MAPPING OF FOLIAR DISEASE RESISTANCE GENES AND GENES FOR AGRO-MORPHOLOGICAL TRAITS IN *Lens culinaris* Medik.

By

GOPESH CHANDRA SAHA

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Crop and Soil Sciences

DECEMBER, 2009

To the Faculty of Washington State University:

The members of the committee appointed to examine the dissertation of GOPESH CHANDRA SAHA find it satisfactory and recommend that it be accepted.

Fred J. Muehlbauer

Weidong Chen

Stephen S. Jones

Steven E. Ullrich

Gary H. Thorgaard

Clarice Coyne

ACKNOWLEDGEMENTS

I express my sincere thanks to my advisor and the committee chair Dr. Fred J. Muehlbauer for his guidance, encouragement and support to achieve my long cherished desire for an advanced degree in crop science. His kindness and affection will be remembered through out my life. I would like to express my gratitude to Dr. Weidong Chen, Dr. Stephen S. Jones, Dr. Steve E. Ullrich, Dr. Gary Thorgaard and Dr. Clarice Coyne for their inspiration and creative suggestions to this dissertation.

I would like to give special thanks to Dr. Ashutosh Sarker for his kind effort to formulate the USAID-ICARDA linkage project and introducing me to Dr. Muehlbauer for pursing my Ph.D. research at Washington State University. Thanks are also extended to the Director of Pulse Research Station, Ishurdi, Bangladesh and scientists for their sincere efforts in setting up the experiments and taking good care of the research plots.

I would like to thank research leader of Grain Legume Genetics and Physiology Unit, USDA-ARS, Dr. George Vandemark and the department of Crop and Soil Sciences for allowing me to use laboratory, green house, field research facilities and office space. Special thanks to the lab members and technician: Tony Chen, Sheri Rynearson, Sheri McGrew, Jarrod Pfaff, David White, P.N. Rajesh, Evans Njambere, Renuka Attanayake for their help and for their friendship. Heartfelt thanks to all the friends in the department of Crop and Soil Sciences for their inspiration.

Finally, I would like to acknowledge USAID-ICARDA linkage project and Sclerotinia Initiative for their funding support and financial assistance throughout my Ph.D. program.

I am indebted to my parents who have given me the vision and guidance on my way, and my wife and kids for their sacrifice, encouragement and inspiration for the completion of the degree.

MAPPING OF FOLIAR DISEASE RESISTANCE GENES AND GENES FOR AGRO-MORPHOLOGICAL TRAITS IN *Lens culinaris* Medik.

Abstract

By Gopesh Chandra Saha, Ph.D.
Washington State University
December 2009

Chair: Fred J. Muehlbauer
`

Stemphylium blight (caused by *stemphylium botryosum* Wallr.) and rust (caused by *Uromyces fabae* (Pers.) J. Schroet.) are important foliar diseases of lentil (*Lens culinaris* Medik.). To map the genes for resistance to these two diseases, F_6 derived F_7 recombinant inbred line (RIL) populations were developed by crossing ILL-5888 (susceptible to stemphylium blight and rust) to ILL-6002 (resistant to stemphylium blight) and ILL-4605 (resistant to rust). One population was used to map QTLs for resistance to stemphylium blight and genes for six agro-morphological traits. The other population was used to map the gene for resistance to rust.

The genetic linkage map used to map QTL for resistance to stemphylium blight comprised 139 markers distributed over 14 linkage groups. Three significant QTLs were detected for stemphylium blight using data recorded at the Pulse Research Center (PRC), Ishurdi, Bangladesh in 2009; whereas, one QTL was detected using 2007 data. QTL $QLG4_{80\text{-}81}$ was common to both years and accounted for 25.2% and 46% of the phenotypic variation for blight scores, respectively, for the two years.

Differential rust reactions were observed in two seasons at Bangladesh. Genotyping indicated that the gene for resistance was located on linkage group3 and 7.9cM from SRAP marker F7XEM4a.

The gene rich QTL region ($QLG4_{82\text{-}83}$) accounted for a significant amount of the phenotypic variation for days to flowering, seed diameter and 100 seed weight.Growth habit and cotyledon color are conferred by single genes. Prostrate growth habit was dominant over erect and red cotyledon was dominant over yellow.

The results of these experiments indicate that selection for resistance to stemphylium blight and rust can be made using linked molecular markers. Additional fine mapping of these genes is needed to identify more closely linked markers and improve the prospects for marker assisted selection. Validation of these putative markers for resistance genes is also needed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

To my parents,
Who brought me to light and guided me towards light.

1. INTRODUCTION

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a diploid (2n=2x=14 chromosomes) self-pollinating annual species with a haploid genome size of an estimated 4063 Mbp (Arumuganathan and Earle 1991). It is an important legume crop and an important source of dietary protein in human diets and animal feed throughout West Asia and North Africa, the Indian subcontinent, North America, South America and Australia (Webb and Hawtin 1981, Erskine 1997). World production of lentil is estimated at 3.3 million metric tons from an estimated 3.7 million hectares with an average yield of 887 kg/ha (FAOSTAT, 2009). It is an important component of crop diversification in predominantly cereal based cropping systems in South Asia and also an important legume rotational crop in the US Pacific Northwest. The lentil crop can improve soil nutrient status through symbiotic nitrogen fixation, conserving soil moisture and limiting soil erosion (Muehlbauer et al., 1992). Lentil stubbles that were left standing overwinter and trap snow and reduce the rate of evaporation of soil moisture in spring and prevents erosion (Anonymous, 2008). Moisture conservation is important to soil conservation because the additional moisture improves crop growth in the following year. Numerous factors limit yield and seed quality of lentil, including limited moisture availability, salinity, weeds, insect pests and various diseases. Diseases are major factors that limit yields and cause yield instability. Lentil rust caused by *Uromyces vicia- fabae* (Pers.) J. Schroet. and stemphylium blight caused by *Stemphylium botryosum* Wallr. are the two most important diseases of lentil in many lentil production regions worldwide.

Lentil rust is a wide spread disease in many parts of the world. The disease causes substantial yield loses annually in Bangladesh, India, Ethiopia, Morocco and Pakistan. The disease causes up to 70% yield loss, although occasional complete crop losses were observed

(Erskine and Ashutosh, 1997; Negussie et al., 2005). The incidence of disease varies considerably from year to year depending upon environmental conditions. High humidity, cloudy weather, and temperatures of 20-22^0 C favor disease development. The disease appears in areas with dense crop canopies. The pathogen forms aecial cups on the stem late in the season. In severe cases, the leaves are shed and plants dry prematurely.

Stemphylium blight of lentil is a serious threat to lentil cultivation in South Asia, including Bangladesh, and in North America (ICARDA, 2004; Vandenberg and Morrall, 2002). The pathogen has wide geographic distribution and infects plants in forty-three genera. Symptoms of disease start as pinheaded light brown or colored spots on leaflets in plants in thick populations. The spots enlarge rapidly and within 2-3 days they cover the entire leaflet resulting in defoliation and death of young plants. In severe cases the crop may exhibit a blighted appearance; however, the pods may remain green. To this day the disease is poorly understood and no studies have been undertaken to elucidate the genetics of resistance (Kumar et al., 2004).

Agro-morphological traits have immense importance in breeding crops. Breeders select breeding lines for specific purpose because of its market value and demand. Some of the traits are easy to select due to its visual nature but its scientific understanding is important for its manipulation. The timing of flowering is a very important determinant of crop duration. The optimal flowering response differs between regions (Erskine et al., 1994b). Lentil is quantitative long day plant and flowers in progressively longer days (Summerfield et al., 1984). It has been reported that the flowering time is sensitive to photoperiod and temperature and the complete understanding of genetic control of flowering time is limited (Sarker et al., 1999). Tall plant height is a major breeding goal in the western hemispheres for the suitability of mechanical harvest of lentil. Most lentils grown in the South Asia, Middle East and Africa are land races that

are typically short in plant stature, prostrate in growth habit, lack uniformity of pod maturation, have a high incidence of pod shattering and are low yielding (Muehlbauer, 1981).Lower basal pod position is a major hindrance to successful mechanical harvesting, so there is always need to develop tall upright lentil cultivars with high basal pod positions. Uniformity of size, shape and color is important for marketing of lentil. A wide range of lentil cultivars are used throughout the world with the small diameter red cotyledon type accounting for most of the production followed by the large and small yellow cotyledon type. Seed diameter and seed weight has direct correlation with yield. So, understanding genetics of the agro-morphological characters has immense importance in breeding for the yield and quality characters.

In recent years, application of DNA markers has improved the efficiency and effectiveness of breeding for disease resistance and morphological traits in various crops (Baum et al. 2000). The availability of molecular marker maps has facilitated gene tagging, marker-assisted selection and the positional cloning of genes for disease resistance and other traits. Comprehensive studies of the inheritance and molecular mapping of genes controlling resistance to rust and stemphylium blight diseases of lentil are lacking. But sufficient sources of resistance are available among material originating from West Asia and elsewhere. Therefore, genes that confer resistance to rust and stemphylium blight of lentil need to be identified and used to develop improved disease resistant cultivars.

My specific objectives are to:

i) determine the inheritance and linkage map positions of the genes for resistance to rust and stemphylium blight in lentil,

ii) identify molecular markers for the resistance genes that can be used in marker assisted selection,

iii) map the genes for agro-morphological traits to provide a better understanding of the genetics of important traits.

2. REVIEW OF LITERATURE

2.1. Origin and distribution of lentil

Lentil (*Lens culinaris* Medik.) is a member of the Leguminosae family (Webb and Hawtin, 1981) and is characterized as herbaceous annuals with slender stems and branches. The Near East arc and Asia Minor are believed to be the center of origin of cultivated lentils and the commonly accepted site of domestication which is estimated to have taken place over 8000 years ago (Zohary, 1972, Williams et al., 1974). Although lentil originated the Mediterranean region, it is well adapted and grown in temperate and semi-arid regions on all continents (Muehlbauer et al., 2009). The crop is cultivated in West Asia and North Africa, the Indian subcontinent, North America, South America and Australia (Webb and Hawtin 1981, Erskine, 1997).

2.2. Taxonomy and classification based on seed size

The Latin name of the species, *Lens culinaris,* was first published by Medikus in 1787 (Hanelt, 2001). Cultivated lentils belong to the genus *Lens* which is associated with other genera of the tribe Vicieae (Kupricha, 1981). Davis and Plitmann (1970) describe *Lens* as intermediate between *Vicia* and *Lathyrus*, but closer to *Vicia*. *Lens* is distinguished from *Vicia* by calyx morphology and stylar characters, and the shape of the pods and seeds (Muehlbauer et al., 1980). *Lens orientalis* is the presumed progenitor of cultivated *L. culinaris* and the two species are fully crossable and produce fully fertile progenies. The genus *Lens* comprises seven taxa in five species (*L. culinaris, L. odemensis, L. nigricans, L. ervoides* and *L. lamottei*). *L. odemensis* and *L. ervoides* are crossable with *L. culinaris* and but there is a high percentage of sterility when *L.*

culinaris is crossed with *L. odemensis. L. ervoides* is crossable with *L. culinaris* but embryo rescue is required to propagate the hybrid plants (Cohen et al., 1984; Ladizinsky et al., 1985; Ferguson and Erskine, 2001; Ferguson et al., 2000). From the standpoint of crossability for use in breeding, the *Lens* species can be divided into two gene pools whereby *L. culinaris, L. orientalis, and L. odemensis* belong to primary gene pool while *L. nigricans, L. ervoides* and *L. lamottei* comprise a secondary gene pool (Ladizinsky, 1999).

Lentil seeds are lens shaped and weight between 20 and 80mg with diameters that range from 2 to 9 mm. Seed size differs according to genotype and researchers frequently follow the classification of Barulina (1930) who grouped lentils as macrosperma with large seeds that range from 6 to 9 mm diameter, and microsperma with smaller seeds that range from 2 to 6 mm in diameter. The macrosperma types are common to the Mediterranean basin and in the western hemisphere, while the microsperma predominate through the Indian subcontinent and in parts of the Near East (Saxena and Hawtin, 1981).

2.3 Biotic and abiotic stresses of lentil:

Several biotic stresses such as fungal and viral diseases, parasitic pests and abiotic stresses such as cold, drought, heat, salinity, nutrient deficiency and nutrient toxicity adversely affect lentil yields worldwide (Monti et al., 1994; Saxena, 1993; Slinkard, 1994).

2.3.1. Biotic stresses of lentil:

Lentil is infected by a wide range of pathogens throughout its geographic distribution. The most serious biotic constraints are foliar diseases such as ascochyta blight (caused by *Ascochyta fabae* Speg. f. sp. *lentis* Gossen, Sheard, Beauchamp and Morrall), rust (caused by *Uromyces vicia- fabae* (Pers.) J. Schroet.), stemphylium blight (caused by *Stemphylium botryosum* Wallr.), sclerotinia white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary) and grey

mould (caused by *Botrytis cinerea* Pers.: Fr.). Rust is the key yield reducer of lentil in Bangladesh, India, Nepal, Morocco, and Ethiopia while ascochyta blight is important in West Asia, Canada and high rainfall areas of India. Stemphylium blight is a major problem in Bangladesh and Nepal and has appeared in fields in North Dakota and Saskatchewan in recent years (Kumar 2007, Holzgang and Pearse, 2001). Fusarium wilt (caused by *Fusarium oxysporum* Schlecht: Fr. f. sp. *lentis* Vasudeva and Srinivasan) and collar rot (caused by *Sclerotium rolfsii* Sacc.) are also important universally; the former is important in dry areas where foliar diseases are of minor importance and the latter is more prominent under humid conditions. Anthracnose caused by *Colletotrichum truncatum* (Schwein.) Andrus and Moore, botrytis grey mould and sclerotinia white mold are major problems in North America (Chongo et al., 2002). Recently, powdery mildew has also been reported on lentil in Canada and USA (Attanayake et al., 2009; Banniza et al., 2004). Root rot caused by *Aphanomyces euteiches* C. Drechsler, *Phythium ultimum* Trow, *Rhioctonia solani* Kühn are the other important fungal pathogens of lentil. Bacterial diseases are unimportant on lentil although root rot caused by bacteria has been reported in the former USSR (Javornokova, 1932). Some important viral diseases, lentil yellows and pea seed borne mosaic virus, have been reported in North America, Africa and South Asia (Boss et al., 1988). Pea enation mosaic virus was observed recently in the exotic lentil lines in Bangladesh (Saha, 2009). The parasitic angiosperm weed, broomrape, is a major threat to lentil production in parts of the Mediterranean region (Bayaa and Erskine 1994a).

2.3.2 Abiotic stresses of lentil:

Abiotic stresses that affect lentil production are cold, drought, heat, salinity, nutrient deficiency and nutrient toxicity. Of these stresses, drought and heat are considered the most important worldwide (Turner et al., 2001). Cold stresses are important in West Asia and North

Africa (WANA). Salinity is an important stress factor in Indian sub-continent and to some extent in WANA. Nutrient deficiency and nutrient toxicity is of lesser importance worldwide but important in localized regions (Buddenhagen and Richards, 1998).

2.4. Concept of disease resistance:

It has been reported that epidermal hairs, thickness of epidermis and cortical layer plays an important role in disease resistance in lentil (Chowdhury et al., 1997). The genetic nature of plant resistance to pathogens has been under study since the late 1800s and many genes contributing to resistance have been identified and bred into crop plants. Disease resistance is qualitatively or quantitatively inherited. Genes that confer qualitative resistance are thought to function according to the gene –for –gene (GFG) model (Flor, 1947), where the resistance phenotype is determined by the interaction between the product of resistant gene in the host and an avirulence gene in the pathogen. The cloning and sequencing of several genes that confer qualitative resistance has led to the belief that they govern the pathogen recognition and trigger the pathway that ultimately causes resistance (Bent, 1996; Hammond –Kosack and Jones, 1997). It has been shown theoretically that when host and pathogen interact through a strict GFG mechanism, polymorphism at the resistance and avirulence loci is not maintained due to sexual reproduction (Parker, 1994). However Parker's model suggests that GFG genes are only part of a larger set of genes making up the pathways leading to the resistance response (Robertson, 1989; Warren et al., 1999). It has been observed that genes previously identified to confer qualitative resistance have also been found to contribute to quantitative resistance (Li et al., 1999). Some genes previously considered to be qualitative can vary their expression according to genetic background (Dirlewanger et al., 1999). Thus there is support for the idea that resistance in plants is determined by the combination of the products of many genes encompassing all sorts of

functions, including secondary compound production, phenology, physiology and other structural features that might affect pathogen accessibility and growth in the host (Mutschler et al., 1995; Lee et al., 1998).

2.5. Lentil Rust:

2.5.1. Taxonomy and morphology of pathogen:

"The causative pathogen of lentil rust is *Uromyces vicia-fabae* (Pers.) Schroet and is a member of the *Pucciniaceae* and order *Uredinales*. It is an autoecious fungus that completes its life cycle in lentil. Spermagonia are sub-epidermal and globoid. Aecia are sub-epidermal in origin, erumpent later. Aeciospores are elliptical, yellowish brown, measuring 14-22 μm in diameter and with a finely warty wall. Uredia are first sub-epidermal, then erumpent. Urediospores are borne singly on pedicels, mostly echinuate, with three to four germination pores and measure 22-28 μm X 19-22 μm. Telia are sub-epidermal with origin, then erumpent on leaves but remain covered by the epidermis on stems for an extended period. Teliospores are borne singly on pedicels, are globose to sub-globose, very rarely ellipsoid or ovoid, one celled, measuring 25-40 μm X 18-26 μm, with a single germination pore; the wall is obviously pigmented" (Bayaa and Erskine, 1998; Viennot-Bourgin, 1949).

2.5.2. Pathogenic race of rust:

Singh and Sokhi (1980) identified six pathotypes of rust on the basis of their differential reactions on different cutivars of lentil, pea and sweet pea. Singh et al. (1995) have reported five races, and Conner and Berrier (1982) detected 11 races of *U. viciae-fabae*. Conner and Berrier, (1982) speculated that *Vicia*, *Lathyrus* and *Pisum* could be another important source of inoculum and perhaps pathogenic variants due to race specific resistance and selection pressure on the pathogen.

2.5.3. Lentil germplasm sources for rust resistance:

ICARDA developed 90 lentil lines resistant to rust, and some of them have combined resistance against a range of biotic and abiotic stresses (Sarker et al., 2002). For breaking the "bottleneck" of narrow genetic base of lentil in South Asia and combating two major diseases of lentil, rust and stemphylium, ICARDA scientists are working with Bangladesh counterparts to introgress desirable genes to improve Bangladeshi land races. These Bangladeshi land races belong to the *glex pilosae* as described by Barulina (1930) as one of the microsperma types. This approach will help to improve resistance to pulse diseases in Bangladesh (ICARDA, 2004). Negussie et al., (2005) reported four cultivars 'Gudo', R-186, FLIP-87-66L and FLIP-89-60L with different levels of rust resistance. Based on final rust severity, area under disease progress curve (AUDPC), area under the pustule density curve (ADPC) and apparent infection rate (r_G) values, Gudo and R-186 were grouped as having a high level of resistance, FLIP-89-60L as moderately susceptible and FLIP-87-66L as intermediate between susceptible and moderately susceptible lines.

2.5.4. Genetics of lentil rust resistance:

Lentil rust is caused by an obligately biotrophic fungal pathogen that infects only a narrow range of living plant hosts for growth and reproduction. For host colonization, the rust pathogen requires the induction of a subset of fungal genes essential for infection. The infection mechanisms are sophisticated, and include the ability to detect stomata, the entry portal for many rust fungi, and to suppress host resistance responses. Genes of the plant host are also induced in response to infection, but the role and expression of the host gene is difficult to dissect because molecular tools such as protocol for transformation and sequenced genomes are lacking for the rusts (Ayliffe, 2002).

Most studies on genetics of rust resistance in lentil have revealed that resistance is monogenic and dominant (Sinha and Yadav, 1989; Singh and Singh, 1992). Kumar et al. (1997) reported that resistance to *Uromyces fabae* in five lentil genotypes (L 2991, L 2981, L 1534, L 178 and HPLC 8868) was governed by single dominant genes; whereas in one genotype, Precoz, it was conditioned by two dominant genes. Chahota et al. (2002) reported that resistance to rust is controlled by duplicate, non-allelic and non linked dominant genes. Although a breakdown of rust resistance in lentil has not been reported, the likelihood of such an event in varieties with monogenic resistance cannot be ruled out (Negussiee et al., 2005). Expression of *fis1* was localized exclusively in leaf mesophyll cells closely surrounding the rust infection sites during the first hour of infection and before the start of the hypersensitive response in the resistant host. Gene expression was correlated within the amount of fungal growth. *Fis 1* encodes pyrroline -5-carboxylate dehydrogenase, an enzyme in the pathway that degrades proline to glutamate. This mechanism not only protects from proline toxicity, but also provides other roles in glutamate and glutamine in rust metabolism (Ayliffe, 2002).

Continuous cultivation of varieties with race specific resistance in large areas increases selection pressure on the pathogen that may lead to the formation of new races capable of infecting the previously resistant varieties. There are several strategies for developing varieties with durable resistance. These include multilines (Marshall, 1977), partial resistance/ slow rusting (Wilcoxson et al., 1975) and gene pyramiding (Green, 1975; Pederson and Leath, 1988).

2.5.5. Disease rating and assessment:

Khare et al. (1993) developed a 9-point disease severity scale. The scale was then categorized according to Singh and Sandhu (1988) as: 1= resistant (no infection), 3= moderately resistant (10% leaf area infected), 5= moderately susceptible (10.1-25% leaf area infected), 7=

susceptible (25.1-50% leaf area with stems also infected), 9= highly susceptible (>50% leaf area with stem and pods heavily infected). Chen, 2007 modified Khare et al.(1993) scale of the disease severity based on field conditions, where 1 = 0-10% leaf area infected, 3 = 11-30% leaf area infected, 5 = 31-50% leaf area infected, 7 = 51-70% leaf area infected, 9 = More than 70% leaf area infected.

2.6. Stemphylium blight:

2.6.1. Taxonomy and morphology of the pathogen:

The asexual stage of the causal organism of stemphylium blight is *Stemphylium botryosum* Wallr.; whereas, *Pleospora herbarum* is the sexual stage. The fungus is commonly referred as anmorph. Stemphylium blight is a ubiquitous, dematiaceous filamentous fungus that belongs to the kingdom Fungi, phylum Ascomycota, class Ascomycetes, order Pleosporales, family Pleosporacea (Inderbitzin et al., 2009). In medical science, the fungus is considered as allergen (Larone, 2002).

Morphological and developmental characters such as size and shape of the conidia, conidiophores, ascospores and the size and time of maturation of pseudothecia were useful for diagnosing species (Camara et al., 2002). “Conidiophores are short, arise singly or in groups and are aseptate and swollen at the apex. After a conidium is produced, the end of the conidiophore grows out and produces a new cell and a new conidium. The conidiophore may grow to a considerable length and have a nodulose appearance. Conidia are olive brown, muriform and echinulate measuring 24-40 µm.X 14-25 µm. Conidia are oblong with three to four septae and often constricted at the center by a medium cross walls. Perithecia are globose, membranous and black, and sometimes have a slender neck. Asci (183-267 µm X 27-37µm) are oblong to clavate with outer and inner walls. Ascospores (32-48 µm X 12-21 µm) are elongate to ovate,

characteristically with seven cross walls and three to five longitudinal septa, and yellowish to brown in color and muriform when mature" (Bayaa and Erskine, 1998). Estimates of the numbers of described *Stemphylium* species vary from around 20 to 30 (Caˆmara et al 2002, Kirk et al 2001) to up to 150 (Wang and Zhang 2006). The phylogenetic relationships of 43 isolates representing 16 species of *Stemphylium* were inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene sequence data (Marcos et al., 2002).

2.6.2. Disease prevalence, development and environment requirements:

Stemphylium blight is a serious problem in some parts of the world especially in West Asia and South Asia, North Africa and widely distributed in Saskatchewan, Canada where it was considered to be a minor disease of lentil. With the increase of lentil production and deployment of resistance to ascochyta blight and anthracnose in new cultivars, stemphylium blight has become a more serious problem (Vandenberg and Morrall, 2002).

