-33	81.0	15.2	LR - 18-101	15.0	6.1	LR - 18-167	21.0	6.5
LR - 18-34	21.0	4.2	LR - 18-102	90.0	8.7	LR - 18-169	77.0	11.0
LR - 18-36	37.0	17.5	LR - 18-106	76.5	21.0	LR - 18-170	87.0	5.7
LR - 18-37	33.5	6.0	LR - 18-109	80.5	15.7	LR - 18-171	83.0	11.0
LR - 18-38	18.0	4.5	LR - 18-110	84.4	4.3	LR - 18-172	77.5	14.1

LR - 18-40	85.0	10.0	LR - 18-111	70.5	8.4	LR - 18-173	78.0	14.0
LR - 18-41	91.0	5.5	LR - 18-113	71.0	13.4	LR - 18-174	11.5	4.2
LR - 18-42	11.0	4.2	LR - 18-114	65.5	12.0	LR - 18-175	50.0	10.0
LR - 18-43	73.0	25.9	LR - 18-115	25.0	9.1	LR - 18-176	82.0	21.1
LR - 18-44	84.0	11.4	LR - 18-117	71.0	19.5	LR - 18-177	78.5	14.1
LR - 18-46	71.0	13.4	LR - 18-118	83.5	7.0	LR - 18-178	12.5	7.1
LR - 18-47	75.0	14.1	LR - 18-119	17.5	7.5	LR - 18-179	18.0	8.4
LR - 18-50	76.0	12.4	LR - 18-121	15.0	7.9	LR - 18-181	18.3	7.6
LR - 18-51	91.3	4.8	LR - 18-122	19.0	8.2	LR - 18-182	63.8	19.3
LR - 18-52	16.5	5.5	LR - 18-123	74.0	13.4	LR - 18-183	14.5	8.0
LR - 18-53	27.0	12.5	LR - 18-124	76.5	15.6	LR - 18-185	22.0	4.5
LR - 18-54	11.0	5.5	LR - 18-125	89.0	5.5	LR - 18-186	65.5	10.7
LR - 18-56	70.5	20.5	LR - 18-126	89.0	8.9	LR - 18-187	19.5	7.6
LR - 18-57	85.0	10.0	LR - 18-127	87.0	13.0	LR - 18-188	91.5	5.5
LR - 18-58	29.0	5.5	LR - 18-130	84.0	21.9	LR - 18-189	68.0	15.7
LR - 18-59	19.0	8.9	LR - 18-132	18.5	8.2	LR - 18-190	15.0	10.0
LR - 18-60	88.0	8.4	LR - 18-133	18.0	4.5	LR - 18-193	32.0	12.0
LR - 18-61	21.3	12.5	LR - 18-135	25.0	12.2	LR - 18-194	26.5	12.4
LR - 18-62	25.0	7.1	LR - 18-136	19.0	6.5	LR - 18-195	73.5	13.9
LR - 18-63	91.0	8.9	LR - 18-137	11.0	5.5	LR - 18-196	78.1	12.8
LR - 18-64	84.0	11.4	LR - 18-138	22.0	13.0	LR - 18-200	15.0	7.9
LR - 18-66	15.0	7.1	LR - 18-139	69.0	15.6	CDC Robin	23.0	2.0
LR - 18-69	18.5	8.9	LR - 18-141	80.0	23.8	964a-46	93.0	3.0

[¥] DS% - average disease severity percentage score; [£]std - standard deviations; [#] highlighted – genotypes not included in QTL mapping

Appendix E. Markers and their LOD scores in the region of QTL conferring resistance to anthracnose race 1 on lentil chromosome 3 detected by multiple QTL model of R/qtl in LR-01 (ILL 1704 × 'CDC Robin') RIL population. (LOD > 3.5, α = 0.05 with 1000 permutations)

Marker	Pos[a]	LOD[b]	Marker	Pos[a]	LOD[b]
Lcu_2RBY_Chr3_5455609	22.71	4.06	Lcu_2RBY_Chr3_36029854	69.34	12.28
Lcu_2RBY_Chr3_5452594	24.42	4.80	Lcu_2RBY_Chr3_36029916	69.84	13.22
Lcu_2RBY_Chr3_4682811	25.44	4.37	Lcu_2RBY_Chr3_38758654	70.83	9.92
Lcu_2RBY_Chr3_4697931	25.44	4.37	Lcu_2RBY_Chr3_43123343	71.34	7.05
Lcu_2RBY_Chr3_5329365	25.99	5.19	Lcu_2RBY_Chr3_39838514	71.34	7.05
Lcu_2RBY_Chr3_5326965	26.53	4.42	Lcu_2RBY_Chr3_36441754	71.34	7.17
Lcu_2RBY_Chr3_14094190	34.06	5.84	Lcu_2RBY_Chr3_36440873	71.34	7.17
Lcu_2RBY_Chr3_14110893	34.57	6.91	Lcu_2RBY_Chr3_37679175	71.34	7.17
Lcu_2RBY_Chr3_16821456	35.13	7.56	Lcu_2RBY_Chr3_47164692	72.87	7.32
Lcu_2RBY_Chr3_12661333	35.67	7.39	Lcu_2RBY_Chr3_48150713	73.94	6.52
Lcu_2RBY_Chr3_12147582	36.19	6.91	Lcu_2RBY_Chr3_48915995	74.96	8.81
Lcu_2RBY_Chr3_12241143	37.25	6.60	Lcu_2RBY_Chr3_49367927	76.01	8.50
Lcu_2RBY_Chr3_12587498	37.25	6.60	Lcu_2RBY_Chr3_48915354	77.03	7.32
Lcu_2RBY_Chr3_11857912	37.79	5.50	Lcu_2RBY_Chr3_55917241	78.02	8.80
Lcu_2RBY_Chr3_12146467	38.35	5.50	Lcu_2RBY_Chr3_56810695	79.56	7.04
Lcu_2RBY_Chr3_19567118	41.22	6.13	Lcu_2RBY_Chr3_72054642	81.51	10.18
Lcu_2RBY_Chr3_19184608	42.32	7.72	Lcu_2RBY_Chr3_81247957	81.51	10.18
Lcu_2RBY_Chr3_19630090	42.87	9.31	Lcu_2RBY_Chr3_80918323	83.09	7.83
Lcu_2RBY_Chr3_19085368	42.87	9.31	Lcu_2RBY_Chr3_56077076	83.58	7.74
Lcu_2RBY_Chr3_19087390	42.87	9.22	Lcu_2RBY_Chr3_52338311	84.09	9.76
Lcu_2RBY_Chr3_19087355	42.87	9.22	Lcu_2RBY_Chr3_51288447	87.08	5.95
Lcu_2RBY_Chr3_19569408	42.87	10.45	Lcu_2RBY_Chr3_59493172	87.61	5.63
Lcu_2RBY_Chr3_19625958	42.87	10.46	Lcu_2RBY_Chr3_59493145	88.12	4.99
Lcu_2RBY_Chr3_22103592	45.04	11.34	Lcu_2RBY_Chr3_56132444	89.65	7.02
Lcu_2RBY_Chr3_22232104	45.56	11.37	Lcu_2RBY_Chr3_82260216	91.22	5.69
Lcu_2RBY_Chr3_24240604	47.11	10.91	Lcu_2RBY_Chr3_83587242	91.75	5.10
Lcu_2RBY_Chr3_25800594	47.62	11.23	Lcu_2RBY_Chr3_84305645	91.75	5.10
Lcu_2RBY_Chr3_24240327	48.14	10.98	Lcu_2RBY_Chr3_83978249	92.26	5.22
Lcu_2RBY_Chr3_27200229	48.14	10.98	Lcu_2RBY_Chr3_83978283	92.26	5.22
Lcu_2RBY_Chr3_25800219	48.64	10.71	Lcu_2RBY_Chr3_83143883	93.23	6.47

Lcu_2RBY_Chr3_23087478	49.67	13.08	Lcu_2RBY_Chr3_84324396	93.23	6.47
Lcu_2RBY_Chr3_23027077	49.67	13.08	Lcu_2RBY_Chr3_83978592	93.74	6.91
Lcu_2RBY_unitig0289_1940685	51.69	15.49	Lcu_2RBY_Chr3_90597556	95.77	5.70
Lcu_2RBY_unitig0289_1940618	51.69	15.49	Lcu_2RBY_Chr3_105998926	96.79	5.10
Lcu_2RBY_Chr3_29313444	53.20	12.88	Lcu_2RBY_Chr3_90597676	97.32	6.08
Lcu_2RBY_unitig2807_231344	53.69	12.85	Lcu_2RBY_Chr3_83880826	99.85	8.00
Lcu_2RBY_unitig2807_231397	54.17	14.15	Lcu_2RBY_Chr3_84831014	99.85	8.00
Lcu_2RBY_Chr3_29308516	54.69	16.19	Lcu_2RBY_Chr3_83977815	100.92	5.55
Lcu_2RBY_unitig0289_1523750	55.23	17.39	Lcu_2RBY_Chr3_85745671	100.92	5.55
Lcu_2RBY_unitig0289_872743	55.80	16.40	Lcu_2RBY_Chr3_170432789	103.82	4.19
Lcu_2RBY_unitig0289_211009	56.33	15.68	Lcu_2RBY_Chr3_175719550	103.82	4.19
Lcu_2RBY_unitig0289_875663	56.33	15.68	Lcu_2RBY_Chr3_169010429	103.82	4.18
Lcu_2RBY_unitig0289_805214	56.33	15.69	Lcu_2RBY_Chr3_160780020	104.79	4.79
Lcu_2RBY_unitig0289_34678	56.33	15.69	Lcu_2RBY_Chr3_160205376	104.79	4.79
Lcu_2RBY_unitig0289_210977	56.33	15.69	Lcu_2RBY_Chr3_157860120	105.30	5.00
Lcu_2RBY_unitig0289_807431	56.83	13.40	Lcu_2RBY_Chr3_157858285	105.81	4.60
Lcu_2RBY_unitig0289_1318349	57.35	14.38	Lcu_2RBY_Chr3_155353613	106.30	4.79
Lcu_2RBY_unitig0289_1027619	57.86	16.50	Lcu_2RBY_Chr3_128901150	107.28	7.02
Lcu_2RBY_unitig0289_1522574	58.37	14.45	Lcu_2RBY_Chr3_108047666	107.28	7.02
Lcu_2RBY_unitig0289_1010665	59.39	12.04	Lcu_2RBY_Chr3_134571607	108.29	6.43
Lcu_2RBY_unitig0289_1477942	59.88	13.98	Lcu_2RBY_Chr3_140819554	108.29	6.44
Lcu_2RBY_unitig0289_1524560	60.41	16.65	Lcu_2RBY_Chr3_140857898	108.29	7.36
Lcu_2RBY_unitig0289_2197223	60.94	24.28	Lcu_2RBY_Chr3_132685080	108.29	7.39
Lcu_2RBY_unitig0289_2195536	60.94	24.28	Lcu_2RBY_Chr3_131009510	108.29	7.42
Lcu_2RBY_unitig0289_1918197	60.94	24.28	Lcu_2RBY_Chr3_145710069	110.87	5.47
Lcu_2RBY_unitig0289_2196838	61.46	22.83	Lcu_2RBY_Chr3_145710040	111.37	5.19
Lcu_2RBY_unitig0289_2195788	61.98	21.67	Lcu_2RBY_Chr3_147760403	112.97	5.66
Lcu_2RBY_Chr3_29721291	62.45	21.42	Lcu_2RBY_Chr3_111033894	112.97	5.54
Lcu_2RBY_Chr3_30301124	62.45	21.27	Lcu_2RBY_Chr4_338983746	113.45	4.11
Lcu_2RBY_Chr3_30227506	62.93	19.59	Lcu_2RBY_Chr3_156791234	113.95	3.58
Lcu_2RBY_Chr3_30704567	64.41	21.26	Lcu_2RBY_Chr3_168231946	113.95	3.58
Lcu_2RBY_Chr3_30704494	64.88	19.03	Lcu_2RBY_Chr3_151014297	114.45	3.80
Lcu_2RBY_Chr3_30704483	65.37	18.62	Lcu_2RBY_Chr3_148831868	114.45	4.02
Lcu_2RBY_Chr3_30644175	65.37	18.55	Lcu_2RBY_Chr3_148782583	114.45	4.02
Lcu_2RBY_Chr3_31706795	65.37	17.35	Lcu_2RBY_Chr3_149236446	114.96	5.45

