Genetic and Agronomic Effects on Milling Qualities of Lentil

(*Lens culinaris* Medik.)

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by

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ABSTRACT

Red lentil (*Lens culinaris* Medikus) is primarily consumed in dehulled form (*dhal*). Canadian red lentils can be variable for dehulling due to adverse weather conditions at harvest, unique crop management practices, and seed genetics. The research objectives were to investigate the effects of harvest aid herbicides, mid-season applied fungicides, and seed coat color on milling quality, and to identify and map genetic associations with milling characteristics. In field experiments from 2012-2014, potential harvest aid herbicides alone and/or tank mixes with glyphosate, three common fungicides (pyraclostrobin, azoxystrobin, and chlorothalonil), and the four- basic seed coat ground colors of red lentil genotypes were examined for milling quality and milling quality traits were mapped using a recombinant inbred lentil population. Diquat applied alone or in combination with glyphosate as desiccants resulted in increased dehulling efficiency (DE) and milling recovery (MR). Seed glyphosate residue was negatively associated with germination, vigor, DE, and MR, but positively associated with football recovery (FR). Single applications of strobilurin or chlorothalonil did not affect seed yield. Pyraclostrobin application significantly increased DE (+3.5%) in one site year. At a stemphylium blight (SB) affected, significant reductions occurred for seed yield (-46%), DE (-29%), MR (-28%) and FR (-16%). Lentil genotypes expressing the recessive *tgc* allele (green and gray seed coats) had higher DE and MR than those expressing the *Tgc* allele (brown or tan seed coat) but had lower FR in two site-years. Genotype, site-year, and their interactions were significant for seed weight, diameter, thickness, and plumpness and for milling quality traits. Broad sense heritability of DE and MR was moderate, and low for FR. Multiple QTLs for milling traits were detected in six of seven linkage groups (LGs). The most stable and significant QTLs associated with DE and MR clustered on LGs 1, 2, 3 and 7. FR QTLs clustered on LGs 4, 5, 6 and 7. Maintaining or improving lentil milling quality can be accomplished by (1) applying diquat alone or in combination with glyphosate, (2) managing SB disease, (3) breeding for uniform seed size with green or gray seed coat color to maximize DE and MR, and with brown seed coat color to maximize FR, and 4) using milling quality markers in genetic improvement. The results of this research could influence future crop management and breeding strategies for minimizing losses in lentil milling.

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DEDICATION

To my late father Rishi Ram Subedi and my mother Eku Maya Subedi who gave me my life and supported and encouraged me with unconditional love

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
σ^2g	Genotypic variance
σ^2gl	Genotype by location interaction variance;
σ^2gly	Genotype by year by location interaction variance
σ^2gy	Genotype by year interaction variance
σ^2P	Phenotypic variance
a.e,	Acid equivalent
a.i.	Active ingredient
AFLP	Amplified fragment length polymorphism
Ave.	Avenue
CDC	Crop Development Centre
CFIA	Canadian Food Inspection Agency
CIM	Composite interval mapping
cM	Centimorgan
DAS	Days after seeding
DE	Dehulling efficiency (%)
Df	Degrees of freedom
DTM	Days to maturity
EST	Expressed sequence tag
FR	Football recovery (%)
G×E	Genotype by environment interaction
ha	Hectare
HPLC	High performance liquid chromatography
IM	Interval mapping
Kg	Kilogram
LG	Linkage group

LOD	Logarithm of odds
LSD	Least significance difference
MAS	Marker Assisted selection
ME	Milling efficiency
MR	Milling recovery (%)
Mt	Million tonnes
NS	Not significant
PCR	Polymerase chain reaction
PPO	Protoporphyrinogen oxidase
QTL	Quantitative trait loci
RAPD	Random amplified polymorphism DNA
RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
RIL	Recombinant inbred line
RPM	Revolutions per minute
SAS	Statistical analysis software
SB	Stemphylium blight
SK	Saskatchewan
SNP	Single nucleotide polymorphisms
TSW	1000-seed weight

CHAPTER 1. INTRODUCTION

Lentil (*Lens culinaris* Medik. ssp. *culinaris*) is an annual, self- pollinating grain legume crop widely grown in the cool climatic regions with an annual production of 4.5 Mt (FAOSTAT, 2016). Lentil seeds are good source of dietary protein, fiber, complex carbohydrates, and minerals (Jood et al., 1998; Xu and Chang, 2009; DellaValle et al., 2013). Canada is the largest lentil producer and exporter in the world. In 2016, lentil was grown on 2.18 million ha in Canada, with an annual production of 3.2 Mt and exports of more than 1.8 Mt. (Statistics Canada, 2016). Saskatchewan produces most of Canada's lentils, usually more than 90% of the production. The two major types of lentil produced are the large green (pale green seed coat, yellow cotyledon, seed weight 60-75 mg) and small red (gray seed coat, red cotyledon, seed weight 35-45 mg) market classes. Over the past 20 years red lentil production area in Canada has increased steadily to meet growing global demand. In 2016, lentil exports were worth more than one billion dollars to the Saskatchewan economy alone (Statistics Canada, 2016).

A major proportion of the red lentil produced in Canada is exported to South Asia and to the Mediterranean regions, where it is consumed in dehulled form (Wang, 2008; Bruce, 2008). Dehulling is a major post-harvest processing step for red lentil. Dehulling results in reduced cooking time, improved palatability, and digestibility, and removal of anti-nutritional factors such as polyphenols (tannins) which are mostly retained in the seed coat fraction (Singh and Singh, 1992). Physical removal of the seed coat of lentils is reported to improve iron bioavailability and cooking quality (Wang, 2005; DellaValle et al., 2013), likely due to the removal of phenolic compounds that interfere with iron nutrition.