The disease has been reported in Bangladesh by Erskine and Sarker (1997) and it was mentioned that it can cause 70% yield loss up to total crop failure in epidemic years. *Stemphylium botryosum* is reportedly spread by air borne conidia. It overwinters on seed and as mycelium on dead stems and leaves in many crops. Limited information is available on whether the pathogen is seed-borne in lentil or not (Bayaa and Erskine, 1998). In alfalfa, it spreads by airborne and water-borne conidia (conidia and ascospore) and by sowing infected seed (Malvick, 1998).

Environment plays a major role in stemphylium blight disease development and that is why understanding the environmental role in disease development is important for effectively controlling the disease. The diverse host range *S. botryosum* that includes leguminous and non leguminous crops in different parts of the world indicates the adaptability of the pathogen to

different environmental conditions (Du Toit and Derie, 2001). Most of the research on infection by *Stemphylium* spp. of different hosts has confirmed that temperature and moisture are the two most important environmental factors. In South Asia, temperatures of 18°-20°C and relative humidity of over 85% have been reported to favor the development of disease (Erskine and Sarker, 1997). The pathogen requires at least 8 hr of wetness at low temperatures (10°C) for successful infection and infection increases with increased leaf wetness for 24h (Mwakutuya 2006). Bashi and Rotem (1974) indicated that *S. botryosum* on tomato has the ability to infect even in dry periods of 24 h, although it is associated with lower disease severity. The leaf wetness requirements and response to interrupted leaf wetness are expected to vary from region to region as the pathogen adapts to prevailing environmental conditions (Jhorar et al., 1998).

2.6.3. Media requirements for laboratory growth:

Stemphylium botryosum colonies grow rapidly on a variety of media and mature with in 5 days at 25°C on potato dextrose agar (Hashemi et al., 2005). On most media, it produces velvety to cottony gray, brown or brownish-black or black colonies (Larone, 2002). The production of conidia in abundance under laboratory conditions is difficult, even when it is grown on PDA and or V8 juice agar under alternate cycle of 12h light and 12h darkness (Chowdhury et al., 1996; Mehta,1998). The use of mycelial suspensions in disease screening has been found to be as efficient as spore suspensions (Hashemi et al., 2005).

2.6.4. Resistant germplasm sources:

It has been reported that Barimasur-4 (developed from a local cultivar of Bangladesh, Utfala) shows significant resistance against rust and stemphylium blight (Erskine and Sarker, 1997). Preliminary screening at the Crop Development Center (CDC) of University of

Saskatchewan showed that 'Crimson', 'Eston' and ILL 4605-2 and ILL-8008 have good resistance against stemphylium blight.

2.6.5. Genetics of stemphylium blight resistance:

Genetics and inheritance of resistance to stemphylium blight of lentil have not been completely elucidated. Based on the frequency distribution of a recombinant inbred line (RIL) population developed from the cross Barimasur-4 X CDC Milestone, Kumar (2007) reported that resistance was quantitatively inherited. *Stemphylium botryosum* resistance in lettuce is reportedly (Netzer et al., 1985) controlled by two genes *Sm1* and *sm2*, one of which is dominant and the other recessive.

2.6.6. Disease ratings and assessments:

Horsfall-Barrat's logarithmic scale had unequal intervals in disease scores and is difficult to use for quantitatively inherited traits. To overcome this problem Hashemi, (2005b) modified this scale to a 0-10 linear semi-quantitative scale. This scale considered disease development pattern consisting of the appearance of chlorotic spots followed by gradual defoliation of plants (0= free of disease, 1= a few tiny tan spots, 2= few small to large chlorotic spots, 3=expanding lesions on leaves to defoliation started, 4=20% nodes on main stem showing necrotic symptoms and defoliation, 5= 40% nodes on main stem showing necrotic symptoms and defoliation, 6= 60% nodes on main stem showing necrotic symptoms and defoliation, 7=80% nodes on main stem showing necrotic symptoms and defoliation, 8=100% leaves defoliate but small green tip recovering, 9=100% leaves defoliate but stem still green, 10= Completely dead). Kumar, (2007) used this scale (0-10) for stemphylium blight screening. A disease rating scale from 1 to 5 was used for scoring leaf spots in alfalfa caused by *S. botryosum* (Salter and Leath, 1991).

Koike et al. (2001) used a sign scale (-= no disease; +=small leaf spot<5mm; ++=medium leaf spot) for scoring spinach leaf spot disease caused by *S. botryosum.*

2.7. Genetics of some important diseases of lentil:

2.7.1. Ascochyta blight

Ascochyta blight, caused by *Ascochyta fabae* Speg. f. sp. *lentis* Gossen, Sheard, Beauchamp and Morrall, is one of the most globally important diseases of lentil. It causes leaf, stem and pod lesions resulting in reductions in seed yield and seed quality (Morrall and Sheppard 1981). Despite extensive agronomic and chemical control studies, no efficient method has been found to control ascochyta blight in lentil. Breeding for resistance is the most effective and efficient method to control this disease (Erskine et al., 1994a, Ye et al., 2000a). Genetic studies of resistance to *A. lentis* demonstrated that either a dominant or a recessive gene controls resistance (Ahmad et al., 1997, Andrahennandi, 1994, 1997; Ford et al., 1999; Nguyen et al., 2001; Tay and Slinkard, 1989; Ye et al., 2000). Andrahennandi (1994) reported that a recessive gene *ral2* conditioned resistance to *A. lentis* in 'Indian head'. Chowdhury et al. (2001) confirmed this finding and identified two random amplified polymorphic DNA (RAPD) markers linked to the *ral2* gene. These two RAPD markers, UBC 227 $_{1290}$ and OPD 10 $_{870}$, flanked and were linked in repulsion phase to the gene *ral2* at 12 cm and 16cm, respectively. Tay and Slinkard (1989) and Ford et al. (1999) reported major dominant gene *AbR 1* that control the resistance to ascochyta blight in line ILL5588. Closely linked RAPD markers (RB 18 and RV 01) were identified as markers for the *AbR1* gene (Ford et al., 1999).

2.7.2. Fusarium wilt

Fusarium wilt is a major constraint to lentil production in West Asia and North Africa and is caused by the soil borne fungus, *Fusarium oxysporum* Schlecht: Fr. f. sp. *lentis* Vasudeva

and Srinivasan. Komboj et al. (1990). Abbas (1995) reported that inheritance of resistance to vascular wilt is controlled by the monogenic dominant gene, $F_{w.}$

2.7.3. Anthracnose

Anthranose caused by *Colletotrichum truncatum* (Schwein.) Andrus and Moore is one of the major diseases of lentil on the Canadian prairies. The disease first discovered in Manitoba in 1987 (Morrall 1988), Saskatchewan in 1990 (Morrall and Pedersen 1991) and North Dakota in 1992 (Venette et al., 1994). The disease is wide spread in Western Canada and lentil growing region of northern plains of the U.S. The disease induces greenish water soaked lesions on the lower stems. In the early flowering stage, necrotic lesions develop on leaves and as a result leaves shed. Limited information is available regarding genetics of resistance to anthracnose in lentil. Chongo and Bernier (1999) reported partial resistance to anthracnose in PI 320937, PI 345629, breeding line 458-57 and cultivar Indianhead of lentil. Buchwaldt (2001) reported two dominant genes and one recessive gene for resistance to anthracnose based on the segregating F_3 population in three crosses where PI 320937 was used as one of the resistant parents. Tullu et al. (2003) reported that a major dominant gene, *LCt-2* was responsible for resistance to race Ct1 and the minor genes influence variations in resistance level. Tullu et al. (2003) also reported that PI 320937 and Indianhead are resistant to race Ct1 but no available cultivars with resistance to race Ct0 were found. To incorporate higher levels of resistance to both races, Fiala et al. (2009) made a cross between susceptible 'Eston' and a resistant accession of *L. ervoides* germplasm, L-01-827A (resistant for Ct0 and Ct1 races) and stated that two recessive genes might be responsible for resistance to anthracnose but conclusive results could not be achieved due to loss of lines in the population due to sterility.

2.8. Agro-morphological traits:

2.8.1. Days to flowering, growth habit and plant height:

"Four developmental phases of flowering were identified: pre-emergence, pre-inductive, inductive and post-inductive. When lentil plants were transferred from short days (either 8 or 10 h) to long days (16 h), or *vice versa*, the first two phases and the last phase are insensitive to photoperiod, but are probably sensitive to temperature. The duration of the inductive phase can be predicted by assuming that its reciprocal is a linear function of both photoperiod and temperature. Critical photoperiod decreases with increase in temperature and that the duration of the inductive phase can be calculated from a summation of the amounts by which successive day lengths exceed the critical photoperiod until a value ('the photoperiodic sum') characteristic of the genotype is reached" (Roberts et al. 1986)

Growth habit has received a great deal of attention from breeders attempting to develop cultivars with more upright stature that are also lodging resistant and adaptable to mechanized harvest. In F_2s from the crosses of *L. culinaris* with *L. orientalis,* Ladizinsky (1979) found erect, intermediate, and prostrate types in a ratio that indicated a single gene with incomplete dominance. The gene symbol of prostrate canopy type is *Gh Gh* and the erect type is *gh gh*. Plant height usually ranges from 25 to 30 cm for a majority of genotypes, but may vary from extremes of 15 to 75 cm depending on genotype and environmental conditions (Saxena and Hawtin, 1981). Most lentils grown in the South Asia, Middle East and Africa are land races that generally have short plant stature, prostrate in growth habit, lack uniformity of pod maturation, have a high incidence of pod shattering, and are low yielding. Lower basal pod position is a major hindrance to successful mechanical harvesting, so there is a need to develop tall upright lentil cultivars with high basal pod positions (Muehlbauer, 1981). Sakar (1983) reported three

genes were responsible for variation in plant height in the cross of two lentil cultivars, Laird and Precoz.

2.8.2. Cotyledon color, seed diameter, seed weight:

Cotyledon color in lentil can be red/orange, yellow or green. No truly green cotyledon lentils are being marketed at the present time although genetic stocks with green cotyledons are available. "Lentils with yellow cotyledons and testa without mottling or other forms of coloration are called "green lentils", a term that is a common market jargon in developed countries. This expression is not used in major lentil producing and consuming region of South Asia. Large green lentils are marketed to countries of southern Europe, particularly Spain, Italy and Greece and small red type is exported to South Asia and the Middle East" (Muehlbauer et al., 2009). Knowledge of the genetics of cotyledon color has progressed significantly with the identification of the genes involved and linked molecular markers. The first report of genetics of lentil were the studies of cotyledon color by Tschermak (1928) and by Wilson et al., (1970) and showed that red/orange cotyledon color was dominant to yellow cotyledons and controlled by a single gene. Singh (1978) and Slinkard (1978) reported that red cotyledon color is completely dominant over green and yellow. Sharma and Emami (2002) detected monogenic and digenic control of cotyledon coloration in lentil. In their study they discovered that crosses between orange and green cotyledon parents showed monogenic segregation with complete dominance of orange over green cotyledons but digenic segregation was observed between light green and dark green cotyledons. Double recessive homozygous condition (*yy bb*) is responsible for light green coloration and the presence of *Dg* gene causes dark green coloration irrespective of homozygous dominant or recessive *Y or B* genes. It has been reported that seed wt. of lentil is controlled by two genes (Sakar, 1983).

2.8.3. Seed coat, flower and epicotyl color:

Monogenic inheritance of seed coat coloration was reported by Kumar et al. (2005). Mottled testa is dominant over non-mottled testa and brown testa over tan. Background color of lentil seed coats is reportedly controlled by two genes (Vandenberg and Slinkard, 1990). Dominant *Ggc* determines grey ground color while the dominant *Tgc* gene produces tan ground color. When both dominant genes are present (*Ggc Tgc*), brown seed coat color is produced. The double recessive (*ggc tgc*) has green seed coat color.

Kumar et al. (2005) reported flower color is controlled by a single gene and purple is dominant over white. Lal and Srivastova (1975) reported that two genes, each with complete dominance, control the flower color of lentil, but Wilson and Hudson (1978) found additive gene action in the inheritance of flower color. Ladizinsky (1979) reported the involvement of three or more genes for the expression of flower color and also mentioned that seed coat color is controlled by several genes with pleotropic effects for epicotyl and flower color.

Epicotyl color was shown by Lanizinsky (1979) to be simply inherited with purple dominant to green epicotyl.

2.8.4. Flowering pattern, leaf tendril and pod characters:

Gill and Malhotra (1980) reported that the flowering pattern is controlled by single dominant gene and obtained two flowered racemes and three flowered racemes in a 3:1 ratio in an F_2 population.

Leaves without tendrils are reportedly controlled by a single recessive gene (Vandenberg and Slinkard, 1989).

Genes that are expressed in the pods are *Glp* and *Grp* (Vandenberg and Slinkard, 1989). The dominant allele of *Glp* produces pod pubescence, while the homozygous recessive allele

(*glp*) produces glabrous pods. The dominant *Grp* gene produces red pods, while the homozygous recessive *grp* allele produces green pods. Ladizinsky (1979) found that pod indehiscence was controlled by a single recessive gene (*pi*).

2.9. Genetic Mapping:

Genetic maps have been developed in almost all the agricultural crops (O'Brien 1993). The first genetic maps of lentil consisted of a small number of markers, mainly isozymes, restriction fragment length polymorphisms (RFLPs) and some morphological markers that covered a relatively small portion of the genome (Havey and Muehlbauer 1989; Weeden et al., 1992; Tahir et al., 1993). In order to maximize the polymorphism for map construction, interspecific mapping populations have been used in *Lens* (Havey and Muehlbauer 1989; Muehlbauer et al., 1989; Weeden et al., 1992; Tahir et al., 1993; Valliancourt and Slinkard 1993; Tahir and Muehlbauer 1994; Eujayl et al., 1998). However, Causse et al. (1994) and Lefebvre et al. (1995) expressed their opinion that maps based on interspecific populations may not represent the true recombination distances of the cultivated species. Genetic maps based on intraspecific crosses have been recommended for mapping of QTL due to minimal segregation distortion (Havey and Muehlbauer 1989).

The first *Lens* map incorporating RFLPs was developed by Havey and Muehlbauer (1989) and was sparsely covered with markers spanning 330 cM of the genome with average marker distances of 12.8± 3.2 cM. Weeden et al. (1992) constructed an expanded linkage map having ten linkage groups covering 560 cM of the genome with average marker distance of 8.75 cM. Tahir et al. (1993) reviewed morphological and molecular markers to develop a preliminary linkage map of lentil that included 7 morphological, 25 isozyme, 38 RFLPs and 6 other loci, in seven tentative linkage groups. The first map of *Lens* involving RAPD markers were developed

by Eujayl et al. (1997) and spanned 206 cM and an extensive linkage map was constructed by Eujayl et al. (1998) based on 86 recombinant inbred lines (RILs) and consisted primarily of 89 RAPDs and 79 AFLPs (Amplified Fragment Length Polymorphisms) with six co-dominant markers, most of the latter being RFLPs. The map covered 1073 cM of the lentil genome with an average distance of 6 cM between adjacent markers. They observed 15 linkage groups, eight of which were small segments. The first intraspecific linkage map developed by Rubeena et al. (2003) was based on an F_2 population and was comprised of 100 RAPDs, 11 ISSRs (Inter Simple Sequence Repeats) and 3 RGA (Resistance Gene Analogs) markers, and revealed nine linkage groups varying in length from 34.9 cM to 134.8 cM. The map spanned a total length of 784.1 cM with an average distance of 6.9 cM between adjacent markers. The *Lens* sp. map developed by Duran et al. (2004) containing 62 RAPDs, 29 ISSRs, 65 AFLPs and 4 morphological and 1 microsatellite marker. The map consisted of ten linkage groups covering 2171.4 cM with an average distance between markers of 15.9 cM. Most recently, a total of 41 microsatellite and 45 AFLP markers were mapped on 86 recombinant inbred lines of lentil (Hamwieh et al. 2005).

2.10. QTL analysis:

Quantitative traits have been a major area of genetic study for over a century (Fisher, 1918; Wright, 1934; Mather, 1949; Falconer, 1960). Early studies of quantitative traits were focused on inferring numbers of genes from the mean, variance, and covariance of progenies, with no knowledge of location of the genes that underlie these traits (Kearsey and Farquhar 1998). PCR-based molecular markers and powerful statistical techniques have allowed the development of methods to investigate the genetic architecture of traits. Based on statistical associations between markers and phenotypes, it is possible to identify many QTL that contribute to the expression of a given trait, their relative magnitude and their location in the genome.

Estimates of the effects of individual QTL and the interaction between them are possible along with their placement on a linkage map. QTL analysis does not imply only polygenic traits, but can also be used to reveal the location of genes for qualitative traits. QTL analysis requires traits under investigation to have a continuous distribution. Continuous variation observed in any trait can be due to environmental or genetic effects.

The simple or multiple regressions could be used for association between phenotype and genotype based on genetic model. So, QTL mapping is modeled as

$$y = \mu + \sum_i G_i a_i + e$$

Where, y is the phenotype, μ the overall mean of the phenotype, G_i the genotype of gene $_i$, a_i the effect of gene i, and e residual error following a normal distribution

$e \sim N(0, \sigma 2)$.

A number of statistical methods have been developed for QTL detection and affect estimation. From a statistical perspective, methods for QTL mapping are based on three broad classes: regression, maximum likelihood, and Bayesian models (Li, 2007). The simplest single marker analysis identifies QTL based on the difference between the mean phenotypic values of different marker groups but can't separate the estimates of recombination fractions and QTL effect. Simple interval mapping (SIM) based on the maximum likelihood parameter and provides a likelihood ratio test for QTL position (Lander and Botstein, 1989). "Regression interval mapping was proposed to approximate maximum likelihood interval; mapping to save computational time at one or multiple genomic positions. The major disadvantage of SIM is that the estimates of location and effects of QTL may be biased when QTL are linked. Composite interval mapping (CIM) combines SIM with multiple marker regression analysis, which controls

effects of QTL on other intervals or chromosomes on to the QTL that is being tested, and thus increase the precision of QTL detection" (Li et al., 2007, Zeng 1993, 1994; Jansen 1993). CIM is one of the most commonly used methods for mapping quantitative trait loci (QTL) with populations derived from biparental crosses. QTL effects at the current testing position and regression coefficients of the marker variables used to control genetic background were estimated simultaneously in an expectation and conditional maximum likelihood algorithm. Thus, the same marker variable may have different coefficient estimates as the testing position moves along the chromosomes. "The algorithm used can not completely ensure that the effect of QTL at current testing interval is not absorbed by the background marker variables, and may result in biased estimation of the QTL effect. In addition, different background marker selection methods may give very different mapping results and the nature of the preferred method is not clear" (Li *et al*., 2007).

CIM cannot be extended for mapping epistasis (Zeng *et al*., 1999). It has been recognized that non-allelic interaction plays an important role in genetic control of quantitative traits. The interacting genes that influence the phenotype of quantitative traits follow a complex phenomenon. Statistical methodology for epistasis mapping is still developing. The interval mapping and regression interval mapping may be extended for mapping epistasis, but the mapping power is low due to not well controlled background genetic variation. "Multiple interval mapping (MIM) fits multiple putative QTL effects and associated epistatic effects simultaneously in one model. However, it requires determining the number of models (main effect and epistasis). As this is usually unknown, various models of different complexities have to be tested. Different MIM model selection method implemented in popular software of QTL cartographer give different, sometimes controversial results. Bayesian models in QTL mapping

have been widely studied in recent years. It estimates the locations and effect of parameters for a pre-specified number of QTL, which is unknown before mapping. To solve this problem, Bayesian method uses reversible jump Markov Chain Monte Carlo (MCMC) algorithm. It is widely accepted due to difficulty and arbitrary in choosing intensive computing requirements and lack of user friendly software".

Li et al. (2007 and 2008) proposed a model to resolve all the problems in QTL mapping. The model is called inclusive composite interval Mapping (ICIM). Marker variables were considered in linear model in ICIM for additive mapping, and both marker variables and marker pair multiplications were simultaneously considered for epistasis mapping. "Two steps were included in ICIM. In the first step, stepwise regression was applied to identify the most significant regression variables but with different probability label for entering and removing variables. In second step, a one dimensional scanning or interval mapping was conducted for mapping additive and a two dimensional scanning was conducted for mapping digenic epistasis. ICIM provides intuitive statistics for testing additive and epistasis, and can be successfully used on experimental population derived from inbred lines. ICIM increase the detection power and reduces false detection and less biased estimate of QTL" (Li et al., 2008).

REFERENCES

Ahmad, M., A.C. Russell and D.L. McNeil. 1997. Identification and genetic characterization of different resistance sources to Aschochyta blight within the genus *Lens*. Euphytica 97: 311 -315.

Andrahennadi, C.P. 1997. RAPD markers for Aschochyta blight resistance, phylogenetic studies and cultivar identification in lentil. Ph.D. Thesis, Univ. of Saskatchewan, Saskatoon, Canada.

Andrahennadi, C.P. 1994. Genetics and linkage of isozyme markers and resistance to seed borne *Aschochyta* infection in lentil. M.Sc. Thesis, Univ. of Saskatchewan, Saskatoon, Canada.

Anonymous, 2008. Agriculture knowledge centre. Saskatchewan agriculture. http://www.agriculture.gov.sk.ca/Default.aspx?DN=1a2fc0cd-ddb4-47b6-8d29-91dff9dde90a

Arumuganathan, K., and E.D. Earle. 1991. Nuclear DNA content of some important plant species. Plant Mol Biol 9: 208-218.

Attanayake, R.N., D.A. Glawe, F.M. Dugan, and W. Chen. 2009. *Erysiphe trifolii* causing powdery mildew of lentil (*Lens culinaris*). Plant Dis 93: 797-803.

Ayliffe, M.A., J.K. Roberts, H.J. Mitchell, R. Zhang, G.J. Lawrence, J.G. Ellis, and J.P. Tony. 2002. A plant gene up-regulated at rust infection sites. Plan Physiol 129: 1–12.

Banniza, S., J.A. Parmelee, R.A.A. Morrall, A. Tullu, and C.J. Beauchamp. 2004. First record of powdery mildew on lentil in Canada. Can Plant Dis Surv 84: 102-103.

Barulina, H. 1930. Lentils of the USSR and other counties. Bull Appl Bot Gen Plant Breed 40: 225-238.

Bashi, E., and J. Rotem. 1974. Adaptation of four pathogens to semi-arid habitats as conditioned by penetration rate and germinating spore survival. Phytopathology 64: 1035-1039.

Baum, M., N.F. Weeden, F.J. Muehlbauer, G. Kahl, S.M. Udupa, I. Eujayl, F. Weigand, M. Harrabi and Z. Bouznad. 2000. Marker technology for plant breeding. In: Knight R (ed) Linkage research and marketing opportunities for pulses in the 21st century. Kluwer, Dordrecht, 421-427.

Bayaa, B., and W. Erskine. 1994: Response of wild lentil to *Ascochyta fabae f. sp. lentis* from Syria. Genet Crop Evol 41: 61-65.

Bayaa, B., and W. Erskine. 1998. Lentil Pathology. Pathology of Food and Pasture Legumes (eds D. Allen and J. Lenné), Commonwealth Agricultural Bureaux International, U.K in association with: International Crop Research Center for the Semi-Arid Tropics, Patancheru 502 324. Andhra Pradesh, India. p. 423-472.

Bent, A. 1996. Function meets structure in the study of plant disease resistance genes. Plant Cell 8: 1757-1771.

Boss, L., R.O. Hampton and K.M. Makkouk. 1988. Virus and virus diseases of pea, lentil, faba bean, and chickpea.: In World Crops: Cool Season Food Legumes. (Ed.): R.J. Summerfield, Kluwar Academic Publishers. p. 591-615.

Buddenhagen, I.W., and R.A. Richards. 1988. Breeding cool season food legumes for improved performance in stress environments. In: World Crops: Cool Season Food Legumes, (Summerfield, R.J., Ed.) Dordrecht, Kluwer Academic. p. 81–95.

Buchwaldt, L., A. Vandenberg, A. Tullu, and C.C. Bernier. 2001. Genetics of resistance to anthracnose (*Colletotrichum truncatum*) in lentil. 4th European Conference on Grain Legumes. Cracow, Poland. p. 242.