Lcu_2RBY_Chr3_31410745	65.37	17.35	Lcu_2RBY_Chr3_148822468	114.96	5.45
Lcu_2RBY_Chr3_31110799	65.88	18.04	Lcu_2RBY_Chr3_170564844	115.93	4.45
Lcu_2RBY_Chr3_32244106	66.36	17.48	Lcu_2RBY_Chr3_189989892	119.94	3.51
Lcu_2RBY_Chr3_32242366	66.36	17.53	Lcu_2RBY_Chr3_190755573	122.71	3.71
Lcu_2RBY_Chr3_32242385	66.86	18.88	Lcu_2RBY_Chr3_190759211	123.23	3.72
Lcu_2RBY_Chr3_35417536	67.86	14.63	Lcu_2RBY_Chr3_211833309	128.78	3.67
Lcu_2RBY_Chr3_35404488	67.86	14.63	Lcu_2RBY_Chr3_208055988	129.85	3.55
Lcu_2RBY_Chr3_35517687	68.36	14.31	Lcu_2RBY_Chr3_208057690	133.37	3.62
Lcu_2RBY_Chr3_35517678	68.36	14.31	Lcu_2RBY_Chr3_221793009	137.48	3.88

[a]Pos – position of the marker; [b]LOD – Logarithm of the odds

Appendix F. Mean anthracnose race 1 severity of the 200 lentil accessions in the GWAS panel evaluated under controlled conditions. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Accession	¥ DS1	£std	¥ DS2	£std	Accession	¥ DS1	£std	¥ DS2	£std
A3156_11_AGL	25.0	10.0	39.0	15.8	PI_297767_AGL	65.0	20.0	82.8	19.2
CDC_Asterix_AGL	81.7	23.1	77.2	18.6	PI_298121_LSP_AGL	81.7	11.5	89.0	9.7
CDC_Cherie_AGL	81.7	23.1	85.0	21.2	PI_298357_LSP_AGL	95.0	0.0	95.0	0.0
CDC_Glamis_AGL	61.7	5.8	65.0	17.3	PI_298631_LSP_AGL	71.7	11.5	85.0	15.0
CDC_Greenstar_AGL	50.0	5.0	34.0	20.8	PI_298644_LSP_AGL	58.3	11.5	74.0	25.1
CDC_Imax_AGL	13.3	7.6	18.0	20.6	PI_298645_AGL	68.3	5.8	85.0	17.6
CDC_Impower_AGL	.	.	71.0	38.9	PI_298922_LSP_AGL	78.3	15.3	86.0	12.0
CDC_KR_1_AGL	55.0	10.0	60.0	18.4	PI_299116_LSP_AGL	95.0	0.0	95.0	0.0
CDC_LeMay_AGL	18.3	11.5	43.0	19.9	PI_299120_LSP_AGL	95.0	0.0	95.0	0.0
CDC_Maxim_AGL	55.0	10.0	66.0	15.2	PI_299121_LSP_AGL	88.3	11.5	93.0	6.3
CDC_QG-1_AGL	80.0	0.0	61.0	38.4	PI_299126_LSP_AGL	95.0	0.0	89.0	13.5
CDC_Red_Rider_AGL	55.0	0.0	48.0	38.9	PI_299163_LSP_AGL	45.0	17.3	75.0	24.0
CDC_Redcoat_AGL	68.3	5.8	83.0	13.2	PI_299164_LSP_AGL	75.0	0.0	79.0	20.1
CDC_Redwing_AGL	65.0	26.5	84.0	19.1	PI_299165_AGL	58.3	15.3	76.0	19.1
CDC_Robin_AGL	20.0	5.0	18.3	7.1	PI_299177_LSP_AGL	55.0	10.0	78.0	22.6
CDC_Rosebud_AGL	65.0	0.0	54.0	30.0	PI_299289_AGL	95.0	0.0	91.0	8.4
CDC_Rosetown_AGL	5.0	0.0	6.1	3.3	PI_299366_LSP_AGL	71.7	5.8	79.0	20.1
CDC_Rosie_AGL	8.3	5.8	8.0	4.8	PI_300250_LSP_AGL	78.3	28.9	90.0	15.8
CDC_Rouleau_AGL	95.0	0.0	74.0	31.1	PI_302398_LSP_AGL	85.0	17.3	89.0	12.6
CDC_Royale_AGL	51.7	5.8	70.0	22.2	PI_308614_LSP_AGL	95.0	0.0	95.0	0.0
CDC_Ruby_AGL	25.0	0.0	32.0	25.0	PI_311107_LSP_AGL	85.0	17.3	87.0	13.2
CDC_Sedley_AGL	68.3	23.1	80.6	23.0	PI_320936_LSP_AGL	81.7	23.1	88.3	14.1
CDC_Vantage_AGL	65.0	30.0	71.0	20.1	PI_320937_LSP_AGL	8.3	5.8	10.0	7.1
CN_105605_AGL	95.0	0.0	95.0	0.0	PI_320945_LSP_AGL	85.0	17.3	92.0	9.5
CN_105732_AGL	58.3	28.9	70.0	24.2	PI_320946_LSP_AGL	81.7	23.1	87.0	14.0
CN_105767_AGL	88.3	11.5	88.0	11.6	PI_320952_LSP_AGL	18.3	5.8	12.8	8.3
CN_105866_AGL	78.3	28.9	89.4	16.7	PI_320953_LSP_AGL	25.0	0.0	29.0	23.2
CN_108369_AGL	75.0	17.3	84.0	18.5	PI_320954_LSP_AGL	75.0	0.0	90.6	8.8
CN_108370_AGL	.	.	47.0	30.1	PI_329157_LSP_AGL	95.0	0.0	95.0	0.0
Crimson_AGL	81.7	23.1	87.2	15.6	PI_339285_AGL	78.3	15.3	89.4	11.3
DPL_62_AGL	95.0	0.0	95.0	0.0	PI_339292_LSP_AGL	95.0	0.0	90.0	10.8
Gudo_AGL	88.3	11.5	93.0	6.3	PI_343026_LSP_AGL	95.0	0.0	95.0	0.0