Current annual lentil dehulling capacity in Western Canada is estimated to be 100,000 to 150,000 Mt per year. By increasing the current volume of dehulling to 500,000 Mt in Western Canada, additional economic activity could be increased substantially. To increase this volume, there is a need to maintain milling quality of Canadian lentils up to the international standard. Milling quality of Canadian red lentils has been found to be lower than that of the major competitors (Agblor, 2006). An important issue that Canadian red lentil producers face is the possibility of adverse and wet climatic conditions during the harvest period compared to global competitors where the crop is mostly harvested in periods of rising temperature and low humidity. This adversity in climatic condition results in higher moisture content in seed at harvest, that subsequently reduces milling efficiency during the decortication process. Previous

research also highlighted that milling efficiency of pulses is influenced by growing environments, crop management techniques and genetics of seeds (Ramakrishnaiah and Kurien, 1983; Erskine et al., 1991b; Wood et al., 2008).

The indeterminate growth habit combined with genetic variability causes a significant variation in crop maturity within lentil genotypes. To synchronize and hasten the seed maturation process in lentils and to facilitate harvest operations, growers usually apply crop harvest aids (desiccants). Desiccant chemistry and application timing are crucial as they may cause loss in yield and leave herbicide residues in the seeds (Wilson and Smith, 2002). Different desiccants used as herbicides have different modes of action and chemistry. Therefore, these products may affect post-harvest seed quality including milling. Pre-harvest aids were shown to have positive impact on lentil dehulling process in years when seed maturation and harvest occurred under wet weather conditions (Bruce, 2008). Although the Canadian red lentil crop is mostly chemically desiccated prior to harvest with different potential harvest-aid herbicides products, the effects of these pre-harvest herbicides on milling quality have not been determined.

In Western Canada, lentil growers often apply foliar fungicides such as strobilurin (azoxystrobin and pyraclostrobin) and chlorothalonil to lentil crop at mid-flowering stage to control fungal diseases such as ascochyta and anthracnose (Saskatchewan Ministry of Agriculture, 2016). These fungicides have different modes of action and activities in plants. Chlorothalonil has broad spectrum protective action with limited translocation in plant tissues while strobilurins which have both protective and preventive activities which are readily translocated in plants (Butzen et al., 2005; Saskatchewan Ministry of Agriculture, 2016). These fungicides also have a beneficial effect on plants, an effect commonly known as 'greening effect' (Petit et al., 2012), that prolongs photosynthetically active green leaves longer, maximizes carbon assimilation process, and increases the grain/pod filling period (Morrison et al., 1999; Kumudini et al., 2001). Prolonging the pod filling period has been noticed to increase seed yield and seed quality in soybean (Mahoney et al., 2015), and milling recovery yield in cereals (Groth, 2006). As lentil is an indeterminate crop, fungicides may control disease at the top and bottom of the canopy differently and have a fungicidal effect on seed development. This differential control of disease potentially affects post-harvest seed and milling quality. However, no literature or research report exists on the effects of fungicides on lentil milling quality. Therefore, research was required to determine the effectiveness of foliar fungicides to control the fungal infection

and to assess how these fungicides could improve post-harvest milling quality characteristics.

Lentil seed coats have a wide genetic variation in color and pattern. Dominant and recessive combinations of two independent loci *Ggc (gray)* and *Tgc* (tan) determine the four-basic seed coat ground colors: brown (*Ggc Tgc*), gray *(Ggc tgc)*, tan (*ggc Tgc*), and green (*ggc tgc*) (Vandenberg and Slinkard, 1990). Differences in composition, amount and types of polyphenolic compounds basically determine seed coat color in lentil (Mirali et al., 2017). Differential seed coat composition, chemistry and color have an impact on seed coat thickness and hardness of pulse seeds (Reichert et al., 1984; Singh et al., 1992), and this can differentiate seed coat removal during the dehulling process. There is no information on how lentil seed coat color genetics affects dehulling process.

Lentil seeds are different in shape and size. A number of studies reported that seed shape impacts milling efficiency. Thin seeds tended to experience greater damage during processing while plumper seeds with greater diameter were subjected to less damage and subsequently increased dehulled yield (Erskine et al., 1991b; Wang, 2008; Shahin et al., 2012). Lentil milling efficiency could be influenced by genes that affect seed coat color, possibly two genes for cotyledon color or quantitative trait loci (QTLs) associated with seed shape (Vandenberg and Slinkard, 1990; Fedoruk et al., 2013). However, genotype and environment effects on milling efficiency have not been assessed. Similarly, there is no information related to molecular marker and specific genomic region for traits associated with milling efficiency. As lentil production is such a big enterprise in Western Canada, it is very important to enhance milling efficiency of red lentil to sustain entrepreneurs in terms of economic growth and trade. The current research aims to investigate the best crop-harvest (desiccant) aids, mid-season fungicides that improve and increase milling recovery yield and quality. This research also aims to evaluate variation of seed coat color genotypes on milling efficiency, as determined by percentages of dehulling efficiency (DE), milling recovery (MR) and football recovery (FR) and to identify and locate the genes associated with milling quality traits.