Caˆmara, M.P.S., N.R. O'Neill, P. van Berkum. 2002. Phylogeny of *Stemphylium spp*. based on ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 94: 660–672.

Causse, M.A., T.M. Fulton, Y.G. Cho, S.N. Ahn, J. Chunwongse, K.W,J. Xiao, Z. Yu, P.C. Ronald, S.E. Harrington, G. Second, S.R. McCouch, and S.D. Tanksley. 1994. Saturated molecular map of the rice genome based on interspecific backcross population. Genetics 138: 1251-1274.

Chahota, P.K., V.P. Gupta and S.K. Sharma. 2002. Inheritance of rust resistance in lentil. Indian J Genet Plant Breed 62: 226-227.

Chongo, G., S. Banniza, and T.Warkentin. 2002. Occurrence of Ascochyta blight and other diseases in Saskatchewan in the 2001 drought year. Can Plant Dis Surv. 83:85–89.

Chongo, G., and C.C. Bernier. 1999. Field and growth chamber evaluation of components of partial resistance to *Colletotrichum truncatum* in lentil. Can J Plant Pathol 21: 58-63.

Chowdhury, M.A., C.P. Andrahennadi, A.E. Slinkard, and A. Vandenberg. 2001. RAPD and SCAR markers for resistance to Ascochyta blight in lentil. Euphytica 118: 331-337.

Chowdhury, A.M., A. Ahmed and M. Zaman. 1997. Studies on the defence structural factors of some susceptible and resistant varieties of lentil plants. Journal of Mycopath Res 35: 35-39.

Chowdhury, A. M., A. Ahmed, M. Zaman and M.A. Bakr. 1996. Sporulation of *Stemphylium botryosum* Wallr. Journal of Mycopath Res 34: 69-71.

Cohen, D., G. Ladizinsky, M. Ziv, and F.J. Muehlbauer. 1984: Rescue of interspecific *Lens* hybrids by means of embryo culture. Plant Cell Tiss.Org. Cult. 3: 343-347.

Conner, R.L., and C.C. Bernier. 1982. Race identification of in *Uromyces vicia-fabae*. Phytopathology 72: 687-689.

Davis, P.E., and U. Plitmann. 1970. Lens MILLER, In: (Ed Davis P.E.) Flora of Turkey. Edinburgh Univ. Press, Edinburgh. 3: 325-328.

Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pronier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach [Prunus persica (L.) Batsch]. Theor Appl Genet 98: 18–31.

Duran, Y., R. Fratini, P. Garcia, and M.V Perez de la, 2004. An intersubspecific genetic map of *Lens.* Theor Appl Genet 108: 1265-1237.

du Toit, L.J., and M.L. Derie. 2001. *Stemphylium botryosum* pathogenic on spinach seed crops in Washington. Plant Dis 85: 920.

Erskine, W., and A. Sarker. 1997. Lentil: the Bangladesh breakthrough. ICARDA, Carvan No. 6.

Erskine, W. 1997. Lessons for breeders from land races of lentil. Euphytica 93: 107-112.

Erskine, W., M. Tufail, A. Russell, M.C. Tyagi, M.M. Rahman and M.C. Saxena. 1994a. Current and future strategies in breeding in lentil for resistance to biotic and abiotic stresses. Euphytica 73: 127-135.

Erskine, W., A. Hussain, M. Tahir, A. Bahksh, R.H. Ellis, R.J. Summerfield, and E.H. Roberts. 1994b. Field evaluation of a model phototermal responses in a world lentil collection. Theor Appl Genet 88: 423–428.

Eujayl, I., M. Baum, W. Powell, W. Erskine and E. Pehu. 1998. A genetic linkage map of lentil (*Lens* sp.) based on RAPD and AFLP markers using recombinant inbred lines. Theor Appl Genet 97: 83-89.

Eujayl, I., M. Baum, W. Erskine, E. Pehu and F.J. Muehlbauer. 1997. Use of RAPD markers for genetic mapping and evaluation of segregation distortion in lentil (*Lens culinaris* Medik.). Euphytica 96: 405-412.

Falconer, D.S. 1960. Introduction to Quantitative Genetics, 1st edn. Oliver and Boyd: Edinburgh.

FAOSTAT, 2009.http://faostat.fao.org/

Ferguson, M., and Erskine, W. 2001. Lentils (*Lens* L). In: Maxted N and Bennett SJ (eds) Plant Genetic Resources of Legumes in the Mediterranean. Dordrecht: Kluwer Academic Publishers, 125–131.

Ferguson, M.E., M. Maxted, M. van Slageren, and L.D. Robertson. 2000. A re-assessment of the taxonomy of Lens Mill. (Leguminosae, Papilionoideae, Vicieae). Bot J Linn Soc 133: 41–59.

Fiala, J.V., A. Tullu, S. Banniza, G. Séguin-Swartz, and A. Vandenberg. 2009. Interspecies Transfer of Resistance to Anthracnose in Lentil (*Lens culinaris* Medic.). Crop Sci 49: 825-830.

Fisher, R.A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Trans R SOC Edinb 52: 399-433.

Flor, H.H. 1947. Inheritance of reaction to rust in flax. J Agric Res 74: 241-262.

Ford, R., E.C.K. Pang, and P.W.J. Taylor, 1999. Genetics of resistance to ascochyta blight (*Ascochyta lentis*) of lentil and the identification of closely linked RAPD markers. Theor Appl Genet 98:93-98.

Gill, A.S., and R.S. Malhotra. 1980. Inheritance of flower color and flower number per inflorescence in lentils. Lens l 7: 15-19.

Green, G.J. 1975. Virulence changes in *Puccinia graminis f. sp. Tritici* in Canada. Can J Bot 53: 1377-1386.

Hammond-Kosack, K.E., and D.G.J. Jonathan. 1997. Plant disease resistance genes. Plant Mol Biol 48: 575–607.

Hamwieh, A., S.M. Udupa, W. Choumane, A. Sarker, F. Dreyer, C. Jung, and M.Baum. 2005. A genetic linkage map of *Lens* sp. based on microsatellite and AFLP markers and the location of fusarium vascular wilt resistance. Theor Appl Genet 110: 669-677.

Hanelt, P.2001. Lens Mill. In: Hanelt P (ed). Mansfeld's Encyclopedia of Agricultural and Horticultural Crops. Vol. 2: 849–852. *Lens culinaris* Medicus Vorl. Churpf. Phys.-Ökon. Ges. 2: 361 (1787).

Hashemi, P., A. Vandenberg, and S. Banniza. 2005. Developing a protocol for large scale inoculation of lentil germplasms with *Stemphylium botryosum.* Abstract: *In* Proceedings of Plant Canada 2005. Edmonton, AB, June 15-18.

Havey, M.H., and F.J. Muehlbauer. 1989. Linkages between restriction fragment length, isozyme and morphological markers in lentil. Theor Appl Genet 77: 839-843.

Holzgang, G., and P Pearse. 2001. Diseases diagnosed on crop samples submitted to the Saskatchewan Agriculture and Food Crop Protection Laboratory in 2000. Can Plant Dis Surv 81: 21-27.

ICARDA, 2004. Celebration as Bangladesh produces more lentil. Caravan 20/21: 11-12.

Inderbitzin, P., J.Y.R. Mehta, and M.L. Berbee. 2009. Pleospora species with Stemphylium anamorphs: a four locus phylogeny resolves new lineages yet does not distinguish among species in the Pleospora herbarum clade. Mycologia. 101(3): 329–339.

Jansen, R.C. 1993. Maximum likelihood in a generalized linear finite mixture model by using the EM algorithm. Biometrics 49: 227–231.

Javornokova, I.P.1932. Bacterial root rot of red clover, lucern and lentils caused by Bacterium radiciperda n.sp. Bulletin of Plant Potection (Leningrad) 5: 161-172.

Jhorar, O.P., D.R. Butler, and S.S Mathauda. 1998. Effects of leaf wetness duration, relative humidity, light and dark on infection and sporulation by Didymella rabiei on chickpea. Plant Pathol. 47: 586-594.

Kearsey, M.J., and A.G.L. Farquhar. 1998. QTL analysis in plants: where are we now? Heredity 80: 137-142.

Khare, M.N., B. Bayaa and S.P.S. Beniwal. 1993. Selection methods for disease resistance in lentil. In: K.B. Singh and M.C. Saxena (Eds.), Breeding for Stress Tolerance in Cool-Season Food Legumes.Wiley, Chichester, U.K. p.107-121.

Kirk, P.M., P.F. Cannon, J.C. David, and J.A. Stalpers. 2001. Ainsworth and Bisby's Dictionary of the Fungi. Wallingford, Oxon,UK: CABI Publishing. P. 655.

Koike, S.T., D.M. Henederson and E.E. Butler. 2001. Leaf spot disease of spinach in California caused by *Stemphylium botryosum*. Plant Dis. 85:126-130.

Kumar, P. 2007. Genetics of resistance to stemphylium leaf blight of lentil (*Lens culinaris*) in the cross Barimasur-4 × CDC Milestone. M.S. Thesis, University of Saskatchewan, Canada.

Kumar Y., S.K. Mishra, M.C. Tyagi, S.P. Singh, B. Sharma. 2005. Linkage between genes for leaf colour, plant pubescence, number of leaflets and plant height in lentil (*Lens culinaris* Medik.). Euphytica 145: 41-48.

Kumar, P., S. Banniza, and A.Vandenberg. 2004. Stemphylium blight of lentil: finding sources of resistance. *In* Proceedings of the 5th Canadian Pulse Research Workshop. London, ON. 28-30 November, 2004.

Kumar, V., B.M. Singh and S. Singh. 1997. Genetics of lentil resistance to rust. Lens 24(1/2): 23-25.

Kew, Richmond, UK. Advances in legume systematics, part 1: 377–381.

Ladizinsky, G., D. Cohen, and F.J. Muehlbauer. 1985. Hybridization in the genus *Lens* by means of embryo culture. Theor Appl Genet 70: 97–101.

Ladizinsky, G. 1999. Identification of lentil's wild genetic stock. Genetic resources and Crop Evolution 46: 115-118.

Ladizinsky, G. 1979. The genetics of several characteristics in lentil as indicated by crosses between *Lens culinaris* and *Lens orientalis*. J. Hered 70: 135-137.

Lal, S., and R.S. Srivastova. 1975. Inheritance of flower color in lentils. Indian Journal of Genetics and Plant Breeding. 35: 29-30.

Lander, E., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199.

Larone, D.H. 2002. Medically Important Fungi: a Guide to Identification, 4th edn. Washington, DC: American Society for Microbiology.

Lee, E.A., P.F. Byrne, M.D. McMullen.1998. Genetic mechanisms underlying apimaysin and maysin synthesis and corn earworm antibiosis in maize (*Zea mays* L.). Genetics 149: 1997–2006.

Lefebvre, V., A. Palloix, C. Caranta and E. Pochard. 1995. Construction of an intraspecific integrated linkage map of pepper using molecular markers and double-haploid progenies. Genome 38: 112-121.

Li, H., G .Ye, and J .Wang. 2007. A modified algorithm for the improvement of composite interval mapping. Genetics 175: 361–374.

Li, H., J-M. Ribaut, Z. Li, and J. Wang. 2008. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116: 243–260.

Li, Z.K., L.J. Luo, R.M.H. Tabien, A.H. Paterson.1999. A‘defeated’ resistance gene acts as a QTL against a virulent strain of *Xanthonomas oryzae* pv *oryzae*. Mol Gen Genet 261: 58–63.

Malvick, D.K. 1998. Leaf and stem diseases of alfalfa. Report on Plant Disease No.301: April 1988. Dept. of Crop Sciences, University of Illinois at Urbana-Champaign.

Marcos, P.S.C., N.R. O'Neil, and P. van Berkum. 2002. Phylogeny of *Stemphylium* spp. based on ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 94: 660–672.

Marshall, D.R. 1977. The advantages and hazards of genetic homogeneity. In: P.R. Day (Ed.), The Genetic Nasis of Epidemics in Agriculture. New York Academy of Sciences, New York, USA. 1-20.

Mather, K. 1949. The genetical theory of continuous variation. P Int Gen C. 8: 376–401.

Mehta, Y. R. 1998. Severe outbreak of Stemphylium leaf blight, a new disease of cotton in Brazil. Plant Dis 82: 333–336. doi:10.1094/PDIS.1998.82.3.333.

Monti, L., A.J. Biddle, M.T. Moreno, and P. Plancquaert. 1994. Biotic and abiotic stresses of pulse crops in Europe. In: F.J. Muehlbauer and K.J. Kaiser (Eds.), Expanding the Production and Use of Cool Season Food Legumes. Kluwer Academic Publishers, Dordrecht, The Netherlands. p. 204–218.

Morrall, R.A.A., and E.A. Pedersen. 1991. Discovery of lentil anthracnose in Saskatchewan in 1990. Can. Plant Dis. Surv. 71: 105–106.

Morrall, R.A.A. 1988. A new disease of lentil induced by *Colletotrichum truncatum* in Manitoba. Plant Dis Rep 72: 994.

Morrall, R.A.A., and J.W. Sheppard. 1981. Ascochyta blight of lentils in western Canada: 1978-1980. Can Plant Dis Sur 61: 7-12.

Muehlbauer, F.J. 2009. Lentil: Improvement in developing countries. Erskine, W., F.J. Muehlbauer, A. Sarker, and B. Sharma. The Lentil: Botany, production and uses. CAB International.137-154.

Muehlbauer, F.J., R.J. Summerfield, W.J. Kaiser, C.M. Boerboom, M.M. Welsh-Maddux, and R.W. Short. 1992. Principles and practice of lentil production. Electronic publication at http://www.ars.usda.gov/is/np/lentis/lentils.htm

Muehlbauer, F.J., N.F. Weeden, and D.L. Hoffman. 1989. Inheritance and linkage relationships of morphological and isozyme loci in lentil (Lens Miller). J Hered 80: 298-303.

Muehlbauer F.J., D.G. Burnell, T.P. Bogyo, and M.T. Bogyo. 1981. Simulated comparisons of single seed descent and bulk population breeding methods. Crop Sci 21: 572-577.

Muehlbauer, F.J., A.E. Slinkard, and V.E. Wilson. 1980. Lentil. In: W. R. Fehr, and H. H. Hadley (eds), Hybridization of Crop Plants. American Society of Agronomy and Crop Science Society of America, Madison, USA. p. 417-426.

Mutschler, E., H. Derendorf, M. Schafer, K. Elrod, and K. Estes. 1995. Drugs Actions. CRC Press, Germany. p. 434-436.

Mwakutuya, E. 2006. Epidemiology of Stemphylium Blight of Lentil (Lens *culinaris*)in Saskatchewan. M.Sc. Thesis. Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan.

Negussie, T., Z.A. Pretorius, and C.M. Bender. 2005. Component of rust resistance in lentil. Euphytica 142: 55-64.

Netzer, D., D. Globerson, C. Weintal, and R. Elyassi. 1985. Sources and inheritance of resistance to stemphylium leaf spot of lettuce. Euphytica 34: 393-396

Nguyen, T.T., P.W.J. Taylor, J.B. Brouwer, E.C.K. Pang, and R. Ford, 2001. A novel source of resistance in lentil (*Lens culinaris* spp. *Culinaris*) to ascochyta blight caused by *Ascochyta lentis*. Aust Plant Pathol 30: 211-215.

O' Brien, S.J. 1993. Genetic maps: locus map of complex genomes, 6th edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Parker, M. A. 1994. Pathogens and sex in plants. Evol. Ecol 8:560–584.

Pederson, W.L., and S. Leath. 1988. Pyrimiding major genes for resistance to maintain residual effects. Annu Rev Phytopathol 26:369-378.

Robertson, D.S. 1989. Understanding the relationship between qualitative and quantitative genetics. In: Development and Application of Molecular Markers to Problems in Plant Genetics (eds Helentjaris T, Burr B). Cold Spring Harbor Press, Cold Spring Harbor, NY. p.81–88.

Roberts E.H., R.J. Summerfield, F.J. Muehlbauer, and R.W. Short. 1986. Flowering in Lentil (*Lens culinaris* Medic.): The Duration of the Photoperiodic Inductive Phase as a Function of Accumulated Daylength above the Critical Photoperiod. Ann Bot 58: 235-248.

Rubeena, R. F., and P.W.J. Taylor. 2003. Construction of an intraspecific linkage map of lentil (*Lens culinaris* ssp. *culinaris*).Theor Appl Genet 107: 910–916.

Saha, G.C. 2009. Research fields visit of PRC, Ishurdi, Pabna, Bangladesh. Ph.D. dissertation, Dept. of Crop and Soil Sciences, Washington State University.

Salter, R., and K. Leath. 1991. Spring blackstem and leafspot resistance. In: Fox, C.C., R. Berberet, F.A. Gray, C.R. Grau, D.L. Jesse, and M.A. Peterson (eds), Standard tests to characterize alfalfa cultivars.) North American Alfalfa Improvement Conference, Beltsville, MD. p.11.

Sakar, D. 1983.Breeding behaviour of characters related to yield and adaptation to mechanical harvesting in lentil (*Lens culinaris* Medik.). Ph.D. dissertation, Dept. of Agronomy and Soils, Washington State University.

Sarker, A., R.S. Malhotra, S. Khalil, and W. Erskine. 2002. Food legume improvement at ICARDA, ICARDA Annual Report, Aleppo, Syria.

Sarker, A., W. Erskine, B. Sharma, and M.C. Tyagi. 1999. Inheritance and linkage relationship of days to flower and morphological loci in lentil (*Lens culinaris* Medikus subsp. *culinaris*) The Journal of Heredity 90 (2): 270-275.

Saxena, M.C. 1993. The challenge of developing biotic and abiotic stress resistance in cool-season food legumes. In: Singh, K.B., and M.C. Saxena (Eds.), Breeding for StressTolerance in Cool-Season Food Legumes. Wiley, Chichester, United Kingdom. 3–14.

Saxena, M.C., and G.C. Hawtin, 1981. Morphology and growth patterns. In: C. Webb and G. Hawtin (Eds), Lentils. Commonwealth Agricultural Bureaux, Slough, UK. p.39–52.

Sharma, B., and M.K. Emami. 2002. Discovery of anew gene causing dark green cotyledons and pathway of pigment synthesis in lentil (*Lens culinaris* Medik). Euphytica 124: 349-353.

Sinha, R.P., and B.P. Yadav.1989. Inheritance of resistance to rust in lentil. Lens 16: 41.

Singh, J.P., and I.S. Singh. 1992. Genetics of rust resistance in lentil (*Lens Culinaris*). Indian J Agric Sci 62: 337-338.

Singh, S.J., and S.S. Sokhi. 1980. Pathogenic variability in *Uromyces viciae-fabae*. Plant Dis 64: 671-672.

Singh, K., H.S. Rawal, S. Singh, and R.K. Gumber. 1995. Physiological specialization in *Uromyces fabae* causing rust of lentil. Lens 22: 46-47.

Singh, T.P. 1978. Inheritance of cotyledon colour in lentil. Indian J Genet 38: 11–12.

Singh, K., and T.S. Sandhu. 1988. Screening of cultivars of lentil for resistance to rust. Lens 15: 28-29.

Slinkard, A.E., G. Bascur, and G. Hernandez-Bravo. 1994. Biotic and abiotic stresses of cool season food legumes in the western hemisphere. In: Muehlbauer, F.J., and K.J. Kaiser (Eds.). Expanding the production and use of cool season food legumes. Kluwer Academic Publishers, Dordrecht, The Netherlands. p.195–203.

Slinkard, A.E. 1978. In heritance of cotyledon color in lentils. J Hered 69: 139-140.

Summerfield, R.J., F.J. Muehlbauer, and E.H. Roberts.1984. Controlled environments as an adjunct to field research on lentils (*Lens culinaris*). III. Photoperiodic lighting and consequences for flowering. Exp Agric 20: 1-18.

Tahir, M., and F.J. Muehlbauer. 1994. Gene mapping in lentil with recombinant inbred lines. J Hered 85: 306-310.

Tahir, M., C.J. Simon, and F.J. Muehlbauer. 1993. Gene map of lentil: a review. Lens 20: 3-10.

Tay, J., and A.E. Slinkard. 1989. Transgressive segregation of Ascochyta resistance in lentil. Can J Plant Sci 69: 547.

Tschemak-Seysenegg, E. 1987. Lentil and field bean crosses. Sityringsber Akad Wiss Wien Math Nat R1 I Abs. 137(3/4):171-181 (cf. Muehlbauer, F.J. and A.E. Slinkard, 1987. BreedingMethodology. In: C. Webb and G. Hawtin (Eds.), Lentils. CAB-ICARDA, Farnham, England. p.69-90.

Tullu, A., L. Buchwaldt, T. Warketin, B. Taran, and A. Vandenberg. 2003. Genetics of resistance to anthracnose and identification of AFLPand RAPD markers linked to the resistance

gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theor Appl Genet 106: 428-434.

Turner, N.C., G.C. Wright, and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water-limited environments. Adv Agron 71: 193–231.

Vaillancourt, R.E., and A.E. Slinkard. 1993. Linkage of morphological and isozyme loci in lentil. *Lens culinaris* L. Can J Plant Sci 73: 917-926.

Vandenberg, A., and R.A.A. Morrall. 2002. Pulse crop variety development strategies in Saskatchewan. Saskatchewan Pulse Growers Pulse Days 2002, Saskatoon.

Vandenberg, A., and A.E. Slinkard. 1989. Inheritance of four new quantitative genes in lentil. J Hered 80: 320–322.

Vandenberg, A., and A.E. Slinkard. 1990. Genetics of seed coat color and pattern in lentil. J. Hered 81: 484-488.

Venette, J.R., H.A. Lamey, R.L. Lamppa. 1994. First report of lentil anthracnose (*Colletotrichum truncatum*) in the United States. Plant Dis 78: 1216.

Viennot-Bourgin, G. 1949. Les champignons parasites des plantes cultive´es, Masson and Cie, France, p.1850.

Wang, Y., and X.G. Zhang. 2006. Three new species of Stemphylium from China. Mycotaxon 96: 77–81.

Warren, R.F., P.M. Merritt, E. Holub, R.W. Innes.1999. Identification of three putative signal transduction genes involved in R gene-specified disease resistance in *Arabidopsis*. *Genetics* 152: 401–412.

Webb, C., and G. Hawtin. 1981. Lentils. Commonwealth Bureau, Page Bros, Norwich.

Weeden, N.F., F.J. Muehlbauer, G. Ladizinsky. 1992. Extensive conservation of linkage relationships between pea and lentil genetic maps. J Hered 83: 123–129

Wilcoxson, R.D., B. Skovmand, and A.A. Atif. 1975. Evaluation of wheat cultivars for the ability to retard development of stem rust. Ann Appl Biol 80: 275-281.

Williams, J.T., A.M.C. Sanchez, and M.T. Jackson. 1974. Studies on lentils and their variation. The taxonomy of the species. Sabrao Journal 6: 133-145.

Wilson, V.E., and L.W. Hudson. 1978. Inheritance of lentil flower color. J Hered 69: 129-130.

Wilson, V.E., A.G. Law, and R.L. Warner. 1970. Inheritance of Cotyledon Color in *Lens Culinaris* (Medic.). Crop Sci 10: 205-207.

Wright, S. 1934. An analysis of variability in number of digits in an inbred strain of guinea pigs. Genetics 19: 506–536.

Ye, G., D.L. McNeil, and G.D. Hill. 2000. Two major genes confer ascochyta blight resistance coming from *Lens orientalis*. NZ Plant Prot 53:109.

Zeng, Z.-B., C.-H. Kao, and C. J. Basten. 1999. Estimating the genetic architecture of quantitative traits. Genet Res 74: 279–289.

Zeng, Z.-B. 1994. Precision mapping of quantitative trait loci. Genetics 136: 1457-1468.

Zeng, Z.-B. 1993. Theoretical basis for separation of multiple linked gene effects in mapping quantitative trait loci. Proc. Natl. Acad. Sci. USA 90:10972-10976.

Zohary, D. 1972. The wild progenitor and place of origin of the cultivated lentil. *Lens culinaris*. Econ Bot 26: 326–332.