IG_1046_AGL	95.0	0.0	95.0	0.0	PI_345627_LSP_AGL	58.3	20.8	62.0	28.7
IG_1959_AGL	95.0	0.0	95.0	0.0	PI_358602_LSP_AGL	78.3	15.3	84.0	19.1
IG_4258_AGL	95.0	0.0	95.0	0.0	PI_368647_LSP_AGL	78.3	15.3	84.0	12.0
IG_4781_AGL	75.0	17.3	87.0	17.5	PI_368651LSP_AGL	88.3	11.5	88.3	14.1
IG_858_AGL	95.0	0.0	91.0	8.4	PI_370481_LSP_AGL	81.7	23.1	90.0	14.1
ILL_10657_AGL	95.0	0.0	85.0	10.5	PI_374116_LSP_AGL	71.7	5.8	89.4	11.3
ILL_11547_AGL	88.3	11.5	70.0	25.9	PI_374117_LSP_AGL	78.3	15.3	83.0	13.2
ILL_11548_AGL	95.0	0.0	91.0	8.4	PI_374118_AGL	71.7	20.8	88.0	14.9
ILL_11555_AGL	81.7	23.1	88.0	14.9	PI_374120LSP_AGL	18.3	11.5	69.0	36.6
ILL_11558_AGL	95.0	0.0	95.0	0.0	PI_374121_AGL	85.0	10.0	83.9	13.6
ILL_1762_AGL	81.7	23.1	87.0	14.0	PI_379368_LSP_AGL	65.0	10.0	79.4	15.9
ILL_1983_AGL	95.0	0.0	95.0	0.0	PI_420924_LSP_AGL	71.7	20.8	86.0	15.2
ILL_213_AGL	95.0	0.0	95.0	0.0	PI_426202_LSP_AGL	85.0	10.0	89.0	13.5
ILL_28_AGL	81.7	23.1	90.6	13.3	PI_426778_LSP_AGL	61.7	5.8	85.0	16.3
ILL_3025_AGL	95.0	0.0	95.0	0.0	PI_426784_LSP_AGL	95.0	0.0	92.8	6.7
ILL_313_AGL	81.7	23.1	87.0	14.0	PI_426797_LSP_AGL	95.0	0.0	95.0	0.0
ILL_3347_AGL	95.0	0.0	95.0	0.0	PI_426807_LSP_AGL	95.0	0.0	95.0	0.0
ILL_358_AGL	88.3	11.5	93.0	6.3	PI_431622_LSP_AGL	95.0	0.0	95.0	0.0
ILL_3597_AGL	95.0	0.0	95.0	0.0	PI_431630_LSP_AGL	95.0	0.0	95.0	0.0
ILL_4400_AGL	58.3	20.8	74.0	24.7	PI_431662_LSP_AGL	95.0	0.0	95.0	0.0
ILL_4605_AGL	95.0	0.0	88.0	11.6	PI_431663_LSP_AGL	88.3	11.5	93.0	6.3
ILL_4609_AGL	88.3	11.5	91.0	8.4	PI_431684_LSP_AGL	88.3	11.5	92.8	6.7
ILL_4665_AGL	70.0	25.0	78.3	20.6	PI_431705_LSP_AGL	95.0	0.0	95.0	0.0
ILL_4768_AGL	85.0	17.3	85.0	16.3	PI_431714_LSP_AGL	88.3	11.5	93.0	6.3
ILL_4782_AGL	88.3	11.5	81.0	20.7	PI_431717_LSP_AGL	88.3	11.5	93.0	6.3
ILL_4783_AGL	65.0	0.0	80.6	18.1	PI_431728_LSP_AGL	95.0	0.0	95.0	0.0
ILL_4804_AGL	95.0	0.0	95.0	0.0	PI_431863_LSP_AGL	88.3	11.5	93.0	6.3
ILL_4875_AGL	78.3	28.9	90.0	15.8	PI_431888_LSP_AGL	81.7	23.1	91.0	12.6
ILL_4956_AGL	81.7	23.1	87.0	14.0	PI_431893_LSP_AGL	95.0	0.0	95.0	0.0
ILL_6002_AGL	95.0	0.0	88.0	11.6	PI_431923_LSP_AGL	75.0	20.0	89.0	13.5
ILL_624_AGL	95.0	0.0	95.0	0.0	PI_432001_LSP_AGL	95.0	0.0	92.0	9.5
ILL_6821_AGL	78.3	15.3	90.0	10.8	PI_432033_LSP_AGL	95.0	0.0	95.0	0.0
ILL_7089_AGL	95.0	0.0	95.0	0.0	PI_432124_LSP_AGL	95.0	0.0	95.0	0.0
ILL_7558_AGL	58.3	11.5	80.6	18.8	PI_432145_LSP_AGL	78.3	15.3	90.0	10.8
ILL_7668_AGL	95.0	0.0	91.0	8.4	PI_432147_LSP_AGL	95.0	0.0	95.0	0.0
ILL_8009_AGL	95.0	0.0	95.0	0.0	PI_432184_LSP_AGL	95.0	0.0	95.0	0.0
ILL_8174_AGL	81.7	23.1	86.0	19.1	PI_432188_LSP_AGL	95.0	0.0	95.0	0.0

ILL_8595_AGL	75.0	34.6	89.0	19.0	PI_432201_LSP_AGL	81.7	11.5	88.3	10.0
ILL_975_AGL	85.0	17.3	92.0	9.5	PI_432236_LSP_AGL	95.0	0.0	95.0	0.0
ILL_9888_AGL	95.0	0.0	95.0	0.0	PI_432245_LSP_AGL	95.0	0.0	95.0	0.0
ILL_9932_AGL	88.3	11.5	93.0	6.3	PI_432271_LSP_AGL	48.3	15.3	74.0	21.8
ILL_9945_AGL	95.0	0.0	95.0	0.0	PI_432286_LSP_AGL	95.0	0.0	93.0	6.3
ILL_9977_AGL	95.0	0.0	95.0	0.0	PI_451763_LSP_AGL	85.0	17.3	73.0	22.0
ILWL_118_AGL	78.3	28.9	90.0	15.8	PI_458503_LSP_AGL	95.0	0.0	95.0	0.0
Indianhead_AGL	8.3	5.8	20.0	10.8	PI_468900_LSP_AGL	38.3	15.3	52.0	20.0
IPL_220_AGL	81.7	23.1	91.0	12.6	PI_468901_AGL	15.0	17.3	16.1	12.7
Laird_AGL	61.7	11.5	61.0	29.5	PI_468902_LSP_AGL	20.0	5.0	22.8	17.9
PI_163589_AGL	61.7	23.1	86.1	20.3	PI_472136_LSP_AGL	95.0	0.0	95.0	0.0
PI_169534_LSP_AGL	75.0	34.6	71.0	20.7	PI_472213_LSP_AGL	95.0	0.0	91.0	8.4
PI_178939_LSP_AGL	88.3	11.5	82.0	14.9	PI_472327_LSP_AGL	95.0	0.0	95.0	0.0
PI_179324_LSP_AGL	71.7	20.8	88.0	14.9	PI_472416_LSP_AGL	95.0	0.0	95.0	0.0
PI_179330_AGL	95.0	0.0	95.0	0.0	PI_472561_LSP_AGL	95.0	0.0	95.0	0.0
PI_181886_LSP_AGL	88.3	11.5	92.8	6.7	PI_472569_LSP_AGL	85.0	17.3	92.0	9.5
PI_193547_LSP_AGL	75.0	20.0	89.0	13.5	PI_472590_LSP_AGL	95.0	0.0	91.0	8.4
PI_207492_LSP_AGL	88.3	11.5	93.0	6.3	PI_490288_LSP_AGL	78.3	28.9	76.1	20.9
PI_209858_LSP_AGL	68.3	5.8	85.0	17.6	PI_490289_LSP_AGL	85.0	17.3	92.0	9.5
PI_212100_LSP_AGL	81.7	23.1	91.0	12.6	PI_518731_LSP_AGL	81.7	23.1	81.7	17.3
PI_212610_LSP_AGL	95.0	0.0	92.8	6.7	PI_518733_LSP_AGL	10.0	5.0	13.9	7.8
PI_217949_LSP_AGL	95.0	0.0	95.0	0.0	PI_518734_LSP_AGL	11.7	5.8	21.0	8.4
PI_238758_LSP_AGL	41.7	20.8	66.0	32.5	PI_533693_LSP_AGL	41.7	5.8	73.9	23.2
PI_250158_LSP_AGL	95.0	0.0	95.0	0.0	PI_612875_AGL	95.0	0.0	91.7	10.0
PI_273664_LSP_AGL	81.7	11.5	84.0	12.0	PI_643451_AGL	95.0	0.0	95.0	0.0
PI_289066_AGL	85.0	17.3	89.4	11.3	PI_643452_AGL	95.0	0.0	95.0	0.0
PI_289073_LSP_AGL	75.0	20.0	84.0	15.2	W6_27754_LSP_AGL	55.0	0.0	66.0	35.4
PI_289079_LSP_AGL	55.0	17.3	67.2	22.2	W6_27760_LSP_AGL	95.0	0.0	89.0	13.5
PI_290716_LSP_AGL	65.0	17.3	82.0	18.9	W6_27763_LSP_AGL	95.0	0.0	91.0	8.4
PI_297285_LSP_AGL	75.0	17.3	87.0	13.2	W6_27766_LSP_AGL	85.0	17.3	92.0	9.5
PI_297754_LSP_AGL	81.7	23.1	91.0	12.6	W6_27767_LSP_AGL	95.0	0.0	95.0	0.0

[¥] DS - average disease severity percentage score at growth chamber (DS1) and polyhouse (DS2) - ; [£]std - standard deviations

Appendix G. SNP markers significant associated with anthracnose race 1 resitance identfied from trials in the growth chamber and polyhouse, and a combined lsmean of disease severity in a set of 200 lentil accessions.

Environment	SNP Marker	Chr	Position (Mb)[#]	*P*.value	MAF	$R^{2\$}$
Combined	Lcu.2RBY.Chr3.33827173	3	33827173	1.38E-06	0.16	0.65
	Lcu.2RBY.Chr3.33827185	3	33827185	2.43E-08	0.15	0.67
	Lcu.2RBY.Chr3.34117023	3	34117023	2.47E-10	0.14	0.69
	Lcu.2RBY.Chr3.35384298	3	35384298	3.24E-06	0.14	0.65
	Lcu.2RBY.Chr3.341261994	3	3.41E+08	4.30E-07	0.23	0.66
	Lcu.2RBY.Chr3.417940994	3	4.18E+08	6.13E-06	0.06	0.64
	Lcu.2RBY.Chr4.442702129	4	4.43E+08	8.32E-08	0.11	0.66
	Lcu.2RBY.Chr4.442702133	4	4.43E+08	8.48E-08	0.11	0.66
	Lcu.2RBY.Chr5.28582530	5	28582530	3.74E-06	0.12	0.65
	Lcu.2RBY.Chr5.28637458	5	28637458	3.74E-06	0.12	0.65
	Lcu.2RBY.Chr5.33721990	5	33721990	1.82E-09	0.21	0.68
	Lcu.2RBY.Chr5.437910070	5	4.38E+08	3.23E-06	0.13	0.65
	Lcu.2RBY.Chr5.437944230	5	4.38E+08	3.95E-06	0.13	0.65
	Lcu.2RBY.Chr6.374326758	6	3.74E+08	8.88E-07	0.11	0.65
Growth chamber	Lcu.2RBY.Chr2.36606480	2	36606480	4.64E-06	0.13	0.61
	Lcu.2RBY.Chr2.57570472	2	57570472	3.08E-06	0.07	0.62
	Lcu.2RBY.Chr2.59440536	2	59440536	3.08E-06	0.07	0.62
	Lcu.2RBY.Chr2.72471727	2	72471727	4.81E-06	0.07	0.61
	Lcu.2RBY.Chr3.29754683	3	29754683	2.86E-06	0.22	0.62
	Lcu.2RBY.Chr3.33827173	3	33827173	5.27E-07	0.16	0.63
	Lcu.2RBY.Chr3.33827185	3	33827185	1.05E-08	0.15	0.65
	Lcu.2RBY.Chr3.34117023	3	34117023	1.86E-10	0.14	0.67
	Lcu.2RBY.Chr3.35384298	3	35384298	4.15E-06	0.14	0.62
	Lcu.2RBY.Chr3.57041703	3	57041703	5.91E-06	0.06	0.61
	Lcu.2RBY.Chr3.162869024	3	1.63E+08	4.47E-06	0.17	0.62
	Lcu.2RBY.Chr3.341261994	3	3.41E+08	1.46E-07	0.23	0.63