1.1 Hypotheses

This research was planned to develop understanding of the appropriate agronomic strategies and genetic strategies to improve lentil production and value by minimizing dehulling losses. The long-term goal of this research initiative is to contribute fundamental scientific knowledge that will help in the development of effective breeding strategies and agronomic management techniques for lentil in Western Canada. This requires examination of current agronomic and disease management techniques, and genetic characterization to potentially develop marker assisted selection techniques to improve milling recovery yield. Considering these issues, the following hypotheses were tested.

1. Different crop-harvest herbicide aids (desiccants) can affect seed germination, seedling vigor, seed dimension, and milling quality characteristics of lentil;
2. Foliar applied fungicides can improve seed and milling quality characteristics of lentil;
3. Growing environment and genotype influence seed and milling quality characteristics;
4. Major genes determining seed coat color and seed dimension affect milling quality characteristics; and
5. Genetic markers associated with milling quality characteristics can be identified in a lentil recombinant inbred line (RIL) population.

1.2 General Objectives

The overall objective of this research study was to determine the effect of unique crop management practices including application of crop harvest aids and mid-season fungicides, and seed genetics on milling quality characteristics of lentil.

1.3 Specific Objectives

In consideration of the above hypotheses and overall goals of project, the following specific research objectives were developed.

1. Determine the effects of newly registered harvest aid herbicides on seed dimension, seed germination, seedling vigor and milling quality characteristics (percent dehulling efficiency, milling recovery, and football recovery) of lentil.
2. Determine the effects of foliar fungicides on seed yield, seed dimension, and milling quality characteristics of lentil.

3. Evaluate how seed coat color genetics affect lentil milling quality characteristics.
4. Determine heritability, and genotype ×environment interaction effects on milling quality of major commercial seed coat colors in a lentil recombinant inbred population.
5. Identify and map genomic regions associated with milling quality characteristics in a recombinant inbred lentil population.

Four studies were conducted to test the hypotheses listed above, namely:

Study 1 determined the effects of newly registered contact and systemic harvest aid herbicides on seed dimension, seed germination, seed vigor, and the milling quality parameters.

Study 2 examined the effects of foliar fungicides on seed quality and milling quality parameters. Two strobilurin and one chlorothalonil fungicides were assessed under field conditions. A follow up experiment tested the impact of a high level of stemphylium blight disease infection on milling quality. This section is placed in the Appendix section.

Study 3 was designed to determine how the four basic background seed coat colors of lentil affect milling quality of lentil.

Study 4 was focused on determining the heritability, the genotype by environment interactions on seed dimension and milling quality characteristics, and determination of genetic associations between seed morphological traits and milling traits. This study also genetically mapped the chromosome regions that affect milling quality in lentil.

These studies are presented in the manuscript format in chapters 3 to 6 of this thesis.

CHAPTER 2. LITERATURE REVIEW

This section focuses on review of literature related to lentil origin, and distribution, major market classes of lentil, agronomic practices and harvest aid herbicides, lentil diseases and their effect on lentil dehulling and milling. It also includes literature related factors that influence the dehulling process, linkage mapping techniques, genetic basis, and genetic molecular marker use in lentil and other related pulse crops.

2.1 Lentil

This section covers origin and domestication of lentil, production and nutritional value, and major commercial classes of lentil.

2.1.1 Origin, taxonomy, distribution, and production of lentil

Lentil (*Lens culinaris* Medik.) is one of the most ancient annual cool season, self-pollinated diploid crops that originated in the Near Eastern Complex (Zohary, 1999). The genome size of lentil is approximately 4,063 Mbp (Arumuganathan and Earle, 1991). Cultivated lentil belongs to the genus *Lens* of the Fabaceae family, which consists of 18,000 species in 700 genera (Trinick, 1982; Muehlbauer, 2011). Davies et al. (2007) categorized lentil into seven species, namely *L. culinaris, L. orientialis, L. lamottei, L. tomentosus, L. ervoides L, odemensis* and *L. nigricans.* Wong et al. (2015) used genotyping by sequencing to place *L. culinaris, L. tomentosus* and *L. orientalis* in the primary gene pool, *L. lamottei* and *L. odemensis* in the secondary gene pool, and *L. ervoides* and *L. nigricans,* respectively, in the tertiary and quaternary gene pools.

Domestication of lentil began around 7000-8000 B.C (Zohary and Hopf, 1973; Erskine and Sarker, 2004; Erskine et al., 2009). Lentil was first domesticated in the Fertile Crescent of western Asia and then introduced to the Indo-Gangetic plain (Lev-Yadun et al., 2000**;** Pearman, 2005). Lentil spread rapidly to Central Asia and the Mediterranean regions, later to North America, it was first cultivated in northwest USA in 1930s. It was introduced to northern temperate Prairies of Canada in the late 1960s (Muehlbauer et al., 1992; Oplinger et al., 1990, Cubero et al., 2009). Lentil is now widely grown in Mediterranean, Sub-Tropical Savannah, and Northern Temperate zones (Tullu et al., 2011) which have different daylength and temperature patterns that limit exchange of germplasms among zones (Khazaei, et al., 2016).