CHAPTER ONE

INHERITANCE AND LINKAGE MAP POSITIONS OF GENES CONFERRING RESISTANCE TO STEMPHYLIUM BLIGHT IN LENTIL

ABSTRACT

Stemphylium blight (caused by *Stemphylium botryosum* Wallr.) is one of the major diseases of lentil (*Lens culinaris* Medik.) in South Asia and North America. In order to determine the inheritance and map the genes for resistance a population of recombinant inbred lines (RILs) was developed from a cross between ILL-6002 (resistant) and ILL-5888 (susceptible). Progeny were advanced from F_2 to F_7 by single seed descent. The resulting 206 F_7 derived RILs were planted in disease screening plots at Ishurdi, Bangladesh in the 2006-2007 and 2008-2009 winter cropping seasons. The results indicated significant variation among the RILs for disease scores and frequency distributions of disease scores indicated complex inheritance. An intraspecific linkage map was constructed that comprised 139 markers; 21 simple sequence repeats (SSR), 27 random amplified polymorphic DNA (RAPD), 89 sequence related amplified polymorphism (SRAP) markers and 2 morpho-physiological markers distributed over 14 linkage groups. One significant QTL was detected based on stemphylium blight disease scores from the 2006-2007 experiment while three significant QTLs were detected from the 2008-2009 experiment. The QTL $QLG4_{80\text{-}81}$ was common in both the years and accounted for 25.2% and 46% variation in 2006-2007 and 2008-2009 experiments, respectively. The other two QTLs of based on 2008-2009 blight scores were $QLG2_{49}$ and $QLG3_3$ and accounted for 6.3% and 5.0 % of the variation, respectively. Two SRAP markers, ME5XR10 and ME4XR16c, and one RAPD marker, UBC34 were significantly associated with the common QTL ($QLG4_{80\text{-}81}$) for both crop years which reside on linkage group 4. ME5XR10, ME4XR16c and UBC34 markers were 1.8-2.8cM, 0.4-0.6cM and 0.6-1.6cM from the common QTL, respectively. After validation, the more tightly linked ME4XR16c marker can be used for marker assisted selection for stemphylium blight resistance.

Key words: Stemphylium blight resistance, SRAP markers, additive and epistatic effect.

INTRODUCTION

Stemphylium blight (caused by *Stemphylium botryosum* Wallr.) is a serious threat to lentil (*Lens culinaris* Medik.) cultivation in some parts of the world, especially in South Asia including Bangladesh, Northeast India and Nepal. This disease has also been reported in Egypt, Syria and North America (Erskine and Sarker, 1997, Bayaa and Erskine, 1998; ICARDA, 2004; Vandenberg and Morrall, 2002).The disease results in yield losses that exceed 60% in severely infected fields. Historically it has been considered a disease of minor importance in the US northern plains (MT and ND) and in Saskatchewan, Canada, but with the increase of lentil production and deployment of new cultivars with resistance to ascochyta blight (caused by *Ascochyta rabiei* Pass. Lab) and anthracnose (caused by *Colletotrichum truncatum* (Schwein.) Andrus and Moore), stemphylium blight has become a more serious problem (Vandenberg and Morrall, 2002).

The pathogen has a wide geographic distribution and infects plants in 43 genera. The diverse host range of *Stemphylium botryosum*, which includes leguminous and non leguminous species in different parts of the world, demonstrates its adaptability to different genotypes and environments (du Toit and Derie, 2001). Symptoms of disease start as pinhead-sized light brown or colored spots on leaflets of plants in dense populations. The spots enlarge rapidly and within 2-3 days they cover the entire leaflet resulting in defoliation and death of young plants. In severe cases the crop may exhibit a blighted appearance causing large scale defoliation; however, the pods may remain green (Erskine and Sarker, 1997).

Environment plays a major role in stemphylium blight disease development. In South Asia temperatures of 18-20ºC with relative humidity over 85% favors disease development (Erskine and Sarker, 1997). High temperatures favor spore germination and presumably infection. Warm and humid weather that prevails in Bangladesh in January also favors disease development (Erskine and Sarker 1997). Occasional rain in winter in Bangladesh may increase the severity of the disease. Jhorar et al. (1998) reported that infection increased with increased leaf wetness up to 24h duration. Leaf wetness requirements and response to interrupted leaf wetness are expected to vary from region to region as the pathogen adapts to prevailing environmental conditions. Studies showed that structural defense factors such as hairs, thickness of the epidermis and cortical layers of lentil contribute significantly to stemphylium blight resistance (Chowdhury et al., 1997).

There has been limited study on genetics of stemphylium blight in lentils. The frequency distribution within a lentil RIL population developed from the cross Barimasur-4 x CDC Milestone in both field and controlled environments revealed quantitative inheritance (Kumar, 2007). Resistance to *S. botryosum* in lettuce is reportedly controlled by a dominant gene, *Sm1,* and one recessive gene, *sm2* (Netzer et al., 1985).

Non-allelic interaction plays an important role in genetic control of quantitative traits. The interacting genes that influence the phenotype of quantitative traits follow a complex phenomenon. The inclusive composite interval mapping (ICIM) provides intuitive statistics for testing additive and digenic epistatic effects and increase the power of detecting QTL, reduces the false positives of QTL, and provides a less biased estimate of QTL effects (Li et al., 2008). So, ICIM model was used to identify the QTL in our mapping population.

The objectives of the present study were (i) to determine the inheritance and linkage map positions of the genes for resistance to stemphylium blight in lentil and (ii) to identify molecular markers for the resistance genes that can be used in marker assisted selection.

MATERIALS AND METHODS

Inheritance study

Crosses were made between resistant line ILL-6002, a pure line selection from Precoz, and ILL-5888, a highly susceptible germplasm line. ILL-5888 named 'Uthfola' was the first lentil cultivar released by the Bangladesh Agricultural Research Institute (BARI), Gazipur in 1991. In addition, backcrosses were made between the F_1s and the parents (F_1x ILL-5888 and F_1x ILL-6002) in order to determine whether resistance was dominant or recessive.

The two parents were screened in a growth chamber (22°C day and 16°C night temperature with 85% RH) at 2 wks, 4 wks, 6 wks, and 8 wks of age against three isolates (I_1, I_2 and I_3) collected from USA. The isolates were cultured in PDA and V8 juice and the mycelium was transferred to potato dextrose broth for 10 days. The mycelium grown on PDA and V8 juice was blended and three replicates of the parents were inoculated. Chowdhury et al. (1996) used similar methods to produce a mycelial suspension and Hashemi et al. (2005) reported that mycelial suspensions for disease screening was as reliable as spore suspension inoculum. The isolate and plant age that showed differential disease reactions between parents were chosen for the study of the inheritance of disease resistance. Eight wk old parents (ILL-5888, ILL-6002), F_1 (ILL-5888 x ILL-6002) and backcross progenies (F_1x ILL-5888 and F_1x ILL-6002) were inoculated with the I_3 isolate and their disease reactions were evaluated. Three replications were used for this evaluation.

Developing recombinant inbred lines

A recombinant inbred line (RIL) genetic mapping population was developed from a cross of ILL-6002 and the susceptible line ILL-5888 by advancing the F_2 population to the F_7 by single seed descent. The resulting 206 F_7 derived RILs were used for phenotyping.

Phenotyping

The F_7 derived RILs were planted in disease plots at the Pulse Research Center (PRC) of Ishurdi, Bangladesh for the 2006-2007 and 2008-2009 cropping seasons using a randomized complete block design with three replications. The individual plots were single rows 1m long and spaced 40cm apart. Debris of stemphylium blight infected lentil plants was collected the previous year and was spread throughout the field and served as the source of primary inoculum. Phenotyping for disease reaction was done using the 1-9 scale developed by Chen (2007) (personal communication) during the late flowering stage where 1 = 0-10% leaf area infected, 3 = 11-30% leaf area infected, 5 = 31-50% leaf area infected, 7 = 51-70% leaf area infected, 9 = More than 70% leaf area infected. The analysis of variance of disease scores for the RILs was conducted using SAS 9.1.

Genotyping

DNA was isolated from the young leaf tissue of the parents and their RILs. DNA was isolated by chloroform- iso-amyl alcohol (24:1) extraction method and precipitated by 95% ethanol and washed in 70% ethanol (Doyle and Doyle 1987). DNA was dissolved in TE buffer for long term storage and use. The two parents were tested for polymorphism using for 156 SSR, 270 SRAP and 181 RAPD markers. Out of 206 inbred lines, 169 lines were randomly chosen for genotyping using the polymorphic markers.

a) SSR markers:

Of the 156 SSR markers used, 30 were obtained from ICARDA and the rest were developed in our laboratory (GLLC-SSR: Grain Legume *Lens Culinaris*-SSR) using the magnetic bead method (Kijas et al. 1994, Kijas et al.1995). PCR reactions consisted of 20µl PCR reaction contained 40ng genomic DNA, 10pmol of each forward and reverse primer, 0.2 M dNTP, 1X Promega PCR buffer and 1U Taq DNA polymerase (Promega). PCR was carried out in a thermocycler with an initial denaturation of 94ºC for 3 min, followed by 35 cycles of 94ºC for 30s, 55ºC for 35s, and 72ºC for 60s, was followed by a final extension at 72ºC for 5 min. The PCR products were electrophoresed on 6% polyacralimide and visualized through silver staining (Tegelstrom, 1992). The LI-COR 4300 DNA analyzer was used for detecting microsatellite polymorphism, primers were labeled with IRDye 700 and IRDye 800 and the amplicons were separated with 6% polyacralimide gel. The band sizes were analyzed using Saga^{GT} software.

b) RAPD PCR

RAPD markers were generated from genomic DNA in 25 µl reactions containing 50 ng DNA, 2.5µl 10X buffer, 1.5µl $MgCl_2$, 0.4µl primer, and 0.2µl Taq DNA polymerases. The thermocycling profile consisted of an initial step of 94°C for 5 min, followed by 40 cycles of 94°C for 20 s, 36°C for 60 s and 72° C for 60 s, and the final elongation was performed at 72^0C for 8 min. PCR products were resolved on 2% agarose gels in 1X TBE buffer (tris, boric acid and 0.5M EDTA at pH=8.0), stained with ethidium bromide, and visualized under ultraviolet light.

c) SRAP markers

Each 20µl reaction contained 50 ng DNA, 4 ul dNTPs @1.25mM each dNTP, 1.5 mM $MgCl_2$, 5µl 5X promega buffer, 2.5µl forward and primer each @15ng/µl, 0.5µl Taq DNA

polymerase (5 units/µl) . The initial denaturation temperature 95º for 60 s followed by 10 cycles of temperature 94ºC, 35ºC and 72ºC respectively for 60 s each. This was followed by 30 cycles of 94ºC for 60 s, 50ºC for 60 s, and 72ºC for 60 s, and a final extension for 7 min at 72ºC. Amplicons were resolved using 2% agarose gels in Tris-borate EDTA (TBE) buffer and stained with ethidium bromide. Parents were initially screened to detect polymorphisms and primer pairs that produced reliable polymrophisms were used to screen 169 RILs.

Linkage analysis

The markers were analyzed by chi-square goodness of fit to the 1:1 expected Mendelian segregation ratio. Linkage analysis was performed using Mapmaker Macintosh V2.0 (Lander et al., 1987). Linkage groups were developed at a LOD score threshold of 4 and a maximum recombination fraction (θ) of 0.25 by two point analysis using group command. The best marker order was identified using the 'compare' function and sets of more than eight markers were ordered using a combination of the 'compare' and 'try' functions. The Kosambi mapping function (Kosambi, 1944) was used to calculate genetic distance.

QTL analysis

QTL analysis was conducted using QTL IciMapping v2.2 and Q gene 4.2.3 software. In the first step, stepwise regression was applied to identify the most significant regression variables with different probability label by entering and removing variables. In the second step, a one dimensional scanning or interval mapping analysis was conducted to detect additive effects and a two dimensional scanning was then conducted to detect digenic epistatic effects (Li et al., 2008).

RESULTS AND DISCUSSION

Analysis of variance of disease reaction

Phenotyping of the 206 RILs at Ishurdi, Bangladesh indicated variation (P=0.001) in blight scores in both crop years (2006-2007 and 2008-2009) with block effects significant in 2008-2009 only (Table 1). The pooled variance showed highly significant variation among RILs. The pooled analysis also demonstrated that line x year effects were significant as was the year effect on disease expression. It has been observed that 2006-2007 and 2008-2009 stemphylium blight score showed significant correlation (r=0.317, P=0.001).

Trait analysis

The mean disease score of the RILs in the two cropping years, 2006-2007 and 2008-2009, were 4.2 ± 2.5 and 4.6 ± 2.4, respectively. The distribution of disease score was partitioned into four different groups, 1-3.1, 3.7-5.1, 5.7-7.1, 7.9-9.0. Most of the RILs in the two cropping years showed disease score that ranged 1-3.1. The resistant parent ILL-6002 and susceptible parent ILL-5888 showed consistent disease reactions in both years, in 2006-2007 they were 1.7 ± 1.2 and 8.3 ± 1.2, respectively, while in 2008-2009 the mean disease score were 1.7 ± 1.2 and 7.7 ± 1.2, respectively.

The frequency distribution of stemphylium blight disease rating of RILs in 2006-2007 and 2008-2009 cropping years (Fig.1:a1, b1) doesn't fall only the resistant (1.0-3.1 disease rating) and susceptible classes (7.7-9.0 disease rating), there are moderately resistant (3.7-5.0 disease rating) and moderately susceptible classes (5.7-7.1 disease rating) present which suggests that more than one gene is involved in conditioning resistance / susceptibility to stemphylium blight. Normality test (Fig.1:a2, b2) showed absence of normal distribution which suggests

involvement of some major and minor genes for disease resistance. Complex inheritance of resistance is also indicated by frequency distributions (Fig.1:c1) and normality test (Fig.1:c2) based on the pooling of data from both cropping years. The higher number RILs were resistant to stemphylium blight in both the cropping years suggest that the resistance is controlled by dominant genes. The inheritance study also confirmed that F_1 moderately resistant and among the backcross progenies F_1x ILL-6002 showed moderately resistant in disease reaction where as F_1x ILL-5888 showed moderately resistant to susceptible reactions.

Genetic Mapping

An intraspecific lentil (*L. culinaris* ssp. *culinaris* x *L. culinaris* ssp. *culinaris*) map was constructed using 21 SSR, 22 RAPD and 60 SRAP primers that generated 161 polymorphic markers including 23 SSRs, 30 RAPDs and 108 SRAPs (Fig.2). The size of the markers varied from approximately 200 to1400 bp. SRAP primers produced the highest number of polymorphic markers (1.8 markers/primer) followed by RAPDs and SSRs. Two morpho-physiological markers, plant type and cotyledon color, were distinctive between the parents and these were also used to construct the linkage map (Table 2).

Marker distortion was detected by chi-square analysis and it was found that 36 markers did not segregate according to the expected Mendelian ratio of 1:1 at $P>0.05$ i.e. about 22% of the total markers. Approximately 43.5% of SSR markers, 20% of RAPD and 18.5% of SRAP markers showed marker distortion. Rubeena et al. (2003) reported 14% segregation distortion [RAPD, ISSR (Inter Simple Sequence Repeat) and RGA (Resistance Gene Analogs)] in F_2 populations of lentil and Hamwieh et al. (2005) observed that 9.5% of 41 SSR markers and 17.8% of 45 AFLP (Amplified Fragment Length Polymorphism) markers showed marker distortion in lentil 86 RILs. In this study, 24 distorted unlinked markers were discarded and the

lentil linkage map was constructed based on 139 markers. The constructed linkage map comprised 14 linkage groups that spanned 1565.2cM (Table 3).

Linkage groups varied in length from 38.4 to 256.2cM and the average number of marker loci in each linkage group was 11.6cM. Six linkage groups (LG1, LG2, LG3, LG4, LG5 and LG6) were greater than 100cM. LG1 covered the longest distance (256.2cM) among the linkage groups detected and also was composed of the most markers (22), while LG14 represented the smallest linkage group in terms of length (38.4cM). The highest marker density was observed for LG14 (5.5cM/marker), whereas the lowest marker density was observed for LG9 (19.5cM/marker).

Rubeena et al. (2003) detected 100 RAPDs, 11 ISSRs and 3 RGA markers in an F_2 lentil (*L. culinaris* ssp. *culinaris* x *L. culinaris* ssp. *culinaris*) population, and revealed nine linkage groups varying in length from 34.9cM to 134.8cM. The map spanned a total length of 784.1cM with an average distance of 6.9cM between adjacent markers. The *Lens* sp. (*L. culinaris* ssp. *culinaris* x *L. culinaris* ssp. *orientalis*) map developed by Duran et al. (2004) has 62 RAPDs, 29 ISSRs, 65 AFLPs, 4 morphological and 1 microsatellite marker. The map consisted of ten linkage groups covering 2171.4cM with an average distance between markers of 15.9cM. Most recently, a total of 41 SSR and 45 AFLP markers were mapped on 86 lentil RILs (Hamwieh et al. 2005).

Most of the markers detected in this study were clustered near the central regions of each linkage group. The SSR204a mapped to LG1, agreed with Hamwieh et al. (1995), while the other locus of this primer, SSR204b, was located in LG8. Marker SSR48 was positioned on LG2 in our map; however, it was located on LG3 on the map of Hamwieh et al. (1995). Most of the GLLC-SSR markers were at the termini of linkage groups. It has been speculated that AT rich

SSRs are frequently evolving from the poly A tail of the *Alu* retrotransposon in plants (Gortner et al., 1998). The localization of microsatellites varies among genomes and sometimes shows non-random distribution in eukaryotes (Gortner et al., 1998). In this study the RAPD and SRAP markers were distributed evenly across the genome.

The curved map (Fig. 2) could be supplemented with additional markers to reduce the number of linkage groups to 7 so that linkage groups can be assigned to individual chromosomes. SSRs are co-dominant markers that are very useful as anchors to transfer information between different maps within a species. Accordingly, in using SSR markers developed by Hamwieh et al. (2005), only two SSRs were polymorphic in the ILL-5888 x ILL-6002 map resulting in the resolution of three additional loci. A lack of shared polymorphic SSR markers between the two populations limited the ability to join the two maps.

QTL analysis

The LOD in contour profiles and the table showing additive effect clearly indicate the presence of significant QTL for stemphylium blight disease score. The genomic regions with QTLs were observed by light bands in both the axes (Table 4, Fig 3a and 4a). LOD_A in contour profile and interaction table demonstrated combined additive and epistatic effects between digenic QTLs (Table 5, Fig 3b and 4b), whereas LOD_{AA} in contour profile excluded the influence of additive effects and clearly indicated the presence of epistatic effects (Table 5, Fig 3b and 4b). Some of the combined additive and epistatic effect, LOD_A and epistatic effects excluding additivity, LOD_{AA} were significant and showed values >3.

(a) QTL with additive effect

A QTL on the LG4 near to the 80-81cM position was detected from the 2006-2007 and 2008-2009 crop season analysis. This QTL ($QLG4_{80\text{-}81}$) accounted for 25% (with LOD value of

9.6) and 46% (with LOD value 25.4) of the phenotypic variance with additive effect of 1.24 and 1.60 to the disease scores respectively (Table 4, Fig. 5 and 6).

The closest markers in that location are ME5XR10 at the locus 78.2cM, ME4XR16c at the locus 80.4cM and UBC34 at the locus 81.6cM. Two other QTLs, one ($QLG3_3$) located at position 3cM on LG3 and another ($QLG2_{49}$) located at position 49cM on LG2 accounted for 5.0 and 6.3% of the variation in disease scores, respectively.

(b) QTL with interaction effect

In 2006-2007 crop year, the $QLG1_{190}$ x $QLG11_{46}$ interaction resulted in a significant negative epistatic effect (epistatic value= -0.886) that accounted for 12.8% of the total phenotypic variation where as $QLG4_{124}$ x $QLG10_{30}$ had a significant positive epistatic effect (epistatic value=0.727) that accounted for 10% of the variation for disease score (Table 5, Fig 3b and 4b).

Significant epistatic interactions between QTLs were also detected for the 2008-2009 crop year (Table 5). $QLG9_{20}$ x $QLG9_{30}$ (epistatic effect = -1.074) and $QLG2_{202}$ x $QLG3_{104}$ (epistatic effect = 0.650) accounted for 9.7% and 10.3% of phenotypic variation respectively where as $QLG1_{94}$ x $QLG11_{38}$ (epistatic effect = 0.493) accounted 3.6% of the total phenotypic variation, i.e lowest significant variation (Table 5, Fig 3b and 4b).There is a trend that individual QTL with larger effects will show higher LOD scores than QTL that have lesser effects. However, this is not always the case, especially when multiple QTLs are linked. In this study, the $QLG2_{202}$ x $QLG3_{104}$ interaction had a larger effect (10.3%) but a lower LOD value than the following interactions: $QLG1_{94}$ x $QLG11_{38,}$ $QLG1_{206}$ x $QLG3_{16,}$ $QLG1_{208}$ x $QLG3_{18,}$ $QLG1_{210}$ x $QLG3_{20,}$ $QLG1_{212}$ x $QLG3_{24,}$ $QLG4_{140}$ x $QLG5_{104,}$ $QLG5_{104}$ x $QLG9_{6,}$ $QLG8_{34}$ x $QLG9_{36}$, $QLG8_{34}$ x $QLG9_{46}$ and $QLG9_{20}$ x $QLG9_{30}$ (Table 5). This suggests that the interaction of $QLG2_{202}$ x

$QLG3_{104}$ had a repulsion effect with other QTLs and had lower chance of detection, although it accounted for a larger amount of the total phenotypic variation. Interactions between QTLs, $QLG1_{206}$ x $QLG3_{16}$, $QLG1_{208}$ x $QLG3_{18}$ and $QLG1_{210}$ x $QLG3_{20}$ had LOD_A value of 6.61, 6.18 and 5.71 respectively, while the respective LOD_{AA} values for these interactions were 3.13, 3.15 and 3.12 indicating that these loci had both significant additive and epistatic effects. Epistatic interactions were significant while individual additive effects were smaller for the following interactions: $QLG1_{94}$ x $QLG11_{38,}$ $QLG1_{212}$ x $QLG3_{24,}$ $QLG4_{140}$ x $QLG5_{104,}$ $QLG5_{104}$ x $QLG9_{6,}$ $QLG8_{34}$ x $QLG9_{36,}$ $QLG8_{34}$ x $QLG9_{46}$ and $QLG9_{20}$ x $QLG9_{30}$ (Table 5).

Stemphylium blight disease score for the two years of evaluation showed different epistatic interaction effects. This suggests that tri or tetragenic locus interactions may more effectively explain these observed epistatic effects than digenic interactions.

CONCLUSION

We have identified significant QTLs associated with stemphylium blight resistance based field data of two cropping seasons. The QTL, $QLG4_{80\text{-}81}$ is very closely linked to the disease resistance genes. ME5XR10, ME4XR16c, and UBC34 are about 1.8-2.8cM, 0.4-0.6cM and 0.6-1.6cM respectively, from the identified QTL for stemphylium blight resistance. Marker ME4XR16c could be used for marker assisted selection for resistance/ susceptibility after validation. Marker information may be transferable to other populations and will be used for marker assisted selection. So, we need to increased marker density in the vicinity of the QTL that would permit a more precise placement of the QTL in the genome and in future, we will be able to clone the genes with chromosome walking using BAC libraries. Additive and digenic epistatic effects could explain the QTL more effectively and reduce the detection of false QTL. The

introduction of tri or tetra epistatic locus interaction model can improve the efficiency of the QTL detection and the epistatic QTL effects should be brought under consideration along with additive effects during marker assisted selection.

Acknowledgements: This work was supported by USAID-ICARDA linkage project. We are grateful to Director of Pulse Research Center, Ishurdi, Bangladesh for setting up field experiment in their station. We are also indebted to David White and P.N. Rajesh for providing us GLLC-SSR markers and Sheri Rynearson, Tony Chen and Tracie Anderson for laboratory support.

REFERENCES

Bayaa, B., and W. Erskine. 1998. Lentil Pathology. Pages 423-472, in: Pathology of Food and Pasture Legumes (eds D. Allen and J. Lenné), Commonwealth Agricultural Bureaux International, U.K in association with: International Crop Research Center for the Semi-Arid Tropics, Patancheru 502 324. Andhra Pradesh, India.