	Lcu.2RBY.Chr3.348840074	3	3.49E+08	2.34E-06	0.07	0.62
	Lcu.2RBY.Chr3.349773406	3	3.5E+08	4.77E-07	0.06	0.63
	Lcu.2RBY.Chr3.355843750	3	3.56E+08	5.28E-06	0.08	0.61
	Lcu.2RBY.Chr3.400615790	3	4.01E+08	2.57E-06	0.06	0.62
	Lcu.2RBY.Chr3.405224715	3	4.05E+08	2.98E-06	0.08	0.62
	Lcu.2RBY.Chr3.417940994	3	4.18E+08	2.03E-06	0.06	0.62
	Lcu.2RBY.Chr4.442702129	4	4.43E+08	3.27E-07	0.11	0.63
	Lcu.2RBY.Chr4.442702133	4	4.43E+08	4.95E-07	0.11	0.63
	Lcu.2RBY.Chr5.28377874	5	28377874	5.71E-06	0.31	0.61
	Lcu.2RBY.Chr5.33721990	5	33721990	2.56E-09	0.21	0.65
	Lcu.2RBY.Chr5.427535882	5	4.28E+08	4.50E-06	0.21	0.62
	Lcu.2RBY.Chr5.430533888	5	4.31E+08	5.32E-06	0.12	0.61
	Lcu.2RBY.Chr5.437910070	5	4.38E+08	3.14E-06	0.13	0.62
	Lcu.2RBY.Chr5.437944230	5	4.38E+08	3.90E-06	0.13	0.62
	Lcu.2RBY.Chr2.88737442	2	88737442	2.41E-06	0.07	0.59
	Lcu.2RBY.Chr2.96273445	2	96273445	2.66E-06	0.12	0.59
	Lcu.2RBY.Chr3.33827185	3	33827185	6.83E-07	0.15	0.59
	Lcu.2RBY.Chr3.34117023	3	34117023	3.93E-08	0.14	0.61
	Lcu.2RBY.Chr3.35384298	3	35384298	5.39E-06	0.14	0.58
Polyhouse	Lcu.2RBY.Chr3.174824501	3	1.75E+08	4.58E-06	0.12	0.58
	Lcu.2RBY.Chr4.13724159	4	13724159	3.55E-06	0.08	0.58
	Lcu.2RBY.Chr4.442702129	4	4.43E+08	8.53E-07	0.11	0.59
	Lcu.2RBY.Chr4.442702133	4	4.43E+08	9.07E-07	0.11	0.59
	Lcu.2RBY.Chr5.33721990	5	33721990	1.49E-07	0.21	0.60
	Lcu.2RBY.Chr6.374326758	6	3.74E+08	6.89E-07	0.11	0.59

[#]physical position, [$]explained phenotypic variance per marker

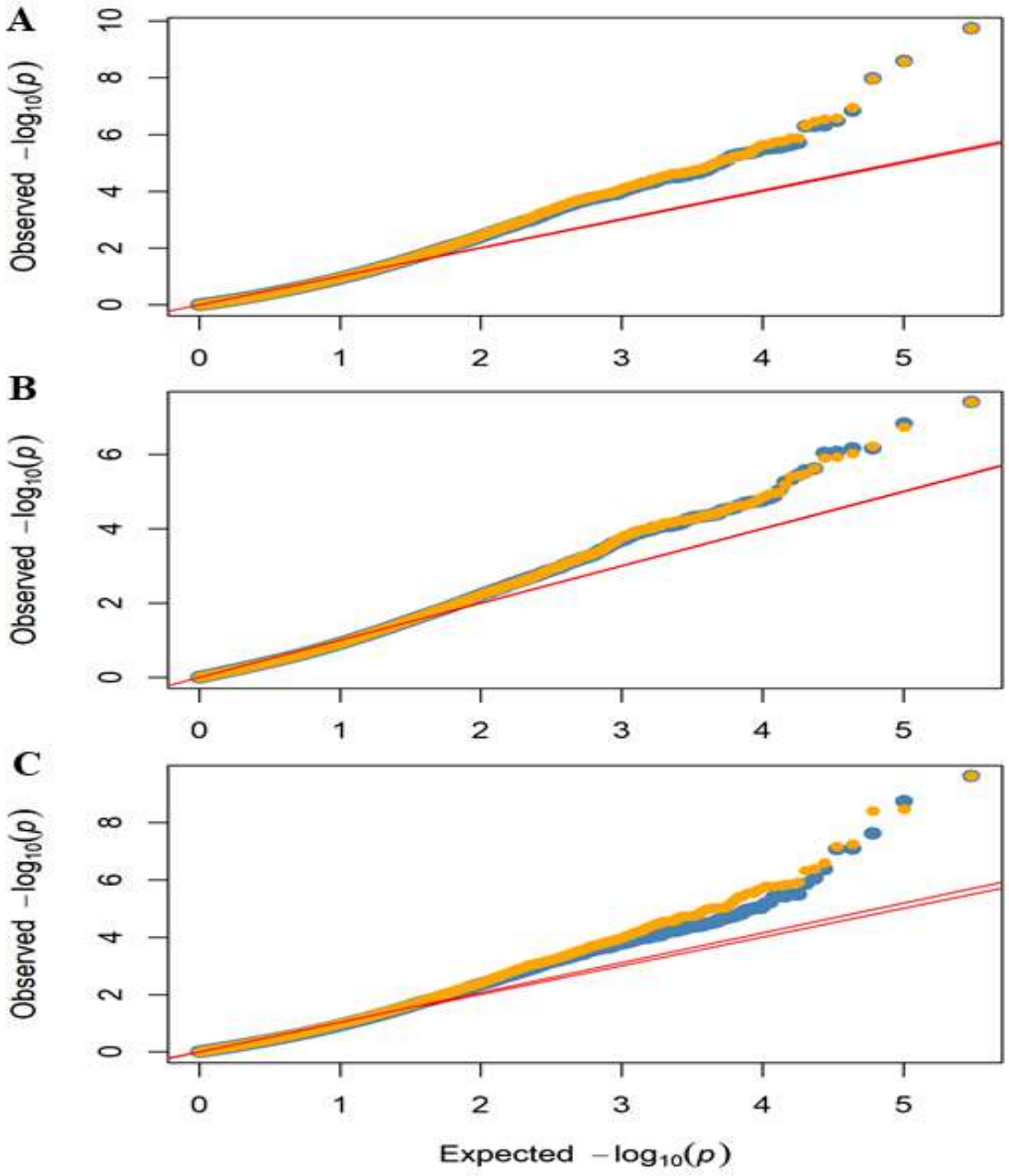
Appendix H. Potential **c**andidate resistance genes associated with anthracnose race 1 resistance in the interval of the QTL detected in RL-01 RIL populations and GWAS regions according to gene annotation of lentil reference genome (v2.0).

Chr[#]	Start (bp)	End (bp)	Gene ID	Annotation
Chr3	30099204	30101106	Lcu.2RBY.3g005210	Thylakoid lumenal 19 kDa protein
Chr3	30175439	30179402	Lcu.2RBY.3g005220	Makorin RING finger protein
Chr3	30217795	30227923	Lcu.2RBY.3g005240	Makorin RING-zinc-finger protein
Chr3	30300254	30301749	Lcu.2RBY.3g005250	Anthocyanin 5-aromatic acyltransferase
Chr3	30457815	30458393	Lcu.2RBY.3g005280	Transmembrane protein
Chr3	30481674	30482215	Lcu.2RBY.3g005290	Transmembrane protein
Chr3	30491797	30492000	Lcu.2RBY.3g005300	Transmembrane protein
Chr3	30641401	30646173	Lcu.2RBY.3g005310	NB-ARC domain disease resistance protein
Chr3	30704762	30705187	Lcu.2RBY.3g005330	Transmembrane protein
Chr3	30735452	30735939	Lcu.2RBY.3g005340	Tubby C 2 protein
Chr3	30735946	30736590	Lcu.2RBY.3g005350	Wall-associated receptor kinase protein
Chr3	30738028	30738585	Lcu.2RBY.3g005360	Transmembrane protein
Chr3	30832778	30837304	Lcu.2RBY.3g005390	Transmembrane protein
Chr3	31031941	31039406	Lcu.2RBY.3g005400	Transmembrane protein
Chr3	31113487	31114027	Lcu.2RBY.3g005460	Transmembrane protein
Chr3	31118273	31118672	Lcu.2RBY.3g005480	Transmembrane protein
Chr3	31195851	31196232	Lcu.2RBY.3g005530	Transmembrane protein
Chr3	31698296	31700380	Lcu.2RBY.3g005650	Anthranilate phosphoribosyltransferase
Chr3	31731103	31731724	Lcu.2RBY.3g005660	ORF1
Chr3	32321603	32324284	Lcu.2RBY.3g005730	Myb transcription factor
Chr3	32693429	32694293	Lcu.2RBY.3g005760	Ulp1 protease family, carboxy-terminal
Chr3	32824221	32824963	Lcu.2RBY.3g005770	Ulp1 protease family, carboxy-terminal
Chr3	32892575	32892931	Lcu.2RBY.3g005790	Subtilisin-like serine protease
Chr3	32993043	32999825	Lcu.2RBY.3g005810	Multidrug and toxic compound extrusion
Chr3	33110296	33115291	Lcu.2RBY.3g005840	ARM repeat CCCH-type zinc finger protein
Chr3	33694640	33695284	Lcu.2RBY.3g005860	Anthranilate N-benzoyltransferase
Chr3	33819256	33828131	Lcu.2RBY.3g005880	Cellulose synthase