Global production of lentil in 2016 was 6.3 Mt from an estimated cultivated area of 4.52 million ha (FAOSTAT, 2016) and Canada, India, Turkey, and Australia are top lentil producers in the world. Canada is the largest lentil exporter and producer. Saskatchewan province alone produced over 3.2 Mt of lentil in 2016 (Statistics Canada, 2016). Lentil acreage increased from less than 600 ha in 1970 (McVicar et al., 2010) to 2.18 million ha in Canada in 2016 (Statistics Canada, 2016). Canada exported lentil to over 100 countries with values about CDN$ 2.5 billion (Saskatchewan Agriculture Exports, 2016). Red cotyledon lentil is the major production and trade market class of lentil in the world. Canada generally exports minimally cleaned red lentils in bulk shipments. A small amount of lentil cleaning and processing occurs in Canada. Some of the processing includes decortication, which involves dehulling, splitting and polishing of mostly red and some green lentil seeds (Pulse Canada, 2016).

2.1.2 Nutritional value of lentil seeds

Lentil seeds are rich sources of dietary protein (22-35%), complex carbohydrates, starch (44-48%), dietary fiber (12-15%), essential minerals (potassium, iron, calcium, phosphorus, and zinc), vitamins, and secondary metabolites (Muehlbauer et al., 1992; Erskine et al., 1985; Mirali et al., 2017). Lentils are also a good source of essential micronutrients like Fe and Zn for people in South Asia (Wang and Daun, 2006). Consumption of lentil provides both nutritional and health benefits to consumers. Health benefits include reduction in coronary heart diseases, preventing iron and zinc deficiency, and stabilizing blood sugar (Leterme, 2002). Lentils are commonly used as soup (*dhal*) and as sprouted grains in salad with rice or *rotis* (Sandhu and Singh, 2007). Prior to consumption in South Asia, red lentil seeds are dehulled, which improves bioavailability of nutrients (DellaValle et al., 2013).

2.1.3 Major commercial groups of Canadian lentils

Lentil seeds vary in shape, size, and color. Seeds are categorized into two major commercial groups, namely: *macrosperma* (6-9 mm in seed diameter with seed weight >40 mg) or *microsperma* (2- 4 mm in seed diameter with seed weight ≤ 40 mg) based on seed size (Barulina 1930, Yadav et al., 2007; Tullu et al., 2011). Two major market classes of Canadian lentils are large green and small red (Vandenberg, 2009). Red lentil seeds typically have gray seed coats with red cotyledons and mean seed weight ranging from 35-45 mg seed^{-1}. Large

green lentil seeds have pale green seed coats, yellow cotyledons, and mean seed weight ranges from 60-75 mg seed^{-1} (McVicar et al., 2010). Many minor market classes, including French green (yellow cotyledon, green seed coat, marbled seed coat pattern), Spanish brown (yellow cotyledon, gray seed coat with dotted pattern), and Beluga (yellow cotyledon with black seed coat), are produced in small quantities. Over the past 10 years, a market has developed for dehulled large green lentils, especially in South Asia and in some European markets (for both large and small yellow cotyledon types) (Vandenberg, personal communication, 2017).

2.2 Major lentil diseases in Canada

In Canada, ascochyta blight caused by *Ascochyta lentis,* fusarium wilt caused by *Fusarium oxysporum* Schlecht: Fr. F. sp. *lentis*; anthracnose caused by *Colletotrichum lentis* Damm, and stemphylium blight caused by *Stemphylium botryosum* Wallr, are the major foliar diseases of lentil. Root rot diseases caused by *Fusarium avenaceum* Corda ex Fr. Sacc, *Pythium* spp. and *Rhizoctonia. solani*, and more recently *Aphanomyces euteiches* Drechsl., are becoming a production problem, particularly in wet growing seasons. These diseases can affect lentil at any growth stage (Morrall, 2003). Across this spectrum of diseases, anthracnose, and more recently, stemphylium blight and *Aphanomyces* root rot have been the most prevalent in lentil fields in the Prairies when warm and humid conditions occur. These diseases are difficult to control due to lack of effective resistant lentil cultivars (Banniza et al., 2013).

2.2.1 Development, symptoms, and effect of stemphylium blight

Stemphylium blight (SB) is an emerging disease of lentil in the Canadian prairies (Morrall et al., 2006), and one of the most devastating lentil diseases in South Asia (Chen et al., 2009; Chen and Sharma, 2011). In 2008, about 25% of the lentil fields in Saskatchewan were affected by SB (Barker, 2009). The fungus can produce both air and seed-borne conidia. These conidia can produce multiple germ tubes that help to penetrate the host through stomata and subsequent invasion of plant foliage and tissues including seeds (Chen et al., 2009). Infected seeds, dead stems, and leaves can be inoculum for the succeeding year's infection (Saha, 2009). The phytotoxin stemphol produced from some isolates of *S. botryosum* are causal agents that form lesions on some hosts (Solfrizzo et al., 1994). Infected plants first develop small, light beige lesions on leaflets. Later, small lesions become large patches with irregular shapes

resulting in blighted, twisted, and rolled leaves. This infection eventually kills entire leaflet and branches. Branches become bare and finally the whole plant die (Isaacs, 2014; Barker, 2009). Prolonged wet weather further expands infections to other plant parts, such as pedicels, flowers, and entire plants eventually become darker and blighted (Chen et al., 2009; Isaacs, 2014). The economic impact of this disease depends upon the potential yield and seed quality loss (Banniza et al., 2006). Caudillo-Ruiz (2016) reported neither seed yield nor seed weight/size was affected by the disease but did notice a significant increase in deformed and stained seed percentage due to the disease infection.