Chen, W. 2007. Stemphylium blight disease scoring in field condition. Research Geneticist, USDA-ARS, Washington State University, Pullman, WA-99164.

Chowdhury, A.M., A. Ahmed and M. Zaman 1997. Studies on the defence structural factors of some susceptible and resistant varieties of lentil plants. Journal of Mycopathological Research 35: 35-39.

Chowdhury, A. M., A. Ahmed, M. Zaman and M.A. Bakr. 1996. Sporulation of *Stemphylium botryosum* Wallr. Journal of Mycopathological Research 34: 69-71.

Dempster, A.P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the *EM* algorithm. Journal of the Royal Statistical Society, Series *B*, 34:1-38.

Doyle, J.J., and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

du Toit, L.J., and M.L.Derie. 2001. *Stemphylium botryosum* pathogenic on spinach seed crops in Washington. Plant Disease 85:920.

Duran, Y., R. Fratini, P. Garcia, and M.V Perez de la , 2004. An intersubspecific genetic map of *Lens.* Theor Appl Genet 108:1265-1237.

Erskine, W. and A. Sarker. 1997. Lentil: the Bangladesh breakthrough. ICARDA Carvan No. 6.

Gortner, G., M. Nenno, K. Weising, D. Zink, W. Nagl, and G. Kahl.1998. Chromosomal localization and distribution of simple sequence repeats and the *Arabidopsis*-type telomere sequence in the genome of *Cicer arietinum* L. Chrom. Res. 6: 97–104.

Hamwieh, A., S. M. Udupa, W. Choumane, A. Sarker, F. Dreyer, C. Jung, and M.Baum. 2005. A genetic linkage map of *Lens* sp. based on microsatellite and AFLP markers and the location of fusarium vascular wilt resistance. Theor Appl Genet 110:669-677.

Hashemi, P., A. Vandenberg and S. Banniza. 2005b. Developing a protocol for largescale inoculation of lentil germplasms with *Stemphylium botryosum. In* Proceedings of Plant Canada 2005. Edmonton, AB, June 15-18. (Abstract)

ICARDA, 2004. Celebration as Bangladesh produces more lentil. Caravan 20/21:11-12.

Jhorar, O.P., D.R. Butler, and S.S Mathauda. 1998. Effects of leaf wetness duration, relative humidity, light and dark on infection and sporulation by Didymella rabiei on chickpea. Plant Pathol. 47: 586-594.

Kijas, J.M.H., J.C.S.Fowler, and M.R.Thomas.1995. An evaluation of sequence tagged microsatellite site markers for genetic analysis within *citrus* and related species. Genome 38:349-355.

Kijas, J.M.H., J.C.S.Fowler, C.A.Garbett and M.R.Thomas.1994. Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin coated magnetic particles. Biotechniques 16:656-662.

Kosambi**,** D. 1944**.** The estimation of map distances from recombination values. *Ann. Eugen.*, 12, 172–175.

Kumar, P. 2007. Genetics of resistance to stemphylium leaf blight of lentil (*Lens culinaris*) in the cross Barimasur-4 × CDC Milestone. M.S. Thesis, University of Saskatchewan, Canada.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174- 181.

Li, H., J-M. Ribaut, Z. Li, and J. Wang. 2008. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243–260.

Netzer, D., D. Globerson, C. Weintal, and R. Elyassi. 1985. Sources and inheritance of resistance to stemphylium leaf spot of lettuce. Euphytica 34: 393-396.

Rubeena, R. Ford, and P.W.J. Taylor. 2003. Construction of an intraspecifi c linkage map of lentil (*Lens culinaris* ssp. *culinaris*).Theor. Appl. Genet. 107: 910–916.

Tegelstrom H. 1992. Detection of mitochondrial DNA fragments. In: Hoelzel AR (ed) Molecular genetic analysis of populations: a practical approach. IRL Press, Oxford, pp 89–114.

Vandenberg, A., and R.A.A. Morrall. 2002. Pulse crop variety development strategies in Saskatchewan. Saskatchewan Pulse Growers Pulse Days 2002, Saskatoon.

Table 1. Analysis of variance of stemphylium blight disease score of the lentil RILs for the year 2006-2007, 2008-2009 and pooled for the crop years.

Source	DF	Sum of Squares	Mean Square	F Value
2006-2007 crop year				
Lines	205	3755.90	18.32	11.64***
Block	2	5.14	2.57	1.16^{ns}
Error	410	645.53	1.57	
Total	617	4406.58		
2008-2009 crop year				
Lines	205	3561.07	17.37	10.35***
Block	2	69.02	34.51	20.56***
Error	410	688.31	1.68	
Total	617	4318.40		
Both years' data pooled				
Lines	205	4426.10	21.59	12.90***
Block	2	31.00	15.50	9.26***
Year	1	95.06	95.06	56.80***
Lines*Year	205	2891.10	14.10	8.43***
Error	823	1377.33	1.67	
Corrected Total	1236	8821.43		

***P < .0001

Table 2. Markers used for the construction of linkage map of lentil.

Marker Type	Number of primers or primer pairs screened	Number of polymorphic primers or primer pairs	Number of polymorphic markers	Number of mapped markers
SSR	156	21 (13.5%)	23 (1.1 markers/ primer)	21
RAPD	181	22 (12.2%)	30 (1.4 markers/ primer)	27
SRAP	270	60 (22.2%)	108 (1.8 markers/ primer)	89
Morpho-physiological marker	2	2	2	2

Table 3. Distribution of markers on different linkage groups of lentil.

Linkage group	Length (cM)	Number of markers	Average distance between markers (cM)
LG1	256.2	22	11.6
LG2	229.8	19	12.1
LG3	183.9	15	12.3
LG4	140.7	11	12.8
LG5	104.1	11	9.5
LG6	102.1	6	17.0
LG7	96.1	7	13.7
LG8	92.1	7	13.2
LG9	77.8	4	19.5
LG10	68.6	8	8.6
LG11	68.1	11	6.2
LG12	54.4	5	10.9
LG13	52.9	6	8.8
LG14	38.4	7	5.5
Total	1565.2	139	11.3

Table 4. QTLs with their additive effects and phenotypic variations.

Stemphylium blight disease in cropping years	QTL	EstAdd	LOD	PVE [+] (%)
Stemphylium blight (2006-2007)	$QLG4_{80}$	1.239	9.63	25.23
	$QLG2_{49}$	0.595	3.21	6.35
	$QLG3_{3}$	0.528	3.71	5.04
Stemphylium blight (2008-2009)	$QLG4_{81}$	1.603	25.4	45.96

[+] phenotypic variation explained

Table 5. Epistatic effects, LOD value at the interaction point (LOD_A), epistatatic effect (LOD_{AA}) and phenotypic variance (PVE%) of different interacting QTL.

Interacting QTL	Epistatic effects	LOD_A	LOD_{AA}	PVE [+] (%)
Stemphylium blight (2006-2007)				
$QLG1_{190}$ x $QLG11_{46}$	-0.886	3.37	3.20	12.78
$QLG4_{124}$ x $QLG10_{30}$	0.727	3.72	3.14	9.99
Stemphylium blight (2008-2009)				
$QLG1_{94}$ x $QLG11_{38}$	0.439	3.48	3.30	3.58
$QLG1_{206}$ x $QLG3_{16}$	-0.465	6.61	3.13	8.08
$QLG1_{208}$ x $QLG3_{18}$	-0.487	6.18	3.15	7.74
$QLG1_{210}$ x $QLG3_{20}$	-0.486	5.71	3.12	6.98
$QLG1_{212}$ x $QLG3_{24}$	-0.495	3.68	3.57	4.33
$QLG2_{202}$ x $QLG3_{104}$	0.650	2.64	2.50	10.25
$QLG4_{140}$ x $QLG5_{104}$	-0.585	6.88	6.57	6.33
$QLG5_{104}$ x $QLG9_{6}$	0.469	3.21	3.03	4.16
$QLG8_{34}$ x $QLG9_{36}$	0.555	4.71	4.51	5.68
$QLG8_{34}$ x $QLG9_{46}$	0.497	4.02	3.81	4.59
$QLG9_{20}$ x $QLG9_{30}$	-1.074	3.63	3.35	9.67

[+] phenotypic variation explained

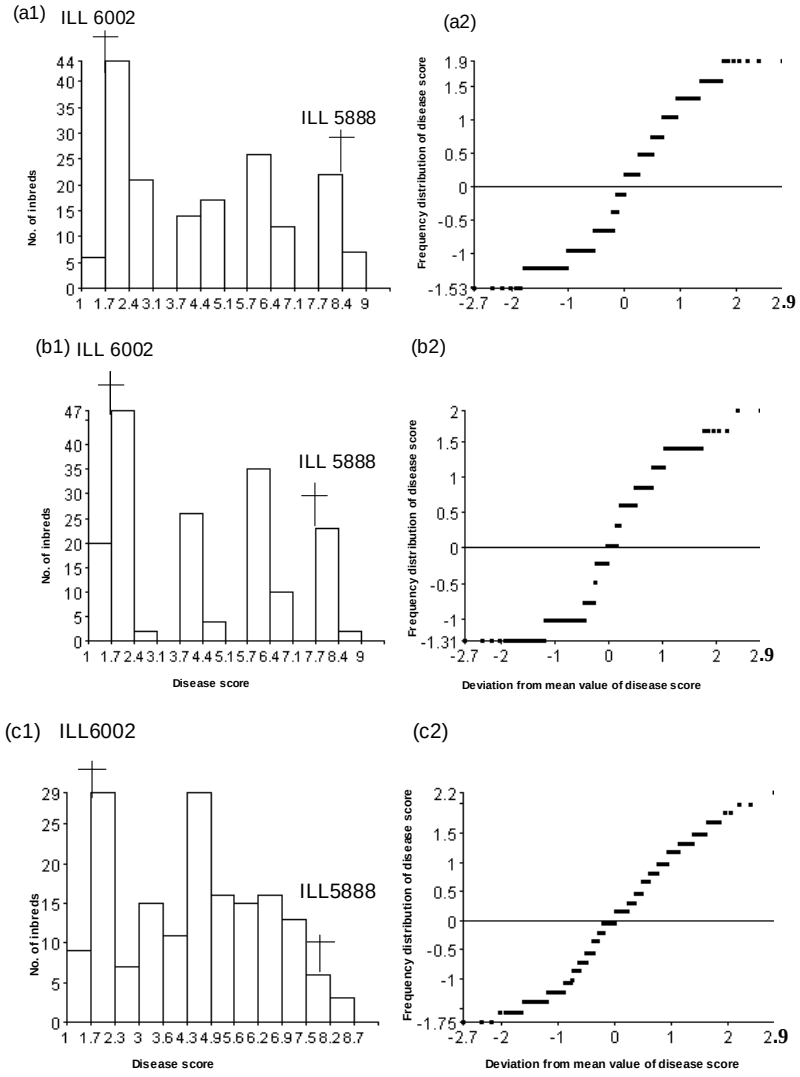

Figure 1. Frequency distribution (a1) 2006-2007 crop year, (b1) 2008-2009 crop year and (c1) pooled data of the two crop years, and normality test (a2) 2006-2007 crop year, (b2) 2008-2009 crop year, (c2)) pooled data of two crop years of stemphylium blight disease in lentil.

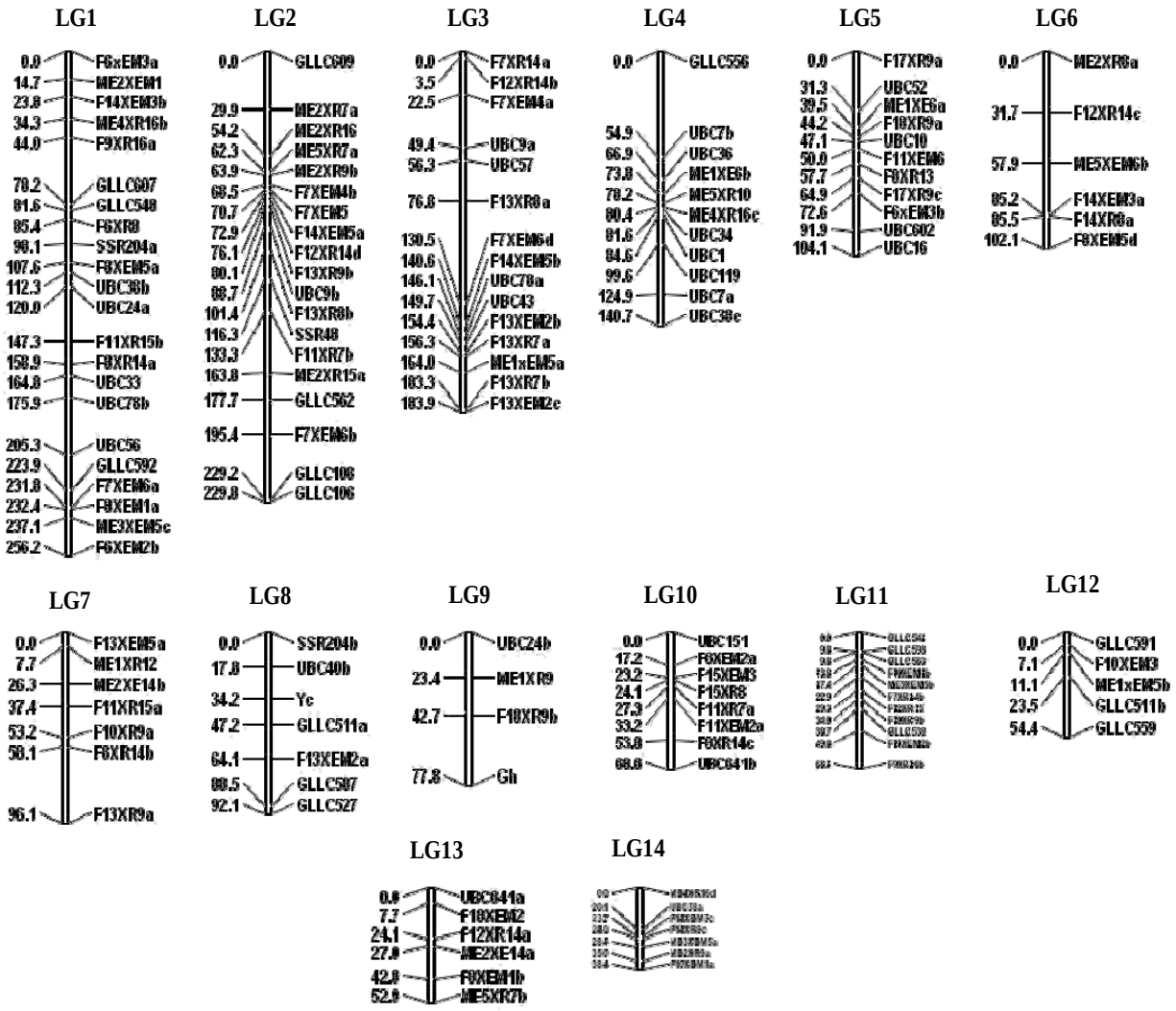

Figure 2. Intraspecific linkage map of lentil at a LOD score of 4.0 and at maximum recombination fraction 0.25. The linkage groups are named (LG1-LG14). Loci names are indicated on the right side and genetic distances are on the left side of the vertical bar. SSR markers are named as SSR or GLLC-SSR, RAPDs are UBC and SRAP are F or ME as forward and R or EM as reverse primer.

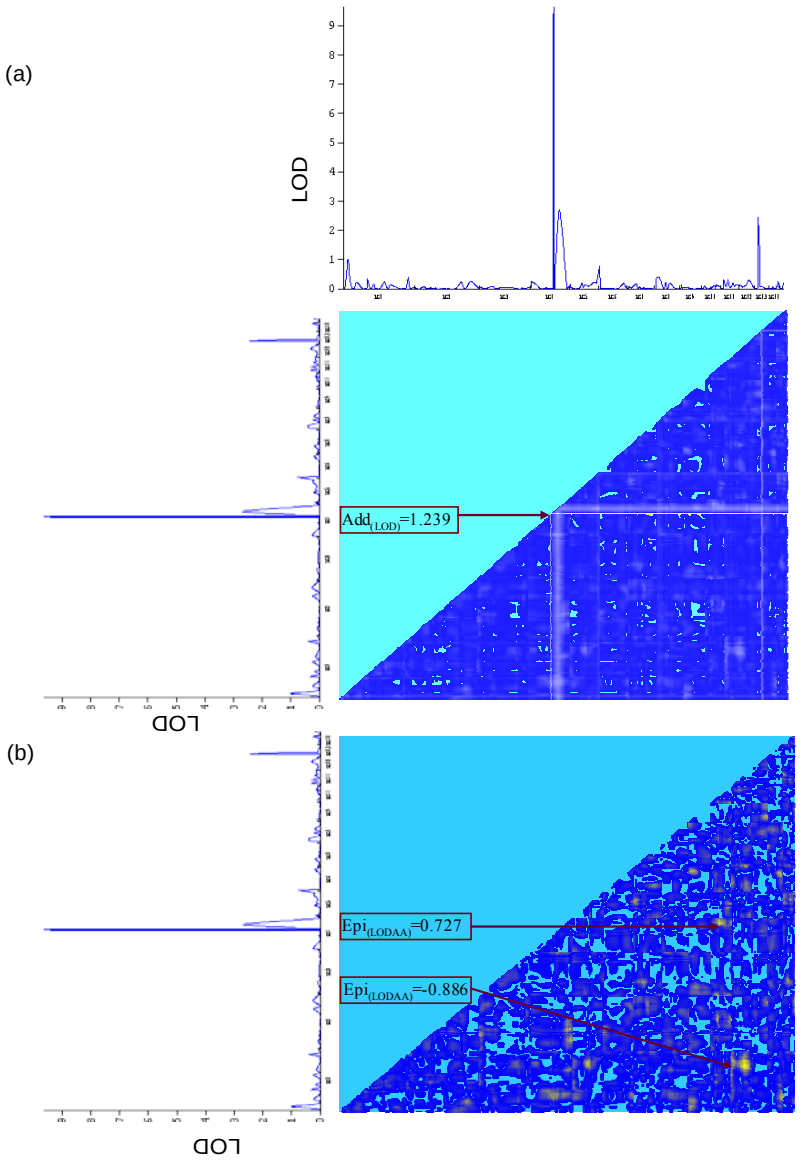

Figure 3. Contour profile showing (a) additive effects: $Add_{(LOD)}$ and (b) interaction effect, epistatic effect: $Epi_{(LODAA)}$ for the 2006-2007 crop year.

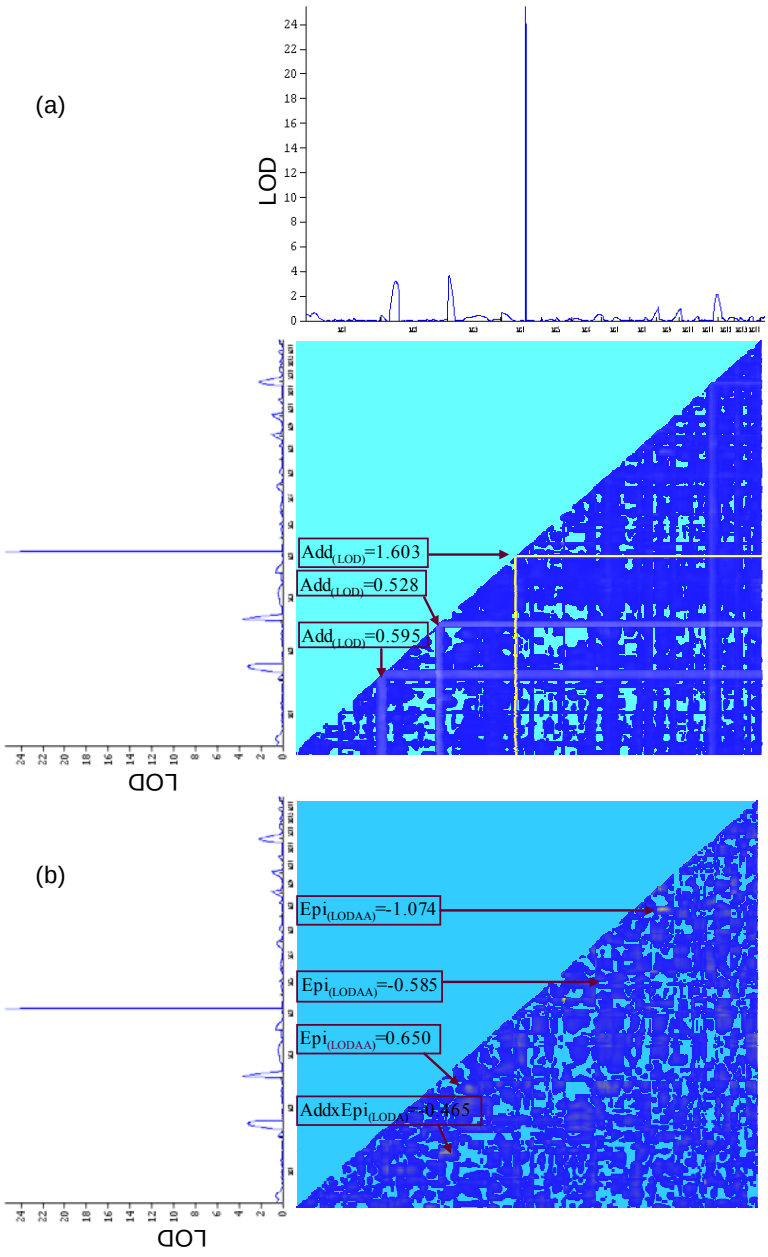

Figure 4. Contour profile showing (a) additive effects: $Add_{(LOD)}$, and (b) Interaction effect, epistatic effects: $Epi_{(LODAA)}$ and combined additive-epistatic effects: $AddxEpi_{(LODA)}$ for the 2008-2009 crop year.

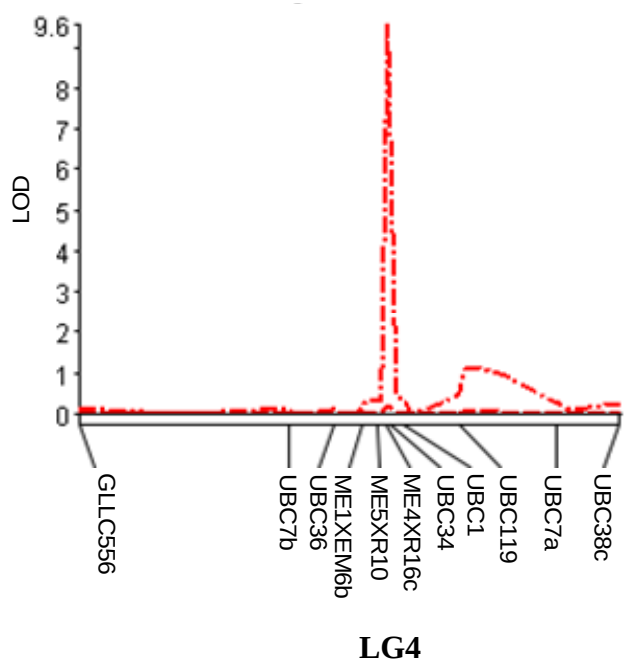

Figure 5. QTL and associated markers that conferred resistance to stemphylium blight for the crop year 2006-2007.

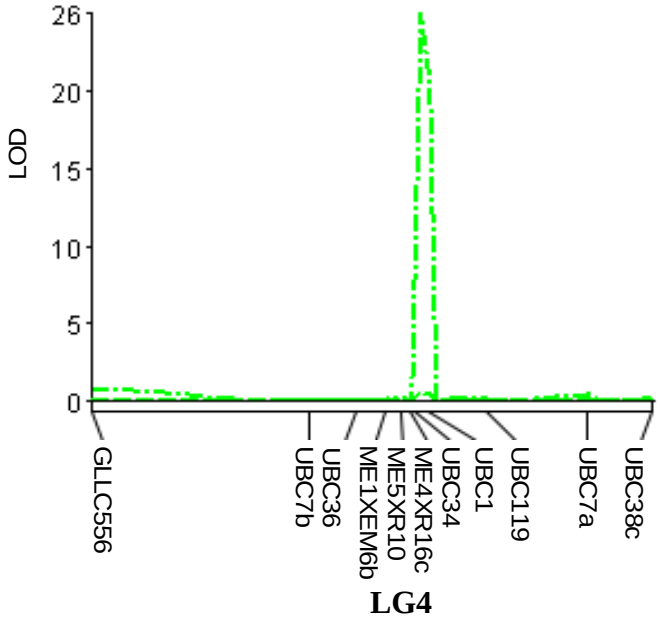

Figure 6. QTL and associated markers confer resistance to stemphylium blight for the crop year 2008-2009.