Chr3	33840742	33843043	Lcu.2RBY.3g005900	F-box SKIP23-like protein
Chr3	34117126	34118497	Lcu.2RBY.3g005910	Anthranilate N-benzoyltransferase
Chr3	34413587	34439978	Lcu.2RBY.3g005930	Ulp1 protease family, carboxy-terminal
Chr3	34608235	34609591	Lcu.2RBY.3g005950	Anthranilate N-benzoyltransferase
Chr3	34618281	34620298	Lcu.2RBY.3g005960	Myb/SANT-like DNA-binding domain
Chr3	34650454	34651468	Lcu.2RBY.3g005970	Ulp1 protease family, carboxy-terminal
Chr3	34722504	34723667	Lcu.2RBY.3g005990	F-box SKIP23-like protein
Chr3	34936710	34937891	Lcu.2RBY.3g006020	F-box SKIP23-like protein
Chr3	35204566	35208726	Lcu.2RBY.3g006030	Polygalacturonase
Chr3	35383081	35387584	Lcu.2RBY.3g006090	TIR-NBS-LRR domain disease resistance
Chr3	35415534	35417750	Lcu.2RBY.3g006110	Zinc finger/RING finger family protein
Chr3	35783673	35784401	Lcu.2RBY.3g006240	MADS-box transcription factor
Chr3	35786719	35787457	Lcu.2RBY.3g006260	Zinc finger, C3HC4 type /RING finger
Chr3	35891715	35892401	Lcu.2RBY.3g006280	Zinc finger, C3HC4 type /RING finger
Chr3	35897123	35904908	Lcu.2RBY.3g006300	DNA topoisomerase II
Chr3	35972988	35973704	Lcu.2RBY.3g006330	LRR & NB-ARC domain disease resistance
Chr3	35974000	35976197	Lcu.2RBY.3g006340	LRR & NB-ARC domain disease resistance
Chr3	35976249	35981698	Lcu.2RBY.3g006350	LRR & NB-ARC domain disease resistance
Chr3	35981709	35982218	Lcu.2RBY.3g006360	NB-ARC domain disease resistance protein
Chr3	35982494	35982865	Lcu.2RBY.3g006370	CC-NBS-LRR resistance protein, putative
Chr3	36028314	36032907	Lcu.2RBY.3g006380	LRR & NB-ARC domain disease resistance
Chr3	36037105	36044777	Lcu.2RBY.3g006390	LRR & NB-ARC domain disease resistance
Chr3	36891988	36892982	Lcu.2RBY.3g006490	Transmembrane protein
Chr3	36920159	36920804	Lcu.2RBY.3g006500	Transmembrane protein
Chr3	37677606	37684302	Lcu.2RBY.3g006580	PPR containing plant-like protein
Chr3	38288475	38295878	Lcu.2RBY.3g006660	LRR & NB-ARC domain disease resistance
Chr3	38538802	38539297	Lcu.2RBY.3g006720	Transmembrane protein
Chr3	38755826	38764156	Lcu.2RBY.3g006750	LRR & NB-ARC domain disease resistance
unitig0289	1917198	1921007	Lcu.2RBY.L001220	LRR & NB-ARC domain disease resistance
unitig0289	1939874	1942161	Lcu.2RBY.L001240	NB-ARC domain disease resistance protein

[#] Chromosome

Appendix I. Quantile-quantile (Q-Q) plots comparing the distribution of observed verses expected p-values for genome-wide association study of 200 lentil accessions evaluated for anthracnose race 1 severity using mixed linear model (MLM) analysis in the: A) growth chamber and B) polyhouse, and C) the combined lsmean from both environments. Orange dots represent the MLM approach using populations structure (K=3) and kinship matrices, and the blue dots represent the model for principal component (PC=3) and kinship.



Appendix J. Mean anthracnose race 0 and race 1 severity of the LR-26 (Eston × IG 72815) interspecific RIL population evaluated under growth chamber and polyhouse conditions. Disease severity was rated on a 0-10 scale, where the disease severity score increased in 10% increments.

Genotype	[G]race 0	[£]std	[P]race 0	[£]std	[G]race 1	[£]std
LR 26-10	95.0	0.0	45.0	8.2	95.0	0.0
LR 26-105	95.0	0.0	85.0	20.0	95.0	0.0
LR 26-107	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-108	95.0	0.0	82.5	15.0	95.0	0.0
LR 26-110	90.0	12.2	82.5	15.0	95.0	0.0
LR 26-111	19.0	8.4	20.0	5.8	17.5	9.6
LR 26-112	27.0	4.2	37.5	18.9	17.5	5.0
LR 26-113	85.0	23.1	25.0	0.0	25.0	0.0
LR 26-115	90.0	12.2	57.5	15.0	80.0	19.1
LR 26-116	95.0	0.0	82.5	15.0	95.0	0.0
LR 26-117	90.0	15.8	30.0	5.8	95.0	0.0
LR 26-118	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-12	90.0	12.2	70.0	19.1	95.0	0.0
LR 26-121	95.0	0.0	55.0	29.4	95.0	0.0
LR 26-122	91.0	8.0	60.0	10.0	95.0	0.0
LR 26-123	91.7	8.2	75.0	16.3	95.0	0.0
LR 26-125	90.0	12.2	82.5	15.0	95.0	0.0
LR 26-127	90.0	12.2	80.0	17.3	95.0	0.0
LR 26-128	58.0	30.6	25.0	0.0	15.0	8.2
LR 26-129	66.0	20.2	27.5	5.0	17.5	9.6
LR 26-13	95.0	0.0	80.0	17.3	95.0	0.0
LR 26-132	95.0	0.0	85.0	11.5	95.0	0.0
LR 26-134	91.7	8.2	80.0	19.1	87.5	15.0
LR 26-135	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-136	91.7	8.2	95.0	0.0	95.0	0.0
LR 26-138	34.0	11.0	25.0	8.2	25.0	27.1

LR 26-139	68.3	13.7	72.5	17.1	73.8	24.2
LR 26-140	83.3	18.3	70.0	17.3	90.0	10.0
LR 26-142	41.0	19.6	45.0	33.7	15.0	8.2
LR 26-145	95.0	0.0	55.0	27.1	95.0	0.0
LR 26-149	76.7	20.4	60.0	23.8	95.0	0.0
LR 26-151	80.0	23.5	77.5	12.6	82.5	15.0
LR 26-152	95.0	0.0	87.5	15.0	95.0	0.0
LR 26-156	95.0	0.0	60.0	10.0	95.0	0.0
LR 26-157	82.0	23.6	27.5	5.0	72.5	28.7
LR 26-16	95.0	0.0	87.5	15.0	95.0	0.0
LR 26-161	95.0	0.0	82.5	15.0	95.0	0.0
LR 26-162	50.0	15.8	47.5	40.3	22.5	5.0
LR 26-163	95.0	0.0	57.5	15.0	95.0	0.0
LR 26-164	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-165	95.0	0.0	87.5	15.0	95.0	0.0
LR 26-169	65.0	25.3	30.0	5.8	25.0	0.0
LR 26-17	90.0	12.2	95.0	0.0	95.0	0.0
LR 26-170	95.0	0.0	77.5	20.6	85.0	17.3
LR 26-171	91.7	8.2	75.0	14.1	95.0	0.0
LR 26-172	88.3	16.3	52.5	34.0	86.1	18.3
LR 26-173	90.0	12.2	45.0	14.1	90.0	10.0
LR 26-175	56.0	21.3	60.0	5.8	45.0	8.2
LR 26-18	67.0	26.6	30.0	5.8	72.5	17.1
LR 26-180	35.0	10.5	47.5	20.6	25.0	0.0
LR 26-181	91.7	8.2	80.0	17.3	95.0	0.0
LR 26-182	90.0	15.8	90.0	10.0	87.5	15.0
LR 26-183	81.7	15.1	90.0	10.0	87.5	15.0
LR 26-184	95.0	0.0	95.0	0.0	87.5	15.0
LR 26-186	95.0	0.0	77.5	20.6	95.0	0.0
LR 26-187	88.3	16.3	95.0	0.0	95.0	0.0
LR 26-188	80.0	16.4	50.3	19.6	95.0	0.0

LR 26-19	85.0	17.6	82.5	15.0	90.0	10.0
LR 26-193	15.0	8.9	25.0	8.2	17.5	9.6
LR 26-194	25.0	4.7	30.0	10.0	10.0	10.0
LR 26-196	95.0	0.0	77.5	12.6	95.0	0.0
LR 26-198	91.7	8.2	95.0	0.0	95.0	0.0
LR 26-20	8.3	5.2	25.0	0.0	5.0	0.0
LR 26-200	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-202	95.0	0.0	85.0	20.0	95.0	0.0
LR 26-203	87.0	17.5	65.0	24.5	95.0	0.0
LR 26-204	95.0	0.0	70.0	33.2	95.0	0.0
LR 26-205	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-206	80.0	24.6	80.0	17.3	95.0	0.0
LR 26-209	69.0	19.6	22.5	5.0	38.3	27.4
LR 26-210	90.0	15.8	53.0	13.1	70.0	30.0
LR 26-215	91.7	8.2	80.0	17.3	87.5	15.0
LR 26-216	91.7	8.2	95.0	0.0	95.0	0.0
LR 26-219	90.0	12.2	95.0	0.0	95.0	0.0
LR 26-22	62.0	23.1	25.0	8.2	57.2	14.8
LR 26-220	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-223	95.0	0.0	77.5	20.6	95.0	0.0
LR 26-224	40.0	10.8	30.0	10.0	27.5	15.8
LR 26-227	91.7	8.2	57.5	5.0	95.0	0.0
LR 26-228	95.0	0.0	85.0	20.0	95.0	0.0
LR 26-23	95.0	0.0	82.5	15.0	87.5	15.0
LR 26-232	86.0	19.1	66.5	24.6	95.0	0.0
LR 26-233	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-235	85.0	16.3	67.5	18.9	87.5	15.0
LR 26-238	90.0	12.2	75.0	24.5	90.0	10.0
LR 26-239	95.0	0.0	60.0	26.5	95.0	0.0
LR 26-240	8.3	5.2	27.5	5.0	7.5	5.0
LR 26-241	95.0	0.0	95.0	0.0	95.0	0.0