2.3 Fungicides used in lentil production in Canada and their effects

Disease management is a priority in lentil production. The short plants create a dense canopy that favors foliar pathogens to develop diseases ((Banniza et al., 2006). In Canada, growers often use foliar fungicides, originally used as a tool to manage ascochyta blight (*Ascochyta lentis*) and anthracnose (*Colletotrichum lentis*) disease in lentil. The fungicides chlorothalonil (Bravo®), pyraclostrobin (Quadris ®), azoxystrobin (Headline®), and boscalid (Cantus^{TM}) have been registered in Saskatchewan to control lentil fungal diseases (ascochyta and anthracnose) (Saskatchewan Ministry of Agriculture, 2016). These fungicides have also been assessed for SB control in lentil (Banniza et al., 2006). Chlorothalonil is a broad spectrum protective fungicide with a limited translocation ability. It reacts with thiol groups in the pathogen's enzymatic systems (Saskatchewan Ministry of Agriculture, 2016).

Azoxystrobin and pyraclostrobin are strobilurin fungicides (Fernández-Ortuño et al., 2010; Gillard et al., 2012a) which have broad spectrum and systemic activity against fungal diseases. Strobilurin fungicides are quinone outside inhibitors that inhibit mitochondrial respiration in fungi by blocking electron transfer at the bc1 complex of cytochrome (Bartlett et al., 2002; Butzen et al., 2005; Mahoney et al., 2014). They have both protective and preventive effects (Grossman and Retzlaff, 1997). These fungicides also prevent the spore germination and mycelial growth of pathogenic, non-pathogenic, and saprophytic fungi (Oerke et al., 2001). No fungicide is registered officially in Canada to control SB of lentil (Dokken-Bouchard, 2010; Government of Saskatchewan, 2016). The optimal time for fungicide application for control of SB in lentil is approximately 9-10 node stages or before canopy closes (Fleury, 2016).

The strobilurin fungicides not only control diseases but also promote physiological plant health called greening effect (Venancio et al., 2003; Fagan, et al., 2010; Mahoney and Gillard, 2014), an ability of strobilurins to maintain the photosynthetically active green leaves for a longer period. This maximizes the grain-filling period, resulting in better yield and quality (Morrison et al., 1999; Kumudini et al., 2001; Muhammad, 2012; Woodward et al., 2016). The underlying physiological causes for greening effects are increased in the production of antioxidants during stress (Wu and Tiedemann, 2002; Zhang et al., 2010); reduction of ethylene production and delaying the senescence of leaves by maintaining chlorophyll content longer and photosynthetic activity of the plants (Grossmann and Retzlaff, 1997); reduction of stomatal opening to conserve water during drought without affecting CO_2 intake (Ko¨hle et al., 1997; Grossmann et al., 1999); and balancing hormone and nitrate reductase activity (Oerke et al., 2001). Increased seed yield due to the application of strobilurin fungicides has been inconsistent (Nelson et al., 2010; Nelson and Meindardt, 2011). Improvement of both seed yield and quality in soybean, wheat, and dry bean following the application of strobilurin fungicides have been reported (Cruz et al., 2010; Nelson et al., 2010; Nelson and Meinhardt, 2011; Weisz et al., 2011; Gillard and Ranatunga, 2013; Mahoney et al. 2014). According to Mahoney and Gillard (2014), strobilurin treatment improved seed quality without affecting seed yield and weight. However, research information related to the efficacy of these fungicides on milling quality in pulse crops, including lentil is lacking or limited.

2.4 Lentil harvesting

Lentil plants are considered mature for harvesting when the bottom third of the pods turn yellow to brown and the seeds inside rattle when shaken (Saskatchewan Pulse Growers, 2015). Uniform seed maturity is impossible in lentil due to indeterminate growth habit. Thus, harvesting is a challenge, particularly in northern climatic regions where lentil harvest begins when temperature drops (Bruce, 2008). Unpredictable rainfall at the mid to late growing seasons on the Canadian Prairies combined with rapid decrease in day length could further delay the lentil crop harvest. Delayed harvesting under cool environment conditions results in decreased seed yield and quality (Riethmuller et al., 2005; Alberta Pulse Growers, 2013). Thus, growers often use pre-harvest treatments, either swathing or chemical desiccation to expedite crop maturity and dry down canopy (Bruce, 2008; Saskatchewan Ministry of Agriculture, 2010; Zhang et al., 2016).

2.4.1 Swathing and desiccation

Some lentil growers prefer swathing (also called windrowing) as a means of accelerating the crop maturity. A self-propelled or tractor powered machine with an oscillating cutter bar cuts the plants close to the ground level and drop plants as a swath (Bruce, 2008; Saskatchewan Pulse Grower, 2015). Swathing can deteriorate seed quality and reduce seed yield by increasing disease infestation, wrinkling, sprouting, and rotting of lentil seeds, if swath remains prolong in the field under unfavorable weather conditions (Saskatchewan Pulse Growers, 2015). Compared to standing crops, swathed lentil crops generally dry slowly, which increases the risk of seed discoloration and sprouting.

Chemical desiccants are harvest aid herbicides used for accelerating crop maturation and to facilitate direct harvesting. These desiccants are either contact or systemic type (Schemenauer, 2011). The desiccants with contact action kill plants immediately when it contacts with no translocation, while systemic desiccants have slow action that enter through leaves, stems or roots and translocate throughout the plant (Ware and Whitacre 2004; Schemenauer, 2011). In Western Canada, diquat, glyphosate, saflufenacil and glufosinate have been registered as desiccants for lentil (Saskatchewan Ministry of Agriculture, 2014; Fleury, 2015).