CHAPTER TWO

IDENTIFICATION OF MARKERS ASSOCIATED WITH GENES FOR RUST RESISTANCE IN *Lens culinaris* Medik.

ABSTRACT

Lentil rust caused by *Uromyces vicia-fabae* (Pers.) Schroet is one of the most important diseases of lentil in South Asia, North Africa and East Africa. This disease is usually observed during late flowering and early podding stages. Early infection accompanied by favorable environmental conditions can result in complete crop failure and huge economic losses. The losses impact human nutrition by reducing the availability of a major protein source in developing countries. Yield losses of 30 to 70% have been reported in some years in research plots. Therefore, breeding for resistance against this pathogen is one of the major challenges for the breeders in those regions. It is important to identify resistance sources and to determine the location of the genes for resistance in the lentil genome. Since field screening is often difficult due to the unpredictable nature of the disease, selectable molecular markers can be useful tools to assist lentil breeding and complement field screening and selection for resistance. To map the genes for resistance, a recombinant inbred line (RILs) population composed of 220 RILs was developed from a cross between a rust resistant line, ILL-4605, and a susceptible line from Bangladesh, ILL-5888. Phenotyping of the RIL population was carried out during 2006-2007 and 2008-2009 cropping seasons at the Pulse Research Center, Ishurdi, Bangladesh. There was a lack of uniformity of disease pressure in the 2006-2007 cropping year causing inconsistencies between replicates. Nevertheless, we were able to choose clearly resistant and clearly susceptible RILs for selective genotyping using markers previously placed on our lentil genetic map. One of the 62 markers used for selective genotyping proved to be linked to the gene for resistance. The identified sequence related amplified polymorphism (SRAP) marker, F7XEM4a, was estimated to be 7.9cm from the gene for resistance. The F7XEM4a marker could be used for marker assisted selection for resistance; however, additional markers closer to the resistance gene are

needed. Also, definitive placement of the resistance gene on the lentil genetic linkage map will require additional mapping using markers transferable from existing lentil linkage maps.

Key words: Rust, Selective genotyping, Linkage, Marker assisted selection.

INTRODUCTION

Lentil rust caused by *Uromyces vicia-fabae* (Pers.) Schroet, an obligate biotrophic fungal pathogen that infects a narrow range of living plant hosts and causes substantial yield losses annually in Bangladesh, India, Ethiopia, Morocco and Pakistan. Erskine and Sarker (1997) report that lentil rust causes up to 70% yield loss, although occasional complete crop losses were observed in epidemic years. The disease appears during the flowering and early podding stages in areas with dense crop canopies. In severe infections the leaves are shed and plants dry prematurely without producing seed or by having shriveled seed.

Lentil seed contaminated with pieces of rust infected debris with teliospores or infested grasses that surrounding fields appear to be sources of primary infection (Khare 1981, Agrawal et al., 1993). The amount of primary inoculum and the degree of buildup of secondary inoculum are important for disease development and severity. In some years, low disease severity was observed due to an environment unfavorable for inoculum growth. Yellow– white pycnidia and aecial cups develop on the lower surface of the leaflet and on pods singly or groups of circular forms. From the aceial cup, aceciospores germinate at 17-22°C and infect plants followed by the formation of secondary aecia or uredia when temperatures increase to about 25°C. Brown uredial pustules circular or oval shaped and 1mm in diameter develop on either surface of the leaves, stems or pods and later coalesce to form larger pustules. Late in the season, dark brown to black

telia develop on the stems and branches and the teliospore can survive at higher temperatures in summer and on dried lentil trash and become a source of infection the following cropping season.

For host colonization, rust requires the induction of a subset of fungal genes essential for infection. The infection mechanisms are sophisticated, and include the ability to detect stomata, the entry portal for many rust fungi, and to suppress host resistance responses. Genes of the plant host are also induced in response to infection, but the role and expression of the host gene is difficult to dissect because molecular tools such as a protocol for transformation and sequenced genomes are lacking for the rusts (Ayliffe, 2002).

Singh and Sokhi (1980) identified six pathotype s of rust on the basis of their differential reactions on different cutivars of lentil, pea and sweet pea. Singh et al. (1995) reported five races, and Conner and Berrier (1982b) detected 11 races of *U. viciae-fabae*. Conner and Berrier, (1982b) speculated that *Vicia*, *Lathyrus* and *Pisum* could be important sources of inoculum and perhaps pathogenic variants due to race specific resistance and selection pressure on the pathogen.

Most studies on genetics of rust resistance in lentil have revealed that resistance is monogenic and dominant (Sinha and Yadav, 1989; Singh and Singh, 1992). Kumar et al. (1997) reported that resistance to *Uromyces fabae* in five lentil genotypes (L 2991, L 2981, L 1534, L 178 and HPLC 8868) was governed by a single dominant gene; whereas in one genotype, Precoz, it was conditioned by two dominant genes. Chahota et al. (2002) reported that resistance to rust is controlled by duplicate, non-allelic and non linked dominant genes. Although a breakdown of rust resistance in lentil has not been reported, the likelihood of such an event in varieties with monogenic resistance cannot be ruled out (Negussiee et al. 2005). The objective of

this study was to determine the genetics of rust resistance in lentil, to locate the resistance genes on the lentil genetic linkage map, and to identify molecular markers that can be used in marker assisted selection for resistance.

MATERIALS AND METHODS

a) Recombinant inbred line development

A population of 220 recombinant inbred lines (RILs) was developed from a cross between a rust resistant line, ILL-4605 from ICARDA, and a susceptible line, ILL-5888, from Bangladesh. Initially, F_1 seeds were planted in pots to produce F_2 seeds which were then planted in flats (51cm X 36cm X 9cm) and advanced to F_7 by single seed descent. Plants were grown with a sandy loam soil mixed in equal proportions with an organic potting mix on a greenhouse bench using a controlled temperature (22°C day and 15°C night), humidity 25-30% and a 17 hour day length. The F_7 RILs were planted at Spillman Agronomy Farm of Washington State University, near Pullman for seed increase. 206 RILs produced a sufficient amount of seeds for phenotyping for rust resistance in Bangladesh at the Pulse research station, Ishurdi, Bangladesh in the 2006- 2007 and 2008-2009 cropping seasons.

b) Phenotyping

The RILs were scored for disease reaction according to qualitative and quantitative scale. The qualitative scale had two distinct classes, resistant and susceptible whereas the quantitative scale developed by Chen (2007) had 1-9 scale, whereby 1 = 0-10% leaf area infected, 3 = 11-30% leaf area infected, 5 = 31-50% leaf area infected, 7 = 51-70% leaf area infected, 9 = More than 70% leaf area infected. The F_7 derived RILs were planted in high Ganges river flood plain calcareous soil at the Pulse Research Station (PRC) , Ishurdi, Bangladesh for phenotyping in

2006-2007 and 2008-2009 crop years using a randomized complete block design with three replications. The individual plots were single rows 1m long and spaced 40cm apart.

c) Markers in identifying putative genes

Six types markers; 16 gene specific markers (EFGC, CysPr1, CTP, 6DCS, UDPGD, CYB6, tRALS, Histone, ATPsynth, Trypinhi, Vicillin, RGA (Resistance Gene Analogs), Lectin, RGA23Q1, isoflasynth1), 156 lentil SSRs (Simple Sequence Repeats) (ICARDA SSRs and GLLC SSRs), 14 pea SSRs (PSMPs), 14 CAPS (Cleaved Amplified Polymorphic Sequence) and dCAPS (Derived Cleaved Amplified Polymorphic Sequence) PCR based RFLP (Restriction Fragment Length Polymorphism) markers (C3, C6, C41, C65, C71, E8, E14, E25, E29, E30, E,31, P14, P57 and P68), 181 RAPD (Random Amplified Polymorphic DNA) and 270 SRAP markers were used to determine polymorphism between parents.

d) Selective genotyping

RILs with clear resistant and susceptible reactions were chosen and genotyped using markers that were polymorphic between the parents. Markers that were clearly associated with resistance in the selective genotyping experiment were used to genotype 196 RILs. The marker segregation between RILs and qualitatively phenotyped disease data were analyzed by chi-square for goodness of fit to the 1:1 expected Mendelian segregation ratio. Linkage between the putative markers and the putative resistance gene was estimated using Mapmaker Macintosh V2.0 (Lander et al., 1987).

RESULTS AND DISCUSSION

Phenotyping of the RILs in the 2006-2007 crop year disease using quantitative scale showed non-significant variation and block effect was also non-significant (Table 1). Some of

the lines escaped infection resulting in inconsistencies in disease score among replicates and the variation among RILs were not detected due to the average effect of the replicates.

Some of the RILs had consistent disease scores among replicates in the 2006-2007 season and those lines were used for selective genotyping to identify markers associated with putative genes for rust resistance. RILs 20, 33, 85, 153, 183, and 188 showed consistent disease reaction across replicates and were similar to the resistant parent, while RILs 51, 61, 92,125,174, 187 showed a consistent susceptible reaction similar to the susceptible parent. The experiment was repeated in 2008-2009 growing season at the same spot at the Pulse Research Center, Ishurdi, Bangladesh, but disease was not wide spread and only a few plants were infected; however, RIL 51, 92 and 187 had disease symptoms but the remaining RILs were free of the disease. Since *Uromyces fabae* is an obligate biotroph, it was speculated that the inoculum was not available in near proximity and inoculum build up was not sufficient to cause wide spread disease. Teliospores in the seed sample mixed with debris might be the cause of disease in 51, 92 and 187 inbred lines.

Six types of markers were used in finding polymorphism between parents. The gene specific markers and RFLPs did not show polymorphism between the parents. Pea SSRs showed the highest number of polymorphism (64%) followed by lentil SSRs (17.3%), SRAP (14.8%) and RAPD (12%) respectively (Table 2). RAPD and SRAP markers were used to identify putative markers and SSRs were used as anchor markers with previously reported lentil linkage maps.

Six consistent resistant and susceptible RILs described previously segregated clearly for the presence or absence of bands, respectively, for marker F7XEM4a. This marker, F7XEM4a is considered as putative marker for the rust disease resistance gene (Fig.1).

After selective genotyping and the identification of F7XEM4a as a possible marker linked to the rust resistance gene, a qualitative assessment of 2006-2007 disease score of the RILs was used to determine the linkage map location of the rust resistance gene. RILs were scored qualitatively as susceptible based on presence of disease in at least one replicate and resistant if there was no disease in any of the replicates. The justification for this scoring procedure was the likelihood of escape from the disease due to uneven inoculum dispersal.

The 196 RILs segregated in the expected 1:1 ratio (93:103) of presence: absence of the F7XEM4a marker (χ^2=0.51*). The RILs segregated in the expected 1:1 ratio (104:92) for rust scores (χ^2=0.73*) (Table 3). The ratio of resistant to susceptible lines is consistent with reports of monogenic control of resistance reported by Negussiee et al. (2005). The linkage analysis between the marker and rust resistance showed that rust resistance gene is located 7.9cM from the F7XEM4a marker (Fig. 2). All resistant RILs had bands at the 600bp region; however, 11 resistant RILs did not have the marker. These latter RILs may actually be susceptible but escaped disease infection in all replicates and were scored as resistant. Additional phenotyping under a more complete rust infection is needed to verify the scoring of the RILs for infection. The F7XEM4a marker is located on our linkage group 3 (Ref. Chapter1) with adjacent and flanking markers F12XR14b and UBC9a. These latter markers were not polymorphic in the rust mapping population.

The F7XEM4a marker may be useful in a marker assisted selection program to facilitate breeding for resistance to rust. However, additional markers in the vicinity of F7XEM4a are needed to confirm the position on the lentil linkage map and to provide additional markers closer to the resistance gene for use in breeding. Confirmation of the F7XEM4a marker for resistance

breeding is needed as well as additional phenotypic data to confirm these results. Placement of the rust resistance gene on a composite linkage map of lentil is also needed.

CONCLUSION

Selective genotyping identified a SRAP marker, F7XEM4a, closely linked to a gene for rust resistance. The marker is located 7.9cM from the resistance gene on our linkage group 3. This marker could be utilized for marker assisted breeding. The linkage map, specifically linkage group 3, should be saturated with co-dominant markers to identify additional markers more closely linked to the gene for resistance. Markers used in other linkage studies of lentil that are polymorphic in our population are possibly a good source of potential markers. Markers identified through selective genotyping will be considered as putative markers and used to increase marker density in the region of the resistance gene. The F7XEM4a SRAP marker can be used for marker assisted selection in a breeding program provided that the breeding populations segregate concurrently for resistance and the marker. Also, increased marker density in the region of the resistance gene will provide additional marker choices for use in a wide range of lentil crosses.

Acknowledgements: This work was supported by USAID-ICARDA linkage project. We are indebted to Sheri Rynearson, Tony Chen and Tracie Anderson for laboratory support and the Director of Pulse Research Center, Ishurdi, Bangladesh for setting up field experiment in their station.

REFERENCES

Ayliffe, M.A., J.K. Roberts, H.J. Mitchell, R. Zhang, G.J. Lawrence, J.G. Ellis, and J.P. Tony. 2002. A plant gene up-regulated at rust infection sites .Plant Physiology 129: 1–12.

Chahota, P.K., V.P. Gupta and S.K. Sharma. 2002. Inheritance of rust resistance in lentil. Indian J Genet Plant Breeding 62: 226-227.

Conner, R.L., and C.C. Bernier. 1982. Race identification of in *Uromyces vicia-fabae*. Phytopathology 72: 687-689.

Erskine, W., and A. Sarker. 1997. Lentil: the Bangladesh breakthrough. ICARDA Carvan No. 6.

Khare, M.N., B. Bayaa and S.P.S. Beniwal. 1993. Selection methods for disease resistance in lentil. In: K.B. Singh and M.C. Saxena (Eds.), Breeding for Stress Tolerance in Cool-Season Food Legumes.Wiley, Chichester, U.K. 107-121.

Kumar, V., B.M. Singh and S. Singh. 1997. Genetics of lentil resistance to rust. Lens Newsl 24(1/2):23-25.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. J. Daly, S. E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174- 181.

Negussie, T., Z.A. Pretorius, and C.M. Bender. 2005. Component of rust resistance in lentil. Euphytica 142: 55-64.

Sarker, A., R.S. Malhotra, S. Khalil, and W. Erskine. 2002. Food legume improvement at ICARDA, ICARDA Annual Report, Aleppo, Syria.

Singh, K., H.S. Rawal, S. Singh, and R.K. Gumber. 1995. Physiological specialization in *Uromyces fabae* causing rust of lentil. Lens Newsl 22: 46-47.

Singh, J.P., and I.S. Singh. 1992. Genetics of rust resistance in lentil (*Lens Culinaris*). Indian J Agric Sci 62:337-338.

Singh, K., and T.S. Sandhu. 1988. Screening of cultivars of lentil for resistance to rust. Lens Newsl 15:28-29.

Singh, S.J., and S.S. Sokhi. 1980. Pathogenic variability in *Uromyces viciae-fabae*. Plant Dis 64: 671-672.

Sinha, R.P., and B.P. Yadav.1989. Inheritance of resistance to rust in lentil. Lens Newsl 16: 41.

Table 1. ANOVA of rust disease scores in RILs of lentil in 2006-2007 cropping year in Pulse Research Center, Ishurdi.

Source	DF	Sum of Squares	Mean Square	F Value
Lines	205	1588.94	7.75	0.76^{ns}
Block	2	0.59	0.29	0.03^{ns}
Error	410	4183.41	10.20	
Total	617	5772.94		

Table 2. Six marker types showing different percentage of polymorphism between parents ILL-5888 and ILL-4605.

Marker type	No. of markers	Polymorphic markers	% polymorphism
Gene specific	15	0	0
SSR	156	27	17.3
RFLP	16	0	0
PSMPs	14	9	64
RAPD	181	22	12
SRAP	270	40	14.8
Total	652	98	15.0

SSRs= Simple Sequence Repeats, RFLP= Restriction Fragment Length Polymorphism, RAPD= Random Amplified Polymorphic DNA, SRAP= Sequence Related Amplified Polymorphism, PSMPs are pea microsatellite markers.

Table 3. Joint segregation of recombinant inbred lines from the ILL-4605 x ILL-5888 for reaction of rust and F7XEM4a SRAP marker.

RILs	SRAP markers		Total	Expected segregation	χ^2 value
	Present (+)	Absent (-)			
Resistant (R)	93	11	104	1:1	0.73*
Susceptible (S)	0	92	92		
Total	93	103		1:1	0.51*
R+: R-: S+: S- = 93: 11: 0: 92				1:1:1:1	155.7^{ns}

* $P < 0.950 > 0.750$

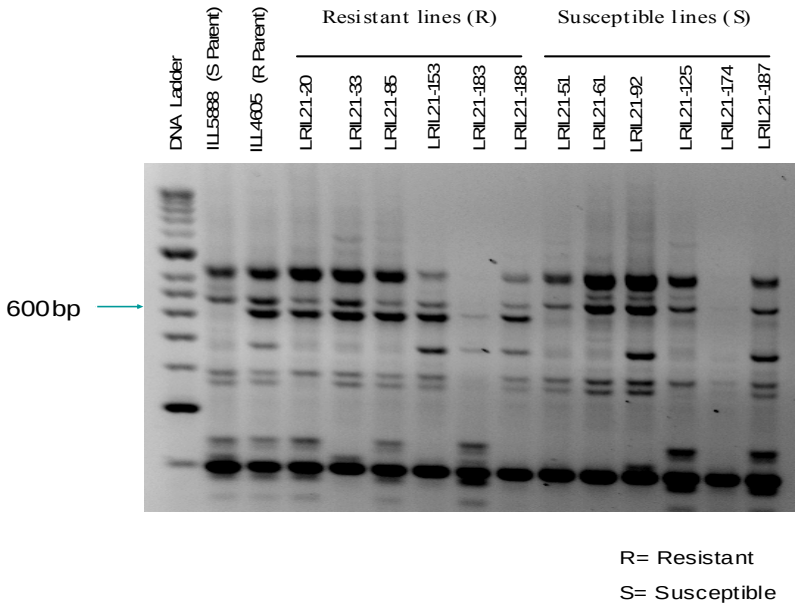

Figure 1. Selective genotyping for identifying putative markers linked to rust resistance. Marker F7XEM4a, (indicated by the arrow at 600bp) is present in the resistant parent and also in the resistant RILs of the (ILL-5888 x ILL-6002).

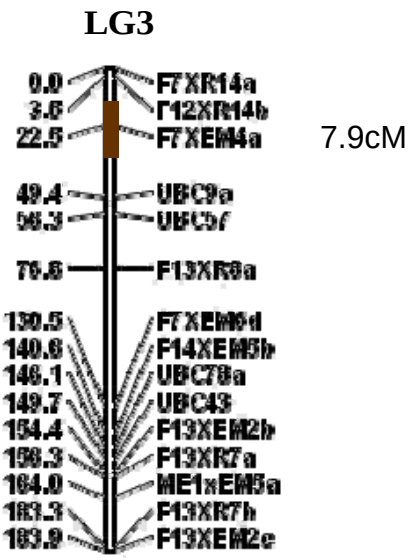

Figure 2. Location of rust resistance genes in lentil in linkage group 3.

CHAPTER THREE

INHERITANCE AND LINKAGE MAP POSITIONS OF GENES CONFERRING AGRO-MORPHOLOGICAL TRAITS IN *Lens culinaris* Medik.

ABSTRACT

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an important legume crop and an important source of dietary protein in human diets and animal feed. Agro-morphological traits have immense importance in breeding lentils for higher yield and yield stability. In order to identify and map the genes for stemphylium blight (caused by *Stemphylium botryosum* Wallr.) resistance, an F_6 derived F_7 RIL population composed of 206 lines was developed by crossing ILL-5888 (Uthfola cultivar name and described as a *pilosae* type) and ILL-6002 (pure line selection from 'Precoz'). The parents contrasted for agro-morphological traits including days to flowering, plant height, seed diameter, 100 seed weight, and cotyledon color and growth habit. Three QTLs for days to flowering were detected with additive and epistatic effects on the trait. One QTL for days to flowering, $QLG4_{83,}$ accounted for an estimated 20.2% of the phenotypic variation, while $QLG1_{24}$ x $QLG13_{52}$ and $QLG4_{84}$ x $QLG13_{8}$ accounted for 15.6% and 24.2% of the variation through additive and epistatic effects. For plant height, epistatic effects accounted for most of the phenotypic variation but the main effect of one QTL, $QLG8_4$, accounted for 15.3% of the phenotypic variation. For seed diameter, three QTLs were detected and one QTL, $QLG4_{82}$, accounted for 32.6% of the phenotypic variation through additive effects. For 100 seed weight, five QTLs were identified with significant additive effects and four with significant interaction effects. The main effect of one QTL, $QLG4_{82}$, also accounted for 17.5% of the phenotypic variation in seed diameter. Therefore, the QTL region at the 82-83cM position of linkage group 4 ($QLG4_{82\text{-}83}$) accommodates three QTLs accounting for 20.2%, 32.6% and 17.5% of the phenotypic variation for days to flowering, seed diameter and 100 seed weight, respectively. In addition, these traits are correlated (r values of days to flowering with seed diameter and 100 seed weight are 0.393 and 0.411, respectively, seed diameter with 100 seed

weight is 0.884). The gene rich QTL region ($QLG4_{82-83}$) was flanked by two random amplified polymorphic DNA (RAPD) markers UBC 34 and UBC1 at 0.4-1.4 and 1.6-2.6cM, respectively. Further, growth habit and cotyledon color are controlled by single genes and prostrate is dominant to erect plant type and red cotyledon was dominant to yellow cotyledon. The QTL information presented here will assist in the selection of breeding lines for early maturity, upright growth habit and improved seed quality.

Key words: Agromorphological traits, epistasis, additive effect, QTL analysis

INTRODUCTION

Agro-morphological traits have immense importance in crop breeding. Crop adaptation, field performance, market value and demands for specific uses are major factors that drive breeding goals. Linkage maps and QTL analysis are valuable tools for plant breeders to improve breeding efficiency by tagging genes with markers and analyzing the association between markers and traits. The inheritance of quantitative traits and tagging genes such as days to flowering, plant height, seed diameter and seed weight, and qualitative trait genes such as growth habit and cotyledon color in lentil (*Lens culinaris* Medikus subsp. *culinaris*) will help breeders in the selection process and understanding interrelationships among traits.

Lentil is quantitative long day plant flowering in progressively longer days (Roberts, et al.1986). It is often hard to determine days to maturity at or near the end of a crop season due to weather conditions. In the Canadian prairies, one of the important breeding goals is to develop varieties that flower within an optimum period of time to provide improved and stable seed production (Tullu et al. 2008). It has been reported that soybean (*Glycine max*) breeders select lines for optimum maturity based on days to flowering (Tulmann and Alves, 1997). Sarker et al.

(1999) reported that flowering time is sensitive to photoperiod and temperature and that a more complete understanding of genetic control of flowering time in lentil is needed. Roberts et al. (1986) proposed four developmental phases of flowering as: pre-emergence, pre-inductive, inductive and post-inductive. When lentil plants were transferred from short days (either 8 or 10 h) to long days (16 h), or *vice versa*, the first two phases and the last are insensitive to photoperiod, but are probably sensitive to temperature. So, it is evident that a complex phenomenon controls the days to flowering in lentil.