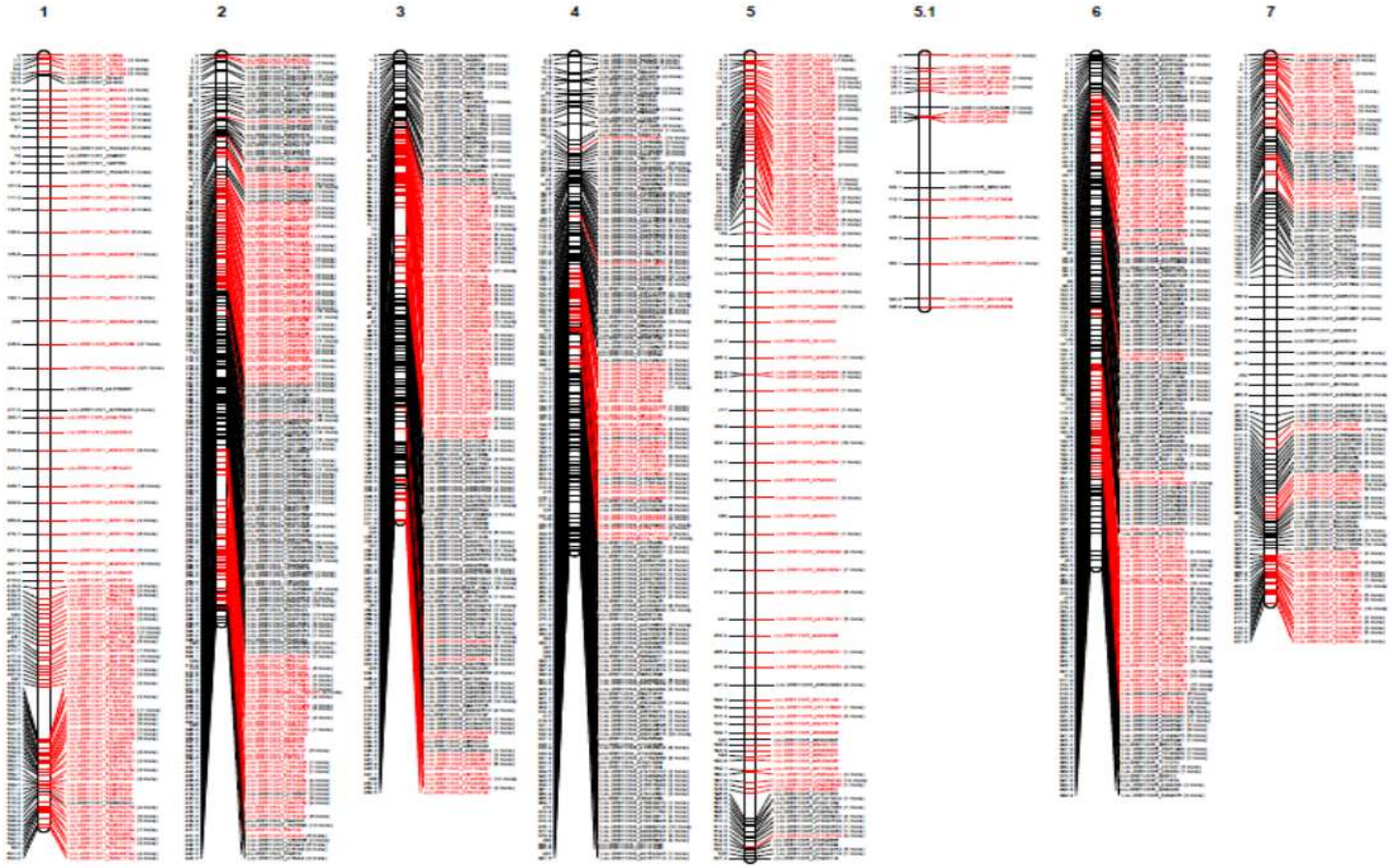
LR 26-243	95.0	0.0	85.0	20.0	95.0	0.0
LR 26-244	76.0	27.7	60.0	30.0	80.0	30.0
LR 26-245	95.0	0.0	95.0	0.0	90.0	10.0
LR 26-246	85.0	15.5	75.0	14.1	90.0	10.0
LR 26-247	61.0	22.7	60.0	10.0	22.5	5.0
LR 26-251	78.3	18.6	67.5	20.6	90.0	10.0
LR 26-252	90.0	12.2	85.0	11.5	80.0	17.3
LR 26-253	90.0	15.8	70.0	17.3	95.0	0.0
LR 26-254	95.0	0.0	35.0	14.1	70.0	37.9
LR 26-256	91.7	8.2	55.0	17.3	87.5	15.0
LR 26-257	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-259	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-261	49.0	12.6	15.0	8.2	26.1	7.8
LR 26-262	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-266	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-267	88.3	16.3	80.0	17.3	85.0	20.0
LR 26-269	90.0	12.2	67.5	5.0	95.0	0.0
LR 26-273	91.7	8.2	77.5	20.6	90.0	10.0
LR 26-274	75.0	23.6	70.0	17.3	82.5	25.0
LR 26-275	76.0	22.8	90.0	10.0	46.1	46.5
LR 26-276	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-280	95.0	0.0	57.5	9.6	95.0	0.0
LR 26-281	95.0	0.0	72.5	5.0	95.0	0.0
LR 26-282	95.0	0.0	50.0	17.3	87.5	15.0
LR 26-283	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-288	95.0	0.0	95.0	0.0	77.5	35.0
LR 26-29	18.3	11.5	22.5	5.0	16.4	9.0
LR 26-290	90.0	12.2	95.0	0.0	95.0	0.0
LR 26-292	90.0	12.2	85.0	11.5	86.1	18.3
LR 26-293	83.0	23.0	72.5	33.0	90.0	10.0
LR 26-294	70.0	34.7	82.5	15.0	72.5	26.3

LR 26-296	95.0	0.0	80.0	10.0	95.0	0.0
LR 26-297	91.7	8.2	90.0	10.0	95.0	0.0
LR 26-298	95.0	0.0	77.5	23.6	95.0	0.0
LR 26-299	70.0	25.9	40.0	23.8	95.0	0.0
LR 26-3	21.7	9.4	22.5	5.0	10.0	10.0
LR 26-30	91.7	8.2	65.0	0.0	87.5	15.0
LR 26-300	59.0	38.6	60.5	11.4	50.6	42.8
LR 26-301	69.4	29.1	60.0	26.5	87.5	15.0
LR 26-303	88.0	22.1	55.0	18.3	95.0	0.0
LR 26-304	28.0	8.2	25.0	8.2	17.5	5.0
LR 26-306	90.0	12.2	95.0	0.0	95.0	0.0
LR 26-307	80.0	25.1	60.0	31.1	95.0	0.0
LR 26-311	88.3	10.3	82.5	15.0	95.0	0.0
LR 26-312	27.2	4.2	38.8	11.1	25.0	0.0
LR 26-32	95.0	0.0	62.5	23.6	95.0	0.0
LR 26-36	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-4	95.0	0.0	75.0	24.5	95.0	0.0
LR 26-41	95.0	0.0	80.0	17.3	95.0	0.0
LR 26-43	95.0	0.0	90.0	10.0	82.5	15.0
LR 26-45	95.0	0.0	82.5	15.0	80.6	31.3
LR 26-47	95.0	0.0	72.5	26.3	90.0	10.0
LR 26-49	83.0	25.3	61.8	24.5	83.9	26.7
LR 26-5	80.0	17.6	57.5	15.0	67.5	18.9
LR 26-54	95.0	0.0	37.5	9.6	80.0	17.3
LR 26-55	88.3	16.3	48.3	10.4	95.0	0.0
LR 26-56	15.0	11.0	37.5	9.6	12.5	9.6
LR 26-57	27.0	7.9	17.5	9.6	15.0	11.5
LR 26-62	29.0	5.2	12.5	9.6	12.5	5.0
LR 26-63	78.3	13.7	82.5	15.0	95.0	0.0
LR 26-64	13.3	7.5	32.5	9.6	7.5	5.0
LR 26-65	95.0	0.0	45.0	14.1	95.0	0.0

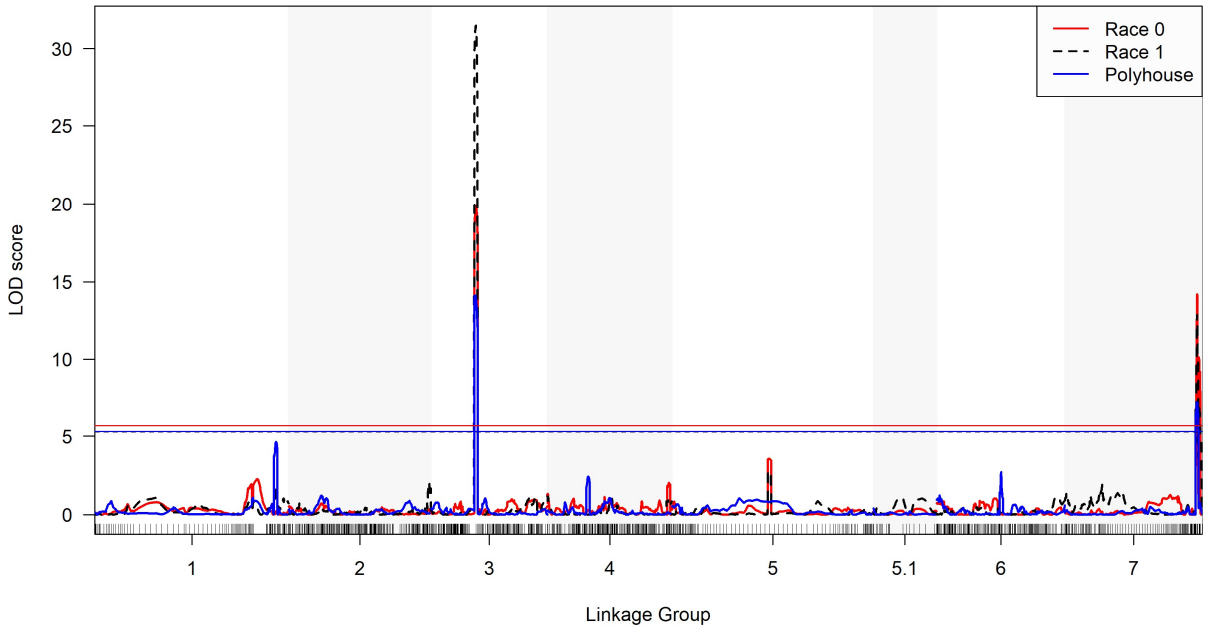
LR 26-66	34.0	12.9	25.0	8.2	10.0	10.0
LR 26-67	95.0	0.0	87.5	15.0	87.5	15.0
LR 26-7	95.0	0.0	68.3	20.5	95.0	0.0
LR 26-70	24.0	15.2	17.5	9.6	7.5	5.0
LR 26-75	39.0	12.6	20.0	12.9	15.0	8.2
LR 26-77	88.3	10.3	80.0	17.3	95.0	0.0
LR 26-78	34.0	11.0	25.0	0.0	15.0	8.2
LR 26-79	95.0	0.0	90.0	10.0	95.0	0.0
LR 26-83	95.0	0.0	67.5	20.6	95.0	0.0
LR 26-84	95.0	0.0	87.5	15.0	95.0	0.0
LR 26-85	60.5	20.1	32.5	5.0	27.5	5.0
LR 26-86	95.0	0.0	77.5	20.6	95.0	0.0
LR 26-87	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-90	78.3	18.6	47.5	17.1	87.5	15.0
LR 26-91	77.0	23.5	22.5	5.0	20.6	10.1
LR 26-95	95.0	0.0	95.0	0.0	95.0	0.0
LR 26-98	88.0	16.4	47.5	23.6	87.5	15.0
LR 26-99	90.0	12.2	72.5	17.1	87.5	15.0

race 0 - average disease severity percentage score for race 0 at growth chamber (^{G}race 0) and polyhouse (^{P}race 0), and for race 1 at growth chamber (^{G}race 1)-; $^{\text{£}}$std - standard deviations

Appendix K. Linkage map of lentil interspecific RIL population LR-26 derived from *Lens culinaris* cv. Eston × *Lens ervoides* IG 72815. SNP markers with significant segregation distortion at a threshold α = 0.00357 are highlighted in red. Significance was declared at α = 0.05 with a Bonferroni correction for genome wide error (0.05/14 = 0.00357), considering at least 14 independent genomic regions (seven pair of chromosomes) in lentil.