Glyphosate [N-(phosphonomethyl) glycine] has a broad spectrum, systemic and non-selective activity in plant (Atkinson and Grossbard, 1985; Baylis, 2000; Hartzler et al., 2006). It can translocate both in phloem and xylem and slowly inhibit plant growth (Devine et al., 1993; Cobb and Reade, 2010; Saskatchewan Ministry of Agriculture, 2014) by interfering the aromatic amino acid synthesis pathways. It disrupts shikimate pathway inhibiting 5 enolpyruvylshikimate-3-phosphate synthase (EPSPS), a crucial enzyme in the pathway used to produce aromatic amino acids that are essential for protein synthesis and plant growth (Reddy et al., 2004; Cobb and Reade, 2010). Inhibition of EPSPS leads plant death due to starvation of aromatic amino acids and carbon, and excessive accumulation of toxic intermediates such as shikimate or shikimate-3-phosphate (Duke and Powles, 2008).

Diquat (1,1'-ethylene-2,2'-bipyridylium ion Reglone®) is widely used as a true desiccant to dry down the lentil crop (Fleury, 2015). It rapidly desiccates plants by affecting the electron transport chain of photosynthesis at Photosystem I that disrupts the protein and lipid formation in internal cell membranes (Fuerst and Norman, 1991; Cobb and Reade, 2010). It has limited translocation in plant, yet it can translocate in the xylem (Cobb and Reade, 2010). Glufosinate

and saflufenacil are newly registered desiccants for lentil in Canada. Glufosinate is a contact, non-selective herbicide that can translocate within plants, but it has less mobility due to rapid phytotoxic activity (Soltani et al., 2010). Glufosinate inhibits glutamine synthetase by binding irreversibly that limits the conversion of glutamate and ammonium into glutamine (Devine et al., 1993; Cox, 1996; Cobb and Reade, 2010). Excessive accumulation of inorganic ammonium or glyoxylate causes plant death because it inhibits RUBISCO and reduces the efficiency of photosynthesis.

Saflufenacil is a fast-acting contact herbicide and relatively new desiccant that translocate primarily through the xylem (Liebl et al., 2008). It inhibits the protoporphyrinogen IX oxidase (PPO) enzyme, which converts protoporphyrinogen IX to protoporphyrin IX (Soltani et al., 2010; Grossmann et al., 2010). The inhibition of the PPO enzyme prevents biosynthesis of chlorophyll and heme (Matringe et al., 1993; Duke et al, 1991)), ultimately leading to cell membrane destruction and necrosis (Duke et al., 1991; Grossman et al., 2010b). Pyraflufen-ethyl and flumioxazin are also potential desiccants in lentil crops. Both herbicides are PPO-inhibitors (Saskatchewan Ministry of Agriculture, 2014). They translocate in the xylem and are used occasionally as harvest aids in cotton (*Gossypium hirsutum* L.), potatoes (*Solanum tuberosum* L.) lentil and dry bean (*Phaseolus vulgaris L*.) (Ivany, 2005; Griffin et al., 2010; Soltani et al., 2013; Zhang et al., 2016). As a desiccant, herbicide is recommended to apply in lentil when sufficient lower pods have turned brown and seed has nearly 30% moisture content (Schemenauer, 2011; Saskatchewan Ministry of Agriculture, 2014; Zhang et al., 2016).

A number of studies illustrated the beneficial impact using herbicides as desiccants. For example, Riethmuller et al. (2005) reported improved lentil yield and quality following the desiccant treatment as compared to the untreated control. Zhang et al. (2016) reported both contact and systemic desiccants had a profound effect on lentil drying without deteriorating yield or seed quality. Similarly, application of diquat, carfentrazone-ethyl, glufosinate, flumioxazin or saflufenacil in tank mixes with glyphosate have no effect on crop yield in soybean and dry bean (Ellis et al., 1998; Soltani et al., 2013). In contrast, Yenish and Young (2000) noted that application of glyphosate as harvest aid in spring wheat (*Triticum aestivum* L.) provided uniform, but poor germination of wheat due to glyphosate translocation in developing seeds (Baur et al., 1977). Therefore, timing of desiccation is important to maintain seed quality (Bond and Bollich, 2007; Zhang et al., 2016).

2.5 Dehulling operations

Milling, primarily known as dehulling of seed, refers to the complete removal of the hull to produce both football (cotyledon together) and split seeds fraction collectively known as *dhal* (Vishwakarma et al., 2017; Wood et al., 2017). Dehulling is a major secondary processing method for the red lentil industry. Dehulled lentils are used to make soup, stews, salad, meat extenders and gluten free-diets for human consumption (Wang, 2008). Dehulling improves nutritional quality and bioavailability, palatability, storability, and digestibility and cooking quality (Narasimha et al., 2003; Wang, 2008; DellaValle et al., 2013). It was found that dehulling improves iron bioavailability in lentil (DellaValle et al., 2013), which may be due to a removal of polyphenol components along with seed coat (Deshpande et al., 1982) that interfere with iron absorption process. About 80-90% of anti-nutritional polyphenols are associated with seed coats in lentil (Mirali, 2016). Several researchers described the term 'dehulling efficiency (DE)' in different ways. For instance, Ehiwe and Reichert (1987) defined DE as the percentage of the hull removed from the cotyledon and the yield of dehulled grain obtained from the process. Wang (2005) and Wood et al. (2012) defined DE as the sum of percentage of whole and split dehulled seeds recovered after decortication. A more precise definition given by Bruce (2008) was the percentage of the total split and un-split cotyledons whose outer surface has over 98% of the hull removed during process.