Plant height of lentil usually ranges from 25 to 30cm for the majority of genotypes, but may vary from extremes of 15 to 75cm depending on genotype and environment (Saxena and Hawtins, 1981). Most lentils grown in South Asia, Middle East and Africa are land races. These are generally short in plant stature, prostrate in growth habit, lack uniformity in pod maturation, have a high incidence of pod shattering and are low yielding. Tall upright lentil cultivars with high basal pod positions are always preferred by farmers for mechanical harvesting (Muehlbauer, 1981). Sakar (1983) reported three genes were responsible for variation in plant height from the cross of the two lentil cultivars, Laird and Precoz. Tullu et al. (2008) reported that both 'Eston' and PI 320937 have genes that contribute to reduced plant height in lentil. QTL for plant height detected from the data of one geographical location was different from the QTLs of another location, though there was non-significant genotype x location interaction. They concluded that more powerful statistical methods are required for the detection of minor QTLs.

Growth habit has received a great deal of attention from breeders attempting to develop cultivars with more upright stature that are also lodging resistant and adaptable to mechanized harvest. Ladizinsky (1979) indicated that a single gene with incomplete dominance controls growth habit in lentil.

Uniformity of seed size, shape and color is important for marketing of lentil. A wide range of lentil cultivars are used throughout the world. Small diameter red cotyledon types accounts for most of the lentil production followed by the large-seeded and small-seeded yellow cotyledon types. Lentil seeds are lens shaped and generally weigh between 20 and 80 mg. Their diameter generally ranges from 2 to 9 mm. Seed size differs according to genotype and researchers frequently follow the classification of Barulina (1930) who grouped lentils as macrosperma with large seeds that range from 6 to 9mm diameter, and microsperma with smaller seeds that range from 2 to 6mm in diameter. The macrosperma types are common to the Mediterranean basin and in the western hemisphere, while the microsperma predominate through the Indian subcontinent and in parts of the Near East (Saxena and Hawtin, 1981). It has been reported that dry seed wt of lentil is controlled by two genes (Sakar, 1983); whereas polygenic control of seed weight was reported by Abbo et al. (1992). Cotyledon color of lentil can be red/orange, yellow or green. Large green lentils with yellow cotyledons are marketed to countries of southern Europe, particularly Spain, Italy and Greece and small red cotyledon type is exported to South Asia and the Middle East (Muehlbauer et al. 2009). The first report of genetics of lentil cotyledon color were studied by Tschermak (1928) and by Wilson et al. (1970) and confirmed that cotyledon color is controlled by a single gene and red/yellow cotyledon is dominant over yellow. Singh (1978) and Slinkard (1978) reported that red cotyledon color is completely dominant over green and yellow. Sharma and Emami (2002) detected monogenic and digenic control of cotyledon coloration in lentil. In their study they discovered that crosses between orange and green cotyledon parents showed monogenic segregation with complete dominance of orange over green pigment but digenic segregation was observed between light green and dark green cotyledon. Double recessive homozygous condition (*yy bb*) is responsible

for light green coloration and the presence of the *Dg* gene causes dark green coloration irrespective of homozygous dominant or recessive *Y or B* genes.

The objectives of the present study were to identify regions of the lentil genome associated with agro-morphological traits including days to flowering, plant height, growth habit, seed diameter, seed weight and cotyledon color that could be utilized in marker assisted breeding and to improve our understanding of the genetics of these traits.

MATERIALS AND METHODS

Development of inbred lines:

In order to identify and map the agro-morphological trait genes, a lentil mapping population [F_6 derived F_7 recombinant inbred lines (RILs)] that was previously developed to determine the genomic locations of the genes for stemphylium blight (caused by Stemphylium botryosum Wallr.) resistance were used. The mapping population is comprised of 206 RILs from the cross ILL-5888 ('Uthfola' the popular name and described as a *pilosae* type, short stature with prostrate growth habit) by ILL-6002 (developed as a pure line selection from Precoz, tall and erect in growth habit). These parents were contrasting for the agro-morphological traits (quantitative and qualitative traits) under study.

Phenotyping:

The parents and the mapping population of 206 RILs were grown at the Washington State University Spillman Agronomy Farm near Pullman, Washington (46º73' N latitude and 117º73' W longitude) in the 2008 cropping year and at Ishurdi, Bangladesh (24º8' N latitude 92º5' E longitude) in the 2008-2009 cropping year. Soil at the Spillman Farm is a silt loam and the soil at Ishurdi, Bangladesh is a high Ganges river flood plain calcareous soil. The experimental design

was a Randomized Complete Block with three replications. Individual plots were single rows 1 meter long and spaced 60cm apart and within row plant spacing of approximately 3cm. Flowering was recorded as number of days from planting to 50% of the plants in the plot with at least one open flower. Plant height was taken at the 50% flowering stage and measured from the soil surface to the tip of the central axis. Seed diameter was measured by using a sliding calipers and 100 seed weight was taken using a Mettler digital balance. Growth habit data were taken based on plant canopy spreading and cotyledon color was determined visually. All the qualitative and quantitative trait data taken in the Spillman farm were used for statistical analysis. The analysis of variance (ANOVA) of the quantitative traits was done using SAS 9.1.

Genotyping, Linkage analysis and QTL mapping:

DNA extraction and genotyping were performed following protocols described in chapter 1. Mapmaker Macintosh V2.0 was used for linkage analysis and QTL analysis for quantitative traits were conducted following inclusive composite interval mapping (ICIM) method with the following software, QTL ICIMapping v2.2 (Li et al., 2008) and Q gene 4.2.3.(Nelson, 1997).

RESULTS AND DISCUSSION

The agro-morphological data of two parents (ILL-5888 and ILL-6002) and 206 RILs were collected at Spillman Agronomy Farm near Pullman in 2008 cropping year and in 2008-2009 at Ishurdi, Bangladesh. It is observed that environment played a major role in flowering at the two test locations. Out of 206 RILs, 44 did not flower at the Ishurdi, Bangladesh; whereas all the RILs flowered at Pullman, WA. Parents and RILs also showed significant differences in flowering time between the two locations (data not shown). The ANOVA showed highly significant differences between the parents and among RILs for the four quantitative traits

analyzed (Table 1). Highly significant (P<0.001) correlations exist between seed diameter and 100 seed weight, and days to flowering and seed diameter and 100 seed weight (Table 2). Plant height was highly significantly correlated with 100 seed weight (P<0.001) and significantly correlated with days to flowering and seed diameter (P<0.05).

To map the genes, an intra-specific lentil map of 14 linkage groups consisted of 23 SSRs (Simple Sequence Repeats), 30 RAPDs and 108 SRAPs (Sequence Related Amplified Polymorphisms) and two morpho-physiological markers (cotyledon color and growth habit) were developed (Ref. Chapter 1). The QTLs and epistatic interactions were identified in different linkage groups, but one of the QTL, $QLG4_{82\text{-}83}$ accounted significant phenotypic variation for days to flowering, seed diameter and 100 seed weight.

I. Qualitative trait loci

a) Cotyledon color:

ILL-5888 and ILL-6002 have red and yellow cotyledon color, respectively. The RILs segregated into a 1:1 ratio of red to yellow (Table-3). The clear 1:1 segregation ratio of the RILs was consistent with reports of the inheritance of cotyledon color by Tschermak (1928) and Wilson et al. (1970) i.e. cotyledon color is controlled by a single gene. In $F_{1,}$ all the seeds were red and the F_2 segregated in a 3:1 ratio of red to yellow (99:32), indicating that red cotyledon is completely dominant over yellow cotyledon.

Singh (1978) and Slinkard (1978) also reported that red cotyledon color is completely dominant over green and yellow. The cotyledon color gene (*Yc*) was positioned on LG8 and flanked by RAPD marker UBC40b and SSR marker GLLC511a at 16.4cM and 13cM from *Yc*, respectively (Fig. 1a).

b) Growth habit:

ILL-5888 is a prostrate variety while ILL-6002 has an erect growth habit. The RILs segregated in a 1:1 ratio (Table 3). Based on segregation of the RILs, it can be inferred that a single gene controls plant growth habit in this population. The F_1s (ILL-5888 x ILL-6002) had a prostrate growth habit indicating that prostrate is dominant over erect. The gene for growth habit (*Gh*) is positioned on LG9 at the 77.8cM position and 35.1cM apart from the F18XR9b SRAP marker, which is considered as loosely linked with the gene (Fig. 1b).

Ladizinsky (1979) made crosses within and between lines of *L. culinaris* and *L. orientalis* that differed for growth habit: erect tall with few branches, erect bushy with many branches, and prostrate. He reported that prostrate growth habit was incompletely dominant over erect growth habit. Emami and Sharma (1999) also showed that prostrate is dominant over erect growth habit. On the contrary, Kumar (2002) and Mishra (2004) reported erect growth habit as completely dominant over prostrate type. It is our speculation that multiple alleles may confer growth habit, so different inheritance patterns may be observed in different crosses.

II. Quantitative traits and QTLs

a) Days to flowering:

ILL-5888 flowered in 53 days compared to 60 days for ILL- 6002. The range for days to flowering among RILs was 47 to 58 days with a mean of 51.4±3.4 days (Table-1). The frequency distribution of the RILs and the normality test of days to flowering showed two discrete classes and the absence of a normal distribution pattern (Fig. 2 a1, b1). Based on the distribution it could be inferred that one major and some minor genes and interactions were important for determining days to flowering in this population.

The ICIM analysis for days to flowering (Table 4, Fig. 3 a1 b1) showed the presence of additive and epistatic interaction effects that accounted for a significant amount of phenotypic variation. Three significant QTLs were detected with two on linkage group 4 and one on linkage group13. The QTL detected at the 83cM position of linkage group 4 ($QLG4_{83}$) showed significant additive effects (1.506) that accounted for 20.2% of the phenotypic variation. The flanking markers, UBC34 and UBC1, were 1.4 and 1.6cM from $QLG4_{83}$. The other two QTLs, $QLG4_7$ and $QLG13_{50}$, accounted for 14.4% and 10.4% of the phenotypic variation, respectively. QTL, $QLG4_7$ was 7cM from the GLLC 556 marker on linkage group 4 and $QLG13_{50}$ was flanked by two SRAP markers, ME5XR7b and F8XEM58b on linkage group 13 separated by 2.9cM and 7.2cM, respectively.

LOD_A determines the significance of total variation at the interaction data point whereas; the LOD_{AA} value indicates epistatic effects between QTLs. The $QLG4_{84}$ x $QLG13_8$ and $QLG1_{24}$ x $QLG13_{52}$ interactions accounted for 24.2% and 15.6% of the phenotypic variation, respectively, and were due to combined additive and epistatic effects. Five other QTL interaction pairs had significant epistatic effects that accounted for 4.6 to 8.0% phenotypic variation each but the additive effects of the individual QTLs were non-significant (Table 4, Fig. 3 b1). It should be noted that the $QLG4_{84}$ x $QLG13_8$ interaction probably involves the main effect QTL $QLG4_{83}$ and the $QLG1_{24}$ x $QLG13_{52}$ interaction probably involves the QTL $QLG13_{50}$.

Erskine et al. (1990 a, 1994) found that lentil germplasm of Indian origin are more sensitive to temperature and less responsive to photoperiod in flowering and so, temperature fluctuations may affect the flowering in Bangladesh experiment. Study of the inheritance of flowering in Precoz under both Indian and Syrian environments determined that a dominant gene *Sn* played a major role in early flowering (Sarker et al. 1999b).

Tahir et al. (1994) identified four QTLs on different linkage groups and Sarker et al. (1999) reported single and polygenic systems of control of days to flowering based on F_2 segregation in different lentil crosses. Tullu et al. (2008) identified two QTLs (LG4 and LG12) from one location data and five QTLs (LG1, LG4, LG5, LG9 and LG12) from another location data for days to flowering.

We could not compare our QTLs for days to flowering with the QTLs identified by Tullu et al. (2008) because the UBC and SSR markers they were using were not polymorphic in our population. But all the QTL mapping studies on lentil indicate the presence of more than two QTLs for days to flowering. In our study, three significant QTLs were detected, $QLG4_{83}$, $QLG4_{7}$ and $QLG13_{50,}$ that have additive effects and accounted for 45% of the phenotypic variation, and one QTL, $QLG4_{83,}$ alone accounted for 20% of the phenotypic variation. The $QLG4_{84}$ x $QLG13_{8}$ and $QLG1_{24}$ x $QLG13_{52}$ interactions accounted for 24.2 and 15.6%, respectively, of the variation through combined additive and epistatic effects. We found that the 83-84cM position on linkage group LG 4 and the 50-52cM position on LG 13 were the major regions and accounted for significant phenotypic variation for days to flowering.

b) Plant height:

The RILs varied in height from 15.7cm to 34.3cm with a mean of 25±3.8 (Table 1). Frequency distribution and the normality test for plant height showed the presence of a normal distribution (Kolmogorov-Smirnov = 0.049) and indicated polygenic control (Fig. 2 a2, b2).

A significant QTL (LOD= 4.9) was detected on linkage group 8 at the 4cM position ($QLG8_4$) and the closest marker, SSR204b, was 4cM away. QTL $QLG8_4$ accounted for an estimated 15.3% of the phenotypic variation for plant height (Table 5, Fig. 3 a2).

The epistatic effects of $QLG3_{76}$ x $QLG8_{36}$, and $QLG3_{82}$ x $QLG4_{6}$ accounted for 17.6 and 17.3% phenotypic variation, respectively, but additive effects of the interacting QTLs were insignificant (Table 5, Fig. 3 b2). The interaction of $QLG1_{44}$ x $QLG8_{14}$ accounted for an estimated 14.6% of phenotypic variation by combined additive and epistatic gene action. Six other pairs of QTLs displayed epistatic interactions each affecting 6.8 to 10.7% of the phenotypic variation.

Epistatic effects of QTLs accounted for a major portion of the phenotypic variation for plant height in our population. Genes close to F13XR8 and *Yc* markers played a major role for the $QLG3_{76}$ x $QLG8_{36}$ interactions, whereas, for the $QLG3_{82}$ x $QLG4_{6}$ interactions, genes close to the same SRAP marker F13XR8 and GLLC 556 accounted for a significant portion of the variation. This raises the question as to whether $QLG3_{76}$ and $QLG3_{82}$ are really separate or distinct QTLs.

Tahir et al. (1994) reported that a gene linked to the *Aat-p* locus was responsible for increased plant height. Tullu et al. (2008) reported that PI 320937 is taller than 'Eston' but both contributed to reduced height of lentil plants and they identified different QTLs of plant height at two locations, though there is a non-significant genotype x environment interaction. They concluded that the chance of simultaneous detection of QTLs at both environments were small due to lack of powerful statistical methods to detect minor QTLs.

c) Seed diameter:

The parents, ILL-5888 and ILL-6002, had seed diameters of 3.7mm and 6.5mm, respectively, and all the RILs were intermediate to the parents. The highest and the lowest seed diameter of the inbred lines were 4.0 and 6.3mm, respectively, with a mean of 4.9±0.5mm (Table 1). Frequency distribution of seed diameter of the RILs is bimodal but continuous (Fig.4 a1, b1)

indicating that a single major gene or QTL along with minor QTLs was involved in determining seed diameter. Three different QTLs, $QLG1_{112}$, $QLG4_{82}$, $QLG5_{98}$, were detected for seed diameter on LG1, LG4 and LG5, respectively (Table 6). $QLG4_{82}$, at the 82cM position of linkage group 4, accounted for 32.6% of the phenotypic variation through large additive effect of 0.293 with a LOD score of 22.2. The $QLG4_{82}$ QTL has the most significant effect by far, and may be responsible for the bimodal frequency distribution of the mapping population observed for this trait (Fig. 4 a1). Two flanking markers, UBC34 and UBC1, were 0.4cM and 2.6cM from the QTL, respectively. The other two QTLs, $QLG1_{112}$ and $QLG5_{98}$ accounted for 4.5 and 3.6% of the phenotypic variation for seed diameter, respectively. Significant epistatic interactions of $QLG2_{62}$ x $QLG2_{132}$, $QLG2_{62}$ x $QLG2_{136}$ and $QLG13_0$ x $QLG14_0$ with relatively minor effects were observed (Table 6, Fig. 5 a1, b1).

Cubero, (1984) reported that a polygenic system governs seed shape and size in faba bean (*Vicia faba*). In chickpea (*Cicer arietinum*), small seed size was found to be dominant in desi x kabuli crosses (Smithson et al., 1985), but Niknejad et al. (1971) found polygenic control of seed size and partial dominance for large seededness. Davis et al., 1985 reported three genes control the seed size in peas (*Pisum sativum*). In our study, one major QTL with two minor QTLs and three minor epistatic interactions indicates that genetic control of seed diameter in lentil is controlled by a combination of genetic effects. Dominant/recessive relationships cannot be determined from our data.

d) 100 seed weight:

Seed weight is a major yield component. The 100 seed weight of ILL-5888 was 2.16 g and ILL-6002 was 5.59 g. The range of 100 seed weight of the RILs was 2.0 to 4.9 g with a mean of 3.2±.06g (Table 1). As with seed diameter, the 100 seed weight of the RILs were

intermediate to the two parents. The frequency distribution showed a skewed distribution toward light seed (Fig 4 a2, b2). Five QTLs for 100 seed weight were identified on four linkage groups (LG1, LG4, LG5 and LG8) that through additively accounted 5.6 to 17.5% of the phenotypic variation. Two QTLs, $QLG4_{82}$ and $QLG1_{113}$ accounted for 17.5% and 12.8% of the phenotypic variation with a LOD score of 15.3 and 11.5, respectively. $QLG4_{82}$ was flanked by UBC34 and UBC1 at 0.4 and 2.6cM, respectively, and $QLG1_{113}$ was flanked by UBC38b and UBC 24a at 1cM and 7cM, respectively. The epistatic interactions of $QLG2_{158}$ x $QLG2_{210}$, $QLG4_{22}$ x $QLG4_{56}$, $QLG5_{74}$ x $QLG5_{78}$ and $QLG5_{76}$ x $QLG5_{84}$ accounted for a significant portion of the phenotypic variation, each accounting for about 9% of the variation (Table 7, Fig. 5 a2, b2).

Genetics of seed weight of common bean (*Phaseolus vulgaris*) has been under investigation since the early studies of Johannsen (1903). According to Motto et al. (1978) based on classical quantitative genetics study, seed weight of common bean is quantitatively inherited and affected by at least ten genes with additive effects . In mungbean (*Vigna radiate)*, seed weight is controlled by genes with additive and non-additive action and low seed weight is dominant (Imrie et al., 1985).). It has been reported that dry seed weight of lentil is controlled by two genes (Sakar, 1983). Abbo et al. (1992) found that seed weight of lentil is under polygenic control with additive and dominant gene action and partial dominance of low seed weight alleles. In our study, two QTLs accounted for relatively high levels of variation supporting Sakar's (1983) findings and the frequency distribution was skewed toward low seed weight and indicated the polygenic nature of the control of seed weight and partial dominance for low seed weight supporting Abbo et al.'s (1992) findings.

CONCLUSION

Agro-morphological traits have immense importance in breeding lentils for high yields, yield stability and market acceptability. The ICIM (Inclusive Composite Interval Mapping) method opens the door for understanding quantitative inheritance with epistatic interactions. Now, it is possible to identify digenic interactions while developing polygenic interaction models to improve the efficiency and accuracy of QTL detection. Taking these interactions into account, it will be possible to formulate breeding and selection procedures for important agronomic and market value traits.

We report here the detection of three significant QTLs ($QLG4_{7}$, $QLG4_{83}$ and $QLG13_{50}$) for days to flowering, one significant QTL ($QLG8_{4}$) for plant height, three significant QTLs ($QLG1_{112}$, $QLG4_{82}$ and $QLG5_{98}$) for seed diameter and five significant QTLs ($QLG1_{107}$, $QLG1_{113}$, $QLG4_{82}$, $QLG5_{55}$ and $QLG8_{34}$) for 100 seed weight.

$QLG4_{82\text{-}83}$ accounted for 20.2%, 32.6%, and 17.5% of the phenotypic variation for days to flowering, seed diameter and 100 seed weight, respectively. $QLG4_{82\text{-}83}$ was flanked by two RAPD markers UBC 34 and UBC1 at 0.4-1.4 and 1.6-2.6cM, respectively. The three traits involved are all positively correlated with each other indicating the possibility for linkage or pleiotrpic effect in this LG 4 QTL region.

Some interacting QTLs accounted for significant phenotypic variation for quantitative traits through additive or epistatic interactions or both. $QLG1_{24}$ x $QLG13_{52}$ and $QLG4_{84}$ x $QLG13_{8}$ accounted for 15.6% and 24.2% through additive and epistatic effects for days to flowering. For plant height, $QLG3_{76}$ x $QLG8_{36}$, $QLG3_{82}$ x $QLG4_{6}$, $QLG4_{66}$ x $QLG14_{16}$ and $QLG5_{72}$ x $QLG11_{68}$ interactions accounted for 17.6%, 17.3%, 10.6% and 10.7% of the

phenotypic variation, respectively. $QLG1_{44}$ x $QLG8_{14}$ both additively as well as epistatically accounted for 14.6% of the phenotypic variation for plant height.

Growth habit and cotyledon color were each controlled by single genes with prostrate growth dominant over erect plant type and red cotyledon dominant over yellow cotyledon.

Understanding genetics of the quantitative traits will help to develop the breeding strategy for selection. The significant correlations among days to flowering, seed diameter, and 100 seed weight and the association between the gene rich QTL region ($QLG4_{82\text{-}83}$) will help the breeders selecting plants for early maturity and improved seed quality. The putative QTLs will be useful to locate the genes in the genome that are important for the traits and provide the guidance for marker assisted selection and cloning the genes.

Acknowledgements: We are indebted to Sheri McGrew, Tony Chen, Jarrod Pfaff and Lorna Burns and Tracie Anderson for the field and lab support.

REFERENCES

Abbo, S., G. Ladizinsky, and N.F.Weeden. 1992. Genetic analysis and linkage study of seed weight in lentil. Euphytica 58: 259-266.

Barulina, H. 1930. Lentils of the USSR and other counties. Bull Appl Bot Gen Plant Breed 40: 225-238.

Cubero, J.I. 1984. Taxonomy, distribution and evolution of faba bean and its wild relative. In: Witcombe, J.R., and W. Erskine, (Eds), Genetic Resources and their explotation: chickpeas, faba beans and lentils. 131-144.

Davies, D.R., G.J. Berry, M.C. Health, and T.C.K. Dawkins. 1985. Pea (*Pisum sativum* L.). In: Grain Legume Crops. Summerfield R.J., and E.H. Roberts (Eds.). Williams Collins Sons and Co. Ltd, London, UK. 147-198.

Emami, M. K., and B. Sharma. 1999. Linkage between three morphological markers in lentil. Plant Breeding. Dec. 118(6): 579-581.

Erskine, W., A. Hussain, M. Tahir, A. Bahksh, R.H. Ellis, R.J. Summerfield, and E.H. Roberts. 1994. Field evaluation of a model phototermal responses in a world lentil collection. Theor Appl Genet 88: 423-428.

Erskine, W., Ellis, R. H., R. J. Summerfield, E. H. Roberts, and A.Hussain.1990. Characterization ofresponses to temperature and photoperiod for time to flowering in a world lentil collection. Theor Appl Genet 80: 193-199.

Imrie, B.C., Z.U. Ahmed, J.P.J. Eerens. 1985. Heritability of seed weight in mungbean. SABRAO J. 17, 173–175.

Johannsen, W. 1903. Om Arvelighed i Samfund ogrene Linier (On the inheritance in populations and pure lines). K. Danske Videnskah. Sr1 skahs Forh 3: 235-294.

Kumar, Y. 2002. Inheritance and linkage of genes for morphological traits in lentil (*Lens culinaris*. Medik.). Ph.D. Thesis, CCS University, Meerut, India.

Ladizinsky, G., 1979. The genetics of several characteristics in lentil as indicated by crosses between *Lens culinaris* and *Lens orientalis*. J. Hered 70: 135-137.

Li, H., J-M. Ribaut, Z. Li, and J. Wang. 2008. Inclusive composite interval mapping (ICIM) for digenic epistasis of quantitative traits in biparental populations. Theor Appl Genet 116:243–260.

Mishra, S.K. 2004. Final technical report on development of comprehensive genetic linkage map in lentil (*Lens culinaris* Medik.). Submitted to ICAR, Krishi Bhawan, New Delhi, India.

Motto, M., G.P. Soressi, and F. Salamini. 1978. Seed size inheritance in a cross between wild and cultivated common beans (*Phaseolus vulgaris* L.).Genetica 49: 31-36.