Appendix L. Whole genome view of LOD profile and location of anthracnose resistance QTL in the LR-26 population detected in the growth chamber with race 0 (red) and race 1 (gray), and in the polyhouse (blue) based on a CIM model run in R/qtl. The X axis represents a linkage map of the seven chromosomes, and the Y axis is LOD scores; the horizontal line represents LOD threshold obtained with 1000 permutation tests (P = 0.05). Data were log10 transformed for normalization prior to analyses.



Appendix M. Trait characteristic of lentil advanced backcross (LABC-01) observed during population advancement and genomic compositions of individual lines of LABC-01 based on 829 SNP markers.

Here the abbreviated table title represents**:** FC- Flower color; SCGC – seed coat ground color; SCP – seed coat pattern; DFL – days to 50% flowering; ANT – average anthracnose race 0 severity in percentage; RP – recurrent parent; DP – Donor parent; Ht – heterozygous; BSS – Best segment size (Mb); # of DS – number of donor segments across the genome.

RILs	FC	SCGC	SCP	DFL	ANT	RP	DP	Ht	BSS (Mb)	# of DS
LABC-01-1	White	gray	absent	43	85.0	85.5	12.5	2.0	1.0	9
LABC-01-2	White	gray	absent	40	75.0	81.6	10.4	8.0	9.2	20
LABC-01-3	Purple	tan	marbled	33	85.0	81.1	17.1	1.8	1.2	18
LABC-01-4	White	gray	absent	40	78.3	77.5	13.0	9.5	0.7	15
LABC-01-5	White	gray	absent	37	65.0	84.0	12.4	3.6	0.6	16
LABC-01-6	White	gray	absent	40	76.0	78.7	16.2	5.1	2.2	15
LABC-01-7	White	gray	absent	37	40.0	66.6	17.4	16.0	14.4	24
LABC-01-8	White	gray	absent	37	73.0	85.8	7.4	6.9	50.4	4
LABC-01-9	White	gray	absent	35	71.7	85.0	8.2	6.8	12.9	11
LABC-01-10	White	gray	absent	37	71.0	83.7	15.8	0.5	4.4	11
LABC-01-11	White	gray	absent	40	72.0	91.4	3.6	5.0	3.0	27
LABC-01-12	White	tan	absent	37	60.0	82.3	14.6	3.1	5.7	8
LABC-01-37	White	gray	absent	37	80.0	84.0	10.5	5.5	3.3	9
LABC-01-38	Purple	gray	marbled	.	52.1	78.1	20.2	1.6	.	18
LABC-01-39	White	gray	absent	37	38.3	76.0	21.7	2.3	22.3	18
LABC-01-40	White	gray	absent	37	61.0	74.8	21.0	4.3	41.4	14
LABC-01-29	White	gray	absent	40	72.0	86.2	13.8	0.0	15.6	8
LABC-01-30	White	tan	absent	37	61.0	77.0	13.0	10.0	13.4	36
LABC-01-31	White	gray	absent	37	71.0	68.5	27.3	4.2	7.9	41
LABC-01-32	White	gray	absent	40	63.0	82.2	15.8	2.0	.	10
LABC-01-33	Purple	gray	marbled	48	67.0	65.5	21.2	13.3	.	57

LABC-01-34	White	tan	absent	37	68.0	67.4	21.8	10.8	7.1	33
LABC-01-35	White	gray	absent	37	86.0	79.2	17.1	3.7	2.7	22
LABC-01-36	White	gray	absent	37	79.0	86.5	7.7	5.7	0.1	4
LABC-01-49	Purple	gray	absent	35	35.0	76.5	20.7	2.8	6.4	22
LABC-01-50	White	tan	absent	37	58.8	88.4	9.4	2.2	2.3	27
LABC-01-51	Purple	gray	marbled	37	87.0	72.7	18.8	8.5	88.7	38
LABC-01-52	White	gray	absent	40	57.0	79.6	13.6	6.7	16.5	15
LABC-01-53	White	gray	absent	43	75.0	86.3	7.0	6.7	.	3
LABC-01-54	White	gray	absent	37	75.0	82.1	7.3	10.6	13.6	17
LABC-01-55	White	gray	absent	43	79.0	83.8	8.7	7.5	29.7	4
LABC-01-56	White	gray	absent	37	42.1	78.8	19.3	1.9	26.4	16
LABC-01-57	White	gray	absent	.	25.0	82.8	9.1	8.0	1.4	14
LABC-01-58	White	gray	absent	37	81.0	86.8	12.6	0.6	.	5
LABC-01-59	White	gray	absent	40	57.2	77.1	8.3	14.7	7.7	17
LABC-01-60	Purple	gray	marbled	33	79.0	71.0	12.1	16.9	.	20
LABC-01-85	Purple	gray	marbled	37	83.0	86.7	10.2	3.1	31.5	10
LABC-01-86	White	gray	absent	43	54.0	73.6	14.7	11.7	2.3	26
LABC-01-87	White	tan	absent	37	72.0	78.0	9.5	12.6	16.6	20
LABC-01-88	White	gray	absent	37	46.0	76.6	19.8	3.6	15.3	23
LABC-01-77	White	gray	absent	33	83.9	81.3	16.6	2.1	11.2	19
LABC-01-78	White	gray	absent	.	77.0	86.0	13.2	0.8	5.6	7
LABC-01-79	White	gray	absent	40	72.0	80.0	18.1	1.9	14.6	17
LABC-01-80	White	gray	absent	37	87.0	87.6	8.5	3.8	7.2	10
LABC-01-81	White	gray	absent	37	30.0	79.9	7.5	12.6	1.9	7
LABC-01-82	White	gray	absent	50	73.0	85.3	7.3	7.4	16.7	5
LABC-01-83	White	gray	absent	40	71.0	85.8	13.1	1.1	.	3
LABC-01-84	White	gray	absent	40	60.0	90.6	3.0	6.4	.	11
LABC-01-97	White	gray	absent	48	49.0	76.6	18.4	5.0	16.6	22
LABC-01-98	Purple	gray	marbled	37	85.0	81.9	14.1	4.0	0.4	24
LABC-01-99	White	gray	absent	40	65.0	85.1	3.0	11.8	65.4	7
LABC-01-100	White	tan	absent	37	91.0	54.2	10.9	34.8	20.7	37

LABC-01-101	White	gray	absent	40	75.0	84.0	13.4	2.6	.	8
LABC-01-102	Purple	gray	marbled	37	91.7	87.0	5.2	7.8	0.2	9
LABC-01-103	White	gray	absent	40	35.0	75.2	11.8	13.0	15.3	16
LABC-01-104	White	gray	absent	48	42.0	70.4	21.7	7.9	2.8	29
LABC-01-105	Purple	gray	marbled	35	66.4	79.3	17.4	3.2	96.1	8
LABC-01-106	White	gray	absent	37	57.0	85.9	3.8	10.2	9.1	5
LABC-01-107	White	gray	absent	37	85.0	84.9	13.3	1.8	11.4	6
LABC-01-108	White	gray	absent	43	73.0	84.4	14.4	1.1	19.5	6
LABC-01-133	White	gray	absent	48	69.0	83.0	14.9	2.1	48.3	17
LABC-01-134	White	gray	absent	.	82.0	83.7	12.5	3.8	1.3	7
LABC-01-135	White	gray	absent	43	33.0	68.9	8.7	22.5	23.1	15
LABC-01-136	White	gray	absent	43	64.0	83.4	13.2	3.4	31.6	7
LABC-01-125	White	gray	absent	51	81.0	69.4	26.4	4.2	19.9	48
LABC-01-126	White	gray	absent	40	76.0	85.3	8.2	6.5	14.4	6
LABC-01-127	White	gray	absent	35	75.0	82.9	9.8	7.2	5.3	9
LABC-01-128	Purple	gray	marbled	37	61.0	72.6	15.4	12.0	24.1	21
LABC-01-129	White	gray	absent	37	80.0	85.4	12.1	2.5	.	5
LABC-01-130	White	gray	absent	43	77.0	73.9	1.4	24.6	41.0	2
LABC-01-131	White	gray	absent	43	69.0	67.8	29.2	3.0	51.5	22
LABC-01-132	White	gray	absent	37	78.0	87.1	12.9	0.1	17.7	7
LABC-01-145	Purple	tan	marbled	40	95.0	70.8	19.4	9.7	90.5	29
LABC-01-146	White	tan	absent	.	70.0	67.7	25.6	6.7	15.3	51
LABC-01-147	Purple	tan	marbled	33	95.0	72.7	14.0	13.3	5.7	37
LABC-01-148	White	gray	absent	33	57.0	78.0	18.2	3.9	2.6	23
LABC-01-149	White	gray	absent	40	55.0	73.1	22.0	4.8	22.5	26
LABC-01-150	White	gray	absent	37	65.0	86.3	7.9	5.9	1.0	7
LABC-01-151	White	gray	absent	37	43.9	87.2	8.8	4.0	14.6	12
LABC-01-152	White	gray	absent	35	73.9	77.0	14.8	8.2	0.2	21
LABC-01-153	Purple	gray	absent	37	82.0	86.1	13.5	0.4	.	4
LABC-01-154	White	gray	absent	37	64.0	82.2	10.6	7.2	3.2	13
LABC-01-155	Purple	gray	marbled	35	25.0	50.2	33.7	16.1	23.6	38