2.5.1 Dehulling methods

Dehulling of pulses is preferably achieved by subjecting the seeds to an abrasive force that decorticates the hull (Sokhansanj and Patil, 2003). In the early 1970s, small-scale, hand-operated stone grinders, vertical stone mills or wooden mills were traditionally used for dehulling of pulse grains in South Asia. These mills resulted in heavy milling loss up to 45% (Ali, 2004). Nowadays, power operated abrasive and attrition type of dehullers are commercially used for pulse dehulling (Kurien, 1984). Attrition type dehullers are suitable for loose seed coat pulse seeds such as peas (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.), whereas abrasive type dehullers are appropriate for tightly adhered seed coat seeds, such as pigeon pea (Kurien, 1984, Tiwari and Singh, 2012).

In attrition type dehullers, seeds pass through a cylindrical head where a drum rubs the seeds against a cylindrical metal screen (Reichert and Youngs, 1976). Abrasive-type dehullers

uses a carborundum stone or emery surface to gradually abrade the seed coat from cotyledon (Reichert et al., 1984). In a continuous operation, seeds are fed into the machine through a hopper located at one end and, after the action of the stones, seeds are released through an overflow outlet (Reichert and Youngs, 1976). One of the abrasive-type dehullers for research studies in Canada is the tangential abrasive dehulling device (TADD), which was developed in the Prairie Regional Laboratory (PRL) of the National Research Council of Canada in Saskatoon (Sokhansanj and Patil, 2003). The average results from the TADD are comparable to those of commercial *dhal* mills in India (Singh, 1995).

Abrasive dehullers operate through friction between seed surface and rollers to separate hulls. Milling efficiency can be optimized by the mill configuration through adjustment of stone speed and texture, diameter, and duration. Rotational speed of rollers is between 600-1100 rpm or higher based on pulse seed features (Vishwakarma et al., 2017). Wang (2005) found that a laboratory Satake testing mill was suitable for dehulling lentils with good reproducibility. This testing mill works with an abrasive stone rotating at variable speeds. The stone, which is surrounded by a screen, crushes the lentils causing the hulls to break and the seeds to split. This mill has been used to study milling of red lentil (Bruce, 2008; Wang, 2008), pigeon pea (Goyal et al., 2010), black gram (Tiwari et al., 2008) and field pea (Black et al., 1998).

2.5.2 Factors affecting milling (dehulling) quality

The outcome or recovery yield after dehulling operations depends upon many factors. These can be intrinsic seed characteristic and seed genetics, seed handling and storage, crop managements and growing environments or dehulling process itself (Ramakrishnaiah and Kurien, 1983; Lucas, 2010; Wang, 2008; Tabil et al., 2010). Formation of broken seeds, more powder and improper dehulled seeds are generally considered as milling loss. Magnitude of milling losses varies between pulses. The amount of loss is lower for easy to mill pulses (such as lentil) (Lucas, 2010) and higher in difficult to mill pulses such as pigeon pea (*Cajanus cajan* L.), and black gram (*Vigna mungo* L.) (Deshpande et al., 2007, Joyner and Yadav, 2015).

2.5.2.1 Seed coat thickness

Seed characteristics, including seed coat thickness and content have been reported to influence dehulling properties of pulses. If there is a higher content of seed coat, the cotyledon

yield will be lower (Singh, 1995). Seed coat thickness and structure differs among pulse species and varieties (Erskine, et al., 1991b; DeSouza and Marcos-Filho, 2001). For example, in lentil, it varies between 6 to 7% of dry seed weight (Erskine et al., 1991b). Canadian lentils, in general, have relatively thick seed coats about 7.3% by dry seed weight (Wang, 2005). A relationship between seed coat thickness and milling efficiency have been studied in number of pulse crops. Ehiwe (1985) found the thicker seed coat of field pea was less susceptible for breakage and damage during process, whereas Wood et al. (2008) observed no association between seed coat content and milling efficiency in desi chickpea.

2.5.2.2 Chemistry of the seed coat and cotyledon

In many legumes, a cementing layer of gum (galactomannans or lignin) binds the seed coat and the cotyledons (Kurien, 1984; Wood et al., 2014b). The quantity, nature of adhesiveness, tackiness, hydration capacity of this layer may differ among species (Kurien, 1984; Ehiwe, 1985). A high amount of arabinoglucan polysaccharides and water-soluble uronic acid (UA) in pigeon pea, and soluble non-starch polysaccharides in chickpea at this layer reduces dehulling efficiency (Ramakrishnaiah and Kurien, 1985; Wood et al., 2014c). High UA in outer cotyledon regions-makes a plausible physical appearance that leads stronger cell wall structure and binds cells and forms a firmer structure (Wood et al., 2014a). Pectin and lignin is also reported to increase the adhesiveness of seed coat to cotyledon. The pectin polysaccharides in the middle lamella of pulses can act to bind cells (Weightman et al., 1994; Tosh and Yada, 2010). Wood et al. (2014c) found that the high amount of pectin in the middle lamella at adjoining layers in seeds of chickpea genotypes made them difficult to dehull.