Muehlbauer, F.J. 2009. Lentil: Improvement in developing countries. Erskine, W., F.J. Muehlbauer, A. Sarker, and B. Sharma. The Lentil: Botany, production and uses. CAB International.137-154.

Muehlbauer, F.J., R.W. Short, R.J. Summerfield, K.J. Morrison and D.G. Swan. 1981. Description and culture of lentils. Cooperative Extension, College of Agriculture, Washington State University and USDA-ARS.

Nelson, J.C., 1997. QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3: 239–245.

Niknejad, M., M. Khosh-khui, and S.R. Ghorashy.1971.Inheritance of seed size in chickpeas (*Cicer arietinum* L.) Crop Sci 11: 768-769.

Roberts E.H., R.J. Summerfield, F.J. Muehlbauer, and R.W. Short. 1986. Flowering in Lentil (*Lens culinaris* Medik.): The Duration of the Photoperiodic Inductive Phase as a Function of Accumulated Daylength above the Critical Photoperiod. Ann Bot 58: 235-248.

Sarker, A., and W. Erskine. 2006. Recent developments in ancient lentil. J Agric Sci 144: 1-11.

Sarker A., W. Erskine, B. Sharma, and M.C. Tyagi. 1999.Inheritance and linkage relationship of days to flower and morphological loci in lentil (*Lens culinaris* Medikus subsp. *culinaris*). J Hered: 90(2).

Sakar, D. 1983.Breeding behaviour of characters related to yield and adaptation to mechanical harvesting in lentil (*Lens culinaris* Medik.). Ph.D. dissertation, Dept. of Agronomy and Soils, Washington State University.

Saxena, M.C., and G.C. Hawtin, 1981. Morphology and growth patterns. In: C. Webb and G. Hawtin (Eds), Lentils. Commonwealth Agricultural Bureaux, Slough, UK. 39–52.

Sharma, B., and M.K. Emami. 2002. Discovery of anew gene causing dark green cotyledons and pathway of pigment synthesis in lentil (*Lens culinaris* Medik). Euphytica 124: 349-353.

Singh, T.P. 1978. Inheritance of cotyledon colour in lentil. Indian J Genet 38: 11–12.

Slinkard, A.E., 1978. Inheritance of cotyledon color in lentils. J Hered 69:139-140.

Smithson, J.B., J.A. Thompson, and R.J. Summerfield. 1985. Chickpea (*Cicer arietinum* L.). In: R.J. Summerfield and E.H. Roberts (eds.), Grain Legume Crops. Collins, London, UK. 312-390.

Tahir, M., and F.J. Muehlbauer. 1994. Gene mapping in lentil with recombinant inbred lines. J Hered 85:306-310.

Tschermak-Seysenegg, E. 1928. Lentil and fieldbean crosses. Sityringsbe Akad Wiss Wien Math Nat R1 I Abs 137(3/4): 171–181 (cf. Muehlbauer, F.J. and A.E. Slinkard, 1987. Breeding Methodology. In: C. Webb and G. Hawtin (Eds.), Lentils, pp. 69–90. CAB-ICARDA, Farnham, England.

Tullu, A., B. Tar'an, T. Warkentin, and A. Vandenberg. 2008. Construction of an Intraspecific Linkage Map and QTL Analysis for Earliness and Plant Height in Lentil. Crop Sci 48: 2254-2264.

Tulmann-Neto, A., M.C. Alves. 1997. Induction of mutations for earliness in the soybean cultivar Paraná. Braz J Genet 20: 45-50.

Wilson, V. E., A.G. Law, and R. L. Warner. 1970. Inheritance of Cotyledon Color in Lens Culinaris (Medic.). Crop Sci 10: 205-207.

Table 1. Statistical parameters of quantitative traits of RILs developed from the ILL-5888 x ILL-6002 cross grown at Pullman, WA.

Statistical parameters	Days to Flowering (Days)	Plant height (cm)	Seed diameter (mm)	100 seed weight (g)
MSE	32.62***	38.609***	0.825***	1.778***
Mean ± sd	51.4±3.4	25.1±3.8	4.9±0.5	3.2±0.6
Range	47-58	15.7-34.3	4.0-6.3	2.0-4.9
ILL-5888	53	18.2	3.7	2.16
ILL-6002	60	27.8	6.5	5.59

MSE= Mean sum of squares and *** P < .001

Table: 2. Correlation between quantitative agro-morphological traits (days to flowering, plant height, seed diameter and 100- seed weight).

Trait	Days to Flowering	Plant height	Seed diameter	100 seed weight
Days to Flowering	1.0	0.217*	0.393***	0.411***
Plant height		1.0	0.207*	0.261***
Seed diameter			1.0	0.885***
100 seed weight				1.0

*** P < 0.001, * P<0.05

Table 3. Chi square tests for goodness of fit to expected segregation ratios for cotyledon color (*Yc/yc*) and growth habit (*Gh/gh*) among RILs developed from the ILL-5888 x ILL-6002 cross grown at Pullman, WA.

RILs/ Parents	Traits and gene symbol	No. of RILs	Expected Segregation ratio	χ^2 value
F_6 derived F_7 RILs	Red cotyledon (*Yc*)	94	1:1	2.14 *
	Yellow cotyledon (*yc*)	75		
Parents: ILL-5888	Red cotyledon (*Yc*)			
ILL-6002	Yellow cotyledon (*yc*)			
F_6 derived F_7 RILs	Prostrate growth habit (*Gh*)	92	1:1	1.73*
	Erect growth habit (*gh*)	75		
Parents: ILL-5888	Prostrate growth habit (*Gh*)			
ILL-6002	Erect growth habit (*gh*)			

* P < 0.250 >0.100

Table 4. Summary of QTL analysis for days to flowering variation in RILs from the ILL-5888 x ILL-6022 cross.

Trait	QTL	Additive effect	LOD	PVE^{+}(%)	
Days to flowering	$QLG4_{7}$	-1.262	4.84	14.38	
	$QLG4_{83}$	1.506	10.24	20.18	
	$QLG13_{50}$	1.078	4.99	10.40	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE^{+}(%)
	$QLG1_{24}$ x $QLG13_{52}$	0.637	13.25	3.48	15.57
	$QLG2_{0}$ x $QLG12_{24}$	-0.914	6.74	6.59	7.26
	$QLG2_{30}$ x $QLG8_{92}$	0.858	6.09	5.89	6.39
	$QLG2_{82}$ x $QLG3_{150}$	-0.937	7.01	6.56	7.98
	$QLG2_{132}$ x $QLG8_{18}$	0.731	4.73	4.43	5.07
	$QLG3_{50}$ x $QLG7_{94}$	0.744	3.81	3.74	4.56
	$QLG4_{84}$ x $QLG13_{8}$	0.912	19.22	6.57	24.16

$^{+}$ phenotypic variation explained

Table 5. Summary of QTL analysis for plant height variation in RILs from the ILL-5888 x ILL-6002 cross.

Trait	QTL	Additive effect	LOD	PVE,$^{+}$(%)	
Plant height	$QLG8_{4}$	-1.478	4.93	15.33	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE^{+}(%)
	$QLG1_{44}$ x $QLG8_{14}$	-0.958	8.42	4.12	14.57
	$QLG1_{44}$ x $QLG8_{22}$	-0.976	4.39	4.36	6.84
	$QLG1_{86}$ x $QLG7_{76}$	1.114	3.26	3.21	8.65
	$QLG1_{224}$ x $QLG5_{30}$	1.21	7.19	7.06	9.92
	$QLG3_{76}$ x $QLG8_{36}$	-1.590	11.52	11.34	17.61
	$QLG3_{82}$ x $QLG4_{6}$	-1.557	6.64	6.58	17.29
	$QLG3_{164}$ x $QLG10_{52}$	-1.103	6.17	5.93	8.84
	$QLG4_{66}$ x $QLG14_{16}$	-1.232	6.48	6.42	10.56
	$QLG5_{72}$ x $QLG11_{68}$	1.267	7.98	6.66	10.69

$^{+}$ phenotypic variation explained

Table 6. Summary of significant QTLs, additive and epistatic effects for seed diameter in RIL population from the ILL-5888 x ILL-6002 cross.

Trait	QTL	Additive effect	LOD	PVE^{+}(%)	
Seed diameter	$QLG1_{112}$	-0.097	3.21	3.64	
	$QLG4_{82}$	0.293	22.23	32.63	
	$QLG5_{98}$	0.109	3.25	4.51	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE^{+}(%)
	$QLG2_{62}$ x $QLG2_{132}$	0.113	3.58	3.139	3.48
	$QLG2_{62}$ x $QLG2_{136}$	0.120	3.59	3.14	3.97
	$QLG13_0$ x $QLG14_0$	0.100	4.47	4.24	3.72

$^{+}$ phenotypic variation explained

Table7. Summary of QTLs, additive and epistatic effects for 100 seed weight in RILs from the ILL-5888 x ILL- 6002 cross.

Trait	QTL	Additive effect	LOD	PVE^{+}(%)	
100 seed weight	$QLG1_{107}$	0.146	5.47	5.63	
	$QLG1_{113}$	-0.219	11.45	12.83	
	$QLG4_{82}$	0.258	15.25	17.52	
	$QLG5_{55}$	0.150	5.40	5.83	
	$QLG8_{34}$	-0.166	7.20	7.32	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE^{+}(%)
	$QLG2_{158}$ x $LG2_{210}$	-0.183	3.47	3.06	9.19
	$QLG4_{22}$ x $QLG4_{56}$	-0.159	3.43	2.63	8.67
	$QLG5_{74}$ x $QLG5_{78}$	-0.278	3.58	3.58	9.54
	$QLG5_{76}$ x $QLG5_{84}$	-0.237	3.01	2.93	9.26

$^{+}$ phenotypic variation explained

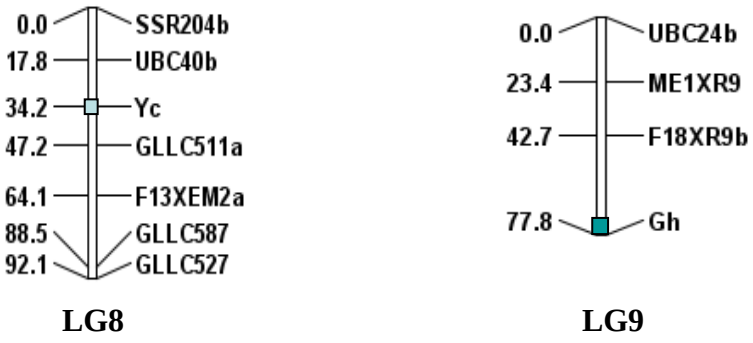

Figure 1. The qualitative agro-morphological traits (a) The linkage group 8 (LG8) having cotyledon color gene (*Yc*) and (b) linkage group 9 (LG9) having *Gh* gene.

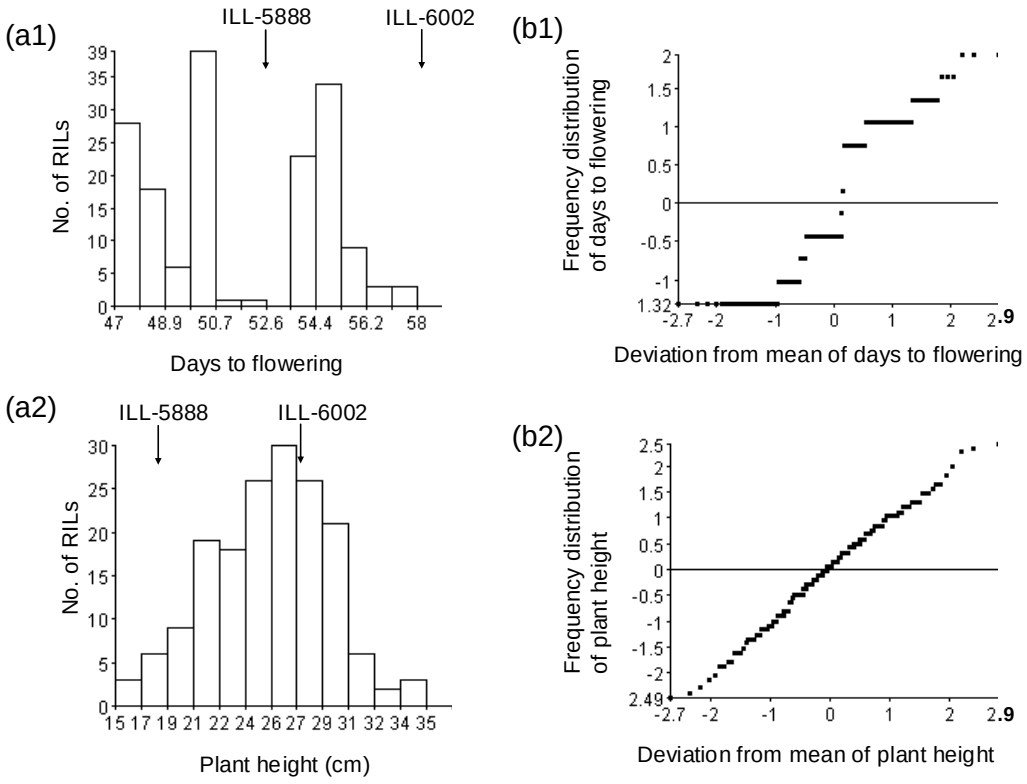

Figure 2. Frequency distribution and normality test of RILs developed from the ILL-5888 x ILL-6002 cross for days to flowering (a1, b1), plant height (a2, b2).

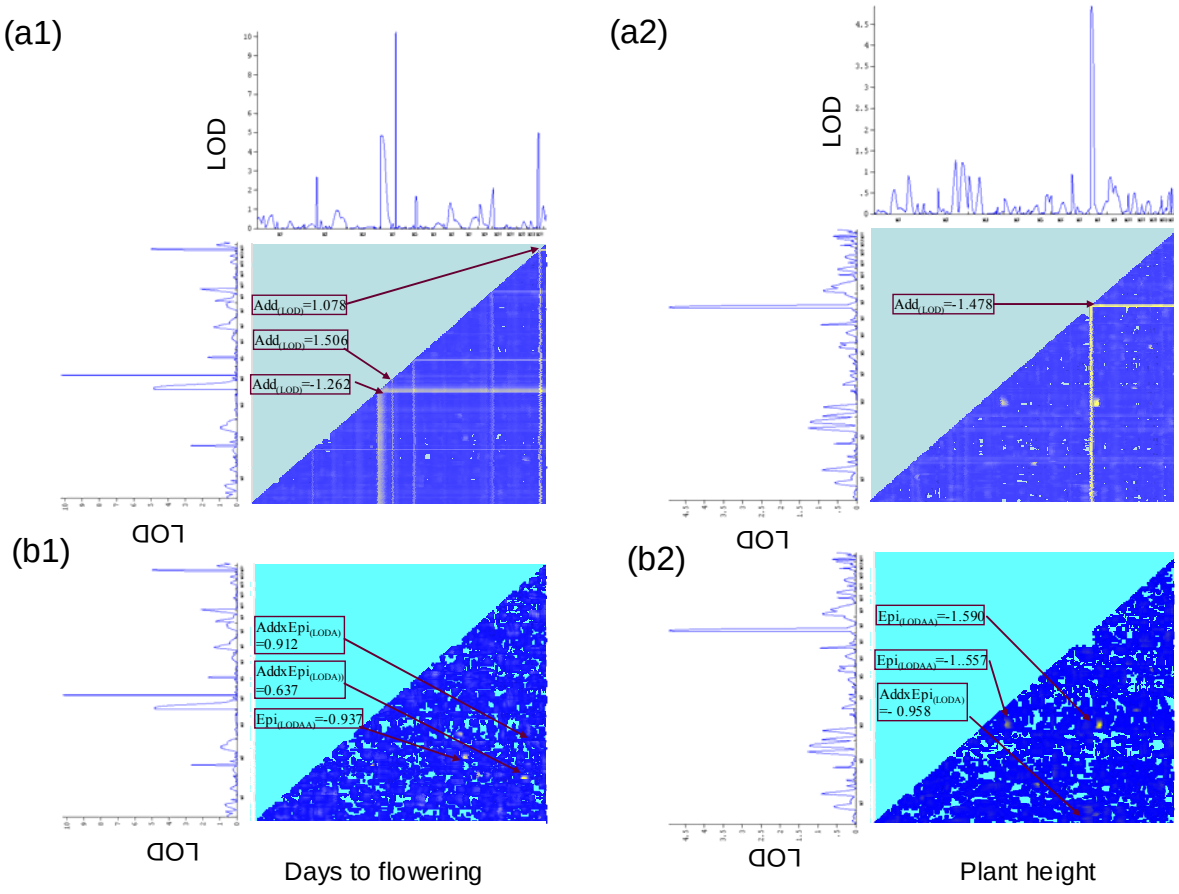

Figure 3. Contour profile showing significant QTLs for days to flowering (a1) additive effect: $Add_{(LOD)}$, (b1) interaction effect; epistatic effect: $Epi_{(LODAA)}$ and combined additive-epistatic effects: $AddxEpi_{(LODA)}$.

Contour profile showing significant QTLs for plant height (a2) additive effect: $Add_{(LOD)}$, (b2) interaction effect; epistatic effect: $Epi_{(LODAA)}$ and combined additive-epistatic effects: $AddxEpi_{(LODA)}$.

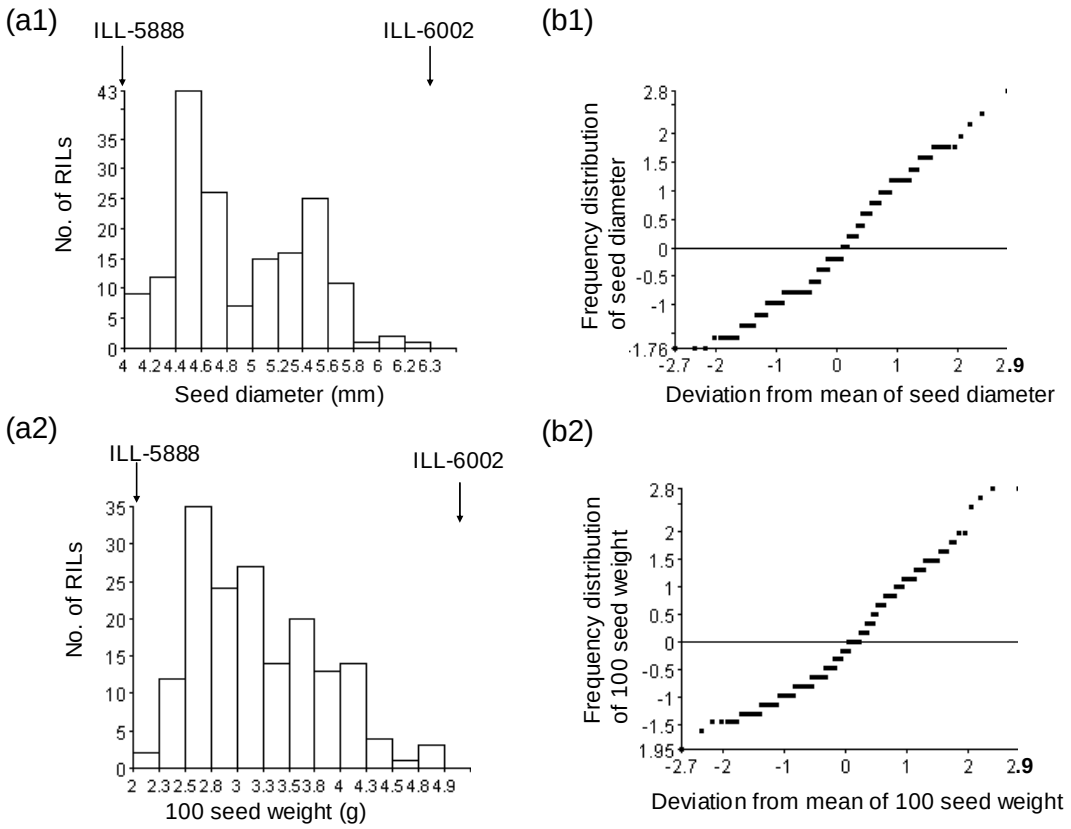

Figure 4. Frequency distribution and normality test of RILs developed from the ILL-5888 x ILL-6002 cross for days to seed diameter (a1, b1) and 100 seed weight (a2, b2).

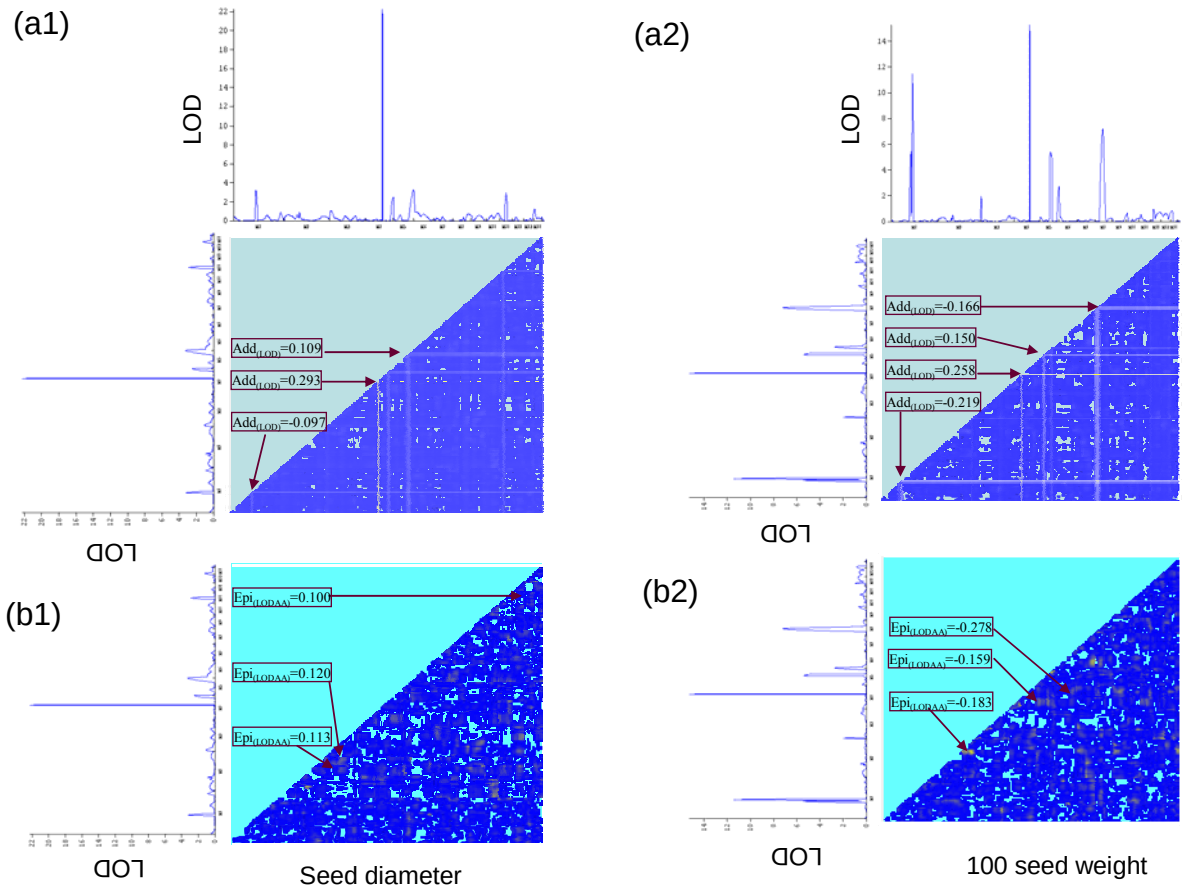

Figure 5. Contour profile showing significant QTLs for seed diameter (a1) additive effect: $Add_{(LOD)}$, (b1) interaction effect; epistatic effect: $Epi_{(LODAA)}$ and combined additive-epistatic effects: $AddxEpi_{(LODA)}$.

Contour profile showing significant QTLs for 100 seed weight (a2) additive effect: $Add_{(LOD)}$, (b2) interaction effect; epistatic effect: $Epi_{(LODAA)}$ and combined additive-epistatic effects: $AddxEpi_{(LODA)}$.

.