LABC-01-156	Purple	gray	marbled	33	84.0	83.2	16.5	0.3	44.8	16
LABC-01-181	White	gray	absent	37	56.0	84.5	11.5	4.1	0.8	22
LABC-01-182	Purple	gray	marbled	33	89.0	93.2	5.4	1.4	1.7	10
LABC-01-183	White	tan	absent	40	85.0	64.6	22.2	13.2	17.6	42
LABC-01-185	White	gray	absent	40	60.0	90.5	9.4	0.1	.	7
LABC-01-173	White	tan	absent	40	79.0	84.0	9.5	6.5	1.4	17
LABC-01-174	White	gray	absent	37	78.0	74.6	19.8	5.6	0.8	18
LABC-01-175	White	gray	absent	40	72.0	78.8	18.0	3.2	19.5	26
LABC-01-176	White	tan	absent	37	91.0	77.5	16.1	6.5	72.3	23
LABC-01-177	White	gray	absent	33	75.0	79.5	12.4	8.2	29.7	16
LABC-01-178	Purple	gray	marbled	35	75.0	78.4	6.1	15.5	18.5	11
LABC-01-179	White	gray	absent	37	50.0	77.1	14.6	8.3	35.8	22
LABC-01-180	White	gray	absent	40	73.0	87.6	10.4	2.0	9.5	9
LABC-01-13	White	gray	absent	35	78.0	56.1	33.6	10.3	50.0	38
LABC-01-14	White	gray	absent	37	81.0	83.3	13.1	3.6	1.9	8
LABC-01-184	White	gray	absent	37	70.0	83.8	11.0	5.2	33.0	21
LABC-01-16	Purple	gray	absent	43	61.0	89.9	8.3	1.8	.	5
LABC-01-17	Purple	gray	marbled	37	71.0	77.3	8.6	14.1	100.7	15
LABC-01-18	Purple	gray	marbled	33	91.0	76.1	17.2	6.7	4.3	13
LABC-01-19	White	gray	absent	43	63.0	90.2	7.8	2.0	.	4
LABC-01-20	White	gray	absent	40	65.0	59.1	24.1	16.8	35.4	33
LABC-01-21	White	gray	absent	43	79.0	60.0	27.5	12.5	81.1	29
LABC-01-22	White	gray	absent	37	71.0	80.5	11.9	7.6	45.4	9
LABC-01-23	White	gray	absent	37	83.0	84.7	14.5	0.8	13.6	9
LABC-01-24	White	gray	absent	37	54.0	70.6	17.3	12.1	13.4	22
LABC-01-25	White	gray	absent	37	74.0	83.9	7.5	8.6	45.3	5
LABC-01-26	White	gray	absent	40	67.0	87.6	10.8	1.6	.	3
LABC-01-27	White	gray	absent	40	68.0	90.9	7.1	2.0	.	4
LABC-01-28	White	gray	absent	48	80.0	86.3	12.3	1.5	8.1	5
LABC-01-41	Purple	gray	marbled	35	63.0	72.0	9.3	18.7	9.5	27
LABC-01-42	White	gray	absent	37	79.0	91.2	2.2	6.6	2.9	7

LABC-01-43	White	gray	absent	33	81.0	71.5	20.3	8.2	9.0	40
LABC-01-44	White	tan	absent	33	82.0	67.3	21.0	11.7	15.7	42
LABC-01-45	White	gray	absent	35	85.0	80.1	19.8	0.1	25.0	14
LABC-01-46	White	tan	absent	37	74.0	85.2	7.2	7.6	31.4	6
LABC-01-47	White	tan	absent	37	25.0	40.5	33.9	25.6	8.9	40
LABC-01-48	White	gray	absent	40	76.0	82.1	15.0	3.0	11.7	13
LABC-01-61	Purple	gray	absent	40	70.0	80.8	5.6	13.6	35.5	21
LABC-01-62	White	tan	absent	37	88.0	68.0	15.7	16.3	11.6	37
LABC-01-63	White	gray	absent	43	83.0	75.3	14.2	10.6	0.2	37
LABC-01-64	White	gray	absent	40	34.0	78.8	13.4	7.7	18.3	16
LABC-01-65	White	gray	absent	37	72.0	70.5	21.5	8.0	10.0	22
LABC-01-66	White	gray	absent	37	28.3	71.2	17.4	11.4	19.0	29
LABC-01-67	White	gray-b	absent	37	17.0	77.6	19.2	3.3	49.5	23
LABC-01-68	White	tan	absent	37	63.0	84.6	14.6	0.8	.	17
LABC-01-69	White	gray	absent	48	85.0	83.9	13.0	3.1	45.9	11
LABC-01-70	White	gray	absent	35	80.0	85.9	11.5	2.6	.	7
LABC-01-71	White	gray	absent	48	54.0	70.6	9.6	19.8	35.7	5
LABC-01-72	White	gray	absent	40	63.0	77.9	16.0	6.1	79.1	15
LABC-01-73	White	gray	absent	40	73.0	80.4	7.8	11.8	15.9	8
LABC-01-74	White	gray	absent	37	80.0	90.8	8.6	0.5	38.9	9
LABC-01-75	White	gray	absent	35	66.1	83.8	6.7	9.5	5.5	10
LABC-01-76	White	gray	absent	37	60.0	83.6	14.8	1.7	6.8	8
LABC-01-89	White	gray	absent	37	45.0	76.5	9.7	13.8	8.4	18
LABC-01-90	White	gray	absent	35	62.0	88.9	5.5	5.7	40.1	6
LABC-01-91	White	gray	absent	37	84.0	79.4	13.9	6.7	31.8	15
LABC-01-92	White	gray	absent	.	69.4	73.3	23.1	3.6	8.0	20
LABC-01-93	White	gray	absent	40	63.0	81.7	10.2	8.0	20.2	16
LABC-01-94	White	tan	absent	37	52.8	72.0	11.9	16.1	11.5	33
LABC-01-95	White	tan	absent	40	77.0	76.8	12.5	10.7	17.5	35
LABC-01-96	Purple	gray	marbled	.	63.0	80.3	17.3	2.3	11.4	18
LABC-01-109	White	gray	absent	35	80.0	84.8	8.1	7.1	1.4	3

LABC-01-110	Purple	gray	marbled	40	89.0	68.2	17.7	14.1	45.6	19
LABC-01-111	White	gray	absent	40	80.0	78.5	19.0	2.5	27.1	9
LABC-01-112	White	gray	absent	37	65.0	74.4	10.7	14.9	26.0	24
LABC-01-113	Purple	gray	marbled	37	71.0	71.5	8.9	19.6	3.6	16
LABC-01-114	White	gray	absent	37	76.0	81.1	11.0	7.9	12.7	15
LABC-01-115	White	gray	absent	40	73.9	78.2	12.7	9.1	8.1	15
LABC-01-116	White	gray	marbled	.	94.0	74.0	16.1	9.9	95.3	27
LABC-01-117	White	gray	absent	.	81.0	84.9	8.0	7.1	12.9	4
LABC-01-118	White	gray	absent	37	88.0	78.2	11.3	10.5	2.0	20
LABC-01-119	White	gray	absent	37	66.0	87.0	12.3	0.8	31.2	4
LABC-01-120	White	gray	absent	40	47.0	71.2	12.1	16.7	37.8	32
LABC-01-121	White	gray	absent	40	80.0	79.0	13.2	7.8	28.3	18
LABC-01-122	White	gray	absent	40	75.0	88.9	4.3	6.8	16.4	13
LABC-01-123	White	tan	absent	40	90.0	73.0	24.5	2.5	37.4	32
LABC-01-124	White	gray	absent	37	73.0	81.7	13.3	5.0	2.5	16
LABC-01-137	White	gray	absent	35	76.0	90.5	8.3	1.2	19.7	6
LABC-01-138	White	gray	absent	40	91.0	81.6	17.2	1.2	72.1	11
LABC-01-139	White	gray	absent	37	85.0	90.4	3.2	6.5	.	3
LABC-01-140	White	gray	absent	43	78.3	78.8	17.7	3.5	13.2	21
LABC-01-141	White	gray	absent	40	80.0	90.8	8.1	1.2	.	2
LABC-01-142	White	gray	absent	33	87.0	77.2	8.7	14.0	.	23
LABC-01-143	White	gray	absent	40	73.0	91.3	7.2	1.5	.	5
LABC-01-144	White	gray	absent	40	66.0	84.3	10.5	5.2	4.1	10
LABC-01-157	Purple	gray	absent	43	66.0	79.1	11.1	9.8	32.7	12
LABC-01-158	White	gray	absent	37	61.7	82.8	13.4	3.8	0.4	19
LABC-01-159	White	gray	absent	33	93.0	80.5	14.3	5.2	3.2	11
LABC-01-160	White	gray	absent	40	83.0	83.6	12.8	3.5	4.5	12
LABC-01-161	White	tan	absent	35	67.0	77.0	15.8	7.2	1.9	24
LABC-01-162	White	gray	absent	40	85.0	83.1	9.0	7.9	16.2	5
LABC-01-163	White	gray	absent	40	58.0	68.5	18.4	13.2	56.7	24
LABC-01-164	Purple	gray	absent	40	56.0	87.1	4.1	8.9	2.4	23

LABC-01-165	White	gray	absent	40	83.9	84.9	7.8	7.3	3.3	13
LABC-01-166	Purple	tan	marbled	35	78.0	56.1	29.6	14.3	35.5	37
LABC-01-167	White	gray	absent	43	84.0	59.4	18.2	22.3	4.6	19
LABC-01-168	White	gray	absent	40	88.0	82.9	8.2	8.8	2.9	8
LABC-01-169	White	gray	absent	40	75.0	83.0	14.3	2.7	.	8
LABC-01-170	Purple	tan	marbled	33	95.0	68.9	18.7	12.4	75.0	23
LABC-01-171	White	gray	absent	37	65.0	92.6	6.6	0.7	184.4	5
LABC-01-172	Purple	gray	absent	37	37.2	88.4	11.6	0.0	8.4	21
LABC-01-189	White	gray	absent	35	35.0	84.4	14.4	1.2	9.3	16
LABC-01-193	White	gray	absent	35	65.0	78.5	14.0	7.6	10.3	14
LABC-01-196	White	gray	absent	43	30.6	83.0	9.4	7.6	2.5	12
LABC-01-200	White	gray	absent	40	47.0	87.2	12.3	0.5	10.9	15
LABC-01-204	White	gray	absent	37	58.0	73.2	21.8	5.1	37.6	21
LABC-01-209	White	gray	absent	40	63.0	77.9	4.0	18.1	25.6	10

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