The high level of lignin (15% by seed weight) in colored lima bean (*Phaseolus lunatus* L.) seeds made it difficult to separate seed coats compared to the low-level lignin (1% by seed weight) content of white colored genotypes (Kannenberg and Allard, 1964). In contrast, easy to dehull chickpea genotypes had high lignin content in seed coat (Wood et al., 2014b). Lignin increases brittleness resulting in easier detachment of seed coat from the cotyledon during processing. Irrespective of condition treatment, high amount of protein, tannin and starch in lentil seeds reduce dehulling efficiency (Wang, 2008). In addition, lentil differs in seed coat color chemistry may affect the milling process. The amount and variability of polyphenolic compounds in seed coats differ seed coat color (Mirali, et al, 2017). Vandenberg and Slinkard

(1990) reported a series of alleles that determine seed coat color from black to solid brown, gray, tan, or green. Mirali et al. (2017) reported both green and gray seed coat genotypes have higher amounts of flavan-3-ols, proanthocyanins, and some flavanol polyphenols than are found in brown or tan seed coat genotypes. These polyphenols may affect separation of seed coat during the dehulling process.

2.5.2.3 Seed shape, size, and hardness

Seed shape, size, hardiness, seed coat surface and structure have been reported to affect the milling process (Reichert et al., 1984; Knights et al., 2011; Wood et al., 2017). A thick and smooth seed coat with highly organized palisade cell structure of cowpea (*Vigna unguiculata* L.) dehulled more efficiently than those, which have roughly arranged cell structure seed coat (Sefa-Dedeh and Stanley, 1979). The difference in epicuticle wax patterns and topography of the cotyledon at the adjoining layer of seed coat and cotyledon differs in chickpea genotypes that are easy and difficult to dehull (Wood et al., 2017).

Seed shape and size are important varietal attributes that are largely controlled by genotype and growing environment (Erskine et al., 1991a; Wood et al., 2012). Legume seed shape ranges from spherical (field pea and pigeon pea), cylindrical (mung bean and black gram) to pyramid (chickpea) or flat (lentil) shape (Narasimha et al., 2003). Rounder seeds in general dehull more efficiently (Kurien, 1984; Reichert et al., 1984). Erskine et al. (1991b) and Wang (2008) reported that plumper and medium size lentil seeds were dehulled more efficiently than very large or very small seeds. Other studies showed plumper lentil seeds from cultivars such as CDC Blaze had higher dehulled yield compared to less plump seeds (Shahin et al., 2012). Round and plumper isogenic lines of chickpea produced 7% more *dhal* (dehulled) than their angular counterparts because rounded seeds have thinner seed coat, more intense seed coat color, faster water absorption and lower hydration capacity. Seed hardness varies with seed orientation (horizontal or vertical) at a given moisture content (Kiani et al., 2008; Vishwakarma et al., 2012), which affects the dehulling process. In general, harder seeds are more difficult to dehull, such as harder pigeon pea and kidney bean, which have significantly lower dehulling yield and cotyledon splitting (Reichert et al., 1984; Singh et al., 1992).

2.5.2.4 Seed moisture content

Optimum seed moisture prior to dehulling is vital for efficient milling of lentil. High or low moisture in pulse seeds can have a negative impact on the dehulling process. For example, in pigeon pea, high moisture results in more broken seeds, and less moisture leads to the seed coat adhering to the cotyledons (Goyal et al., 2010). Seed moisture affects seed coat breakage, hardness, bonding ability between seed and cotyledon, adhesiveness, and friction properties of seeds (Ehiwe et al., 1987; Wood and Malcolmson, 2011; Vishwakarma et al., 2012). The effect of moisture content on milling qualities has been studied in red lentil.

The optimum moisture content for storage of red lentil is 13% on wet basis (Canadian Grain commission, 2006). Optimum recommended moisture content to maximize dehulling efficiency and minimize seed coat adherence in Canadian red lentil is about 12.5% (Wang, 2005). Optimum hydration level may differ among varieties and species of pulses (Gharibzahedi et al., 2014). For example, Wood et al. (2008) equilibrated desi chickpea samples to 10% equilibrium moisture prior to milling. In a study of red lentil milling, Erskine et al. (1991a) found that dehulling efficiency was highest with seed moisture content of approximately 8% followed by immersion in water for 1 min compared to 5, 10 and 30-min immersion times. Dehulling efficiency was inversely associated with seed moisture in pigeon pea (Ramakrishnaiah and Kurien, 1983) and lentil (Erskine et al., 1991b).

2.5.2.5 Seed storage

The way in which lentils are handled and stored affects their dehulling yield. Seed storage temperature, moisture and duration have been found to influence lentil dehulling. After harvest, red lentils are stored for a period before being exported or processed in Canada. While lentils are stored, the weather in Canada goes from freezing and thawing to heating and cooling that causes lentil seeds to undergo variable temperature and humidity cycle known as drying and rewetting cycles (Tabil et al., 2010). These cycles change moisture content in lentil seeds in storage. Drying and rewetting cycles significantly affected dehulling efficiency or milling losses in red lentil (Lucas, 2010; Tabil et al., 2010). High seed moisture content has been shown to decrease the dehulling efficiency of other pulses (Ehiwe et al., 1987). Limited studies have been performed to assess the effect of temperature on dehulling efficiency. Lucas (2010) noticed storage temperature had minor effects on dehulling efficiency in lentil compared to other factors.

Storage time also affected the dehulling characteristics of lentil. For example, Tabil et al (2010) reported that there was a decrease in dehulling efficiency when red lentil samples were stored up to 12 months. Lucas (2010) also reported that storage time affects dehulling characteristics.

2.5.2.6 Crop management and lentil diseases

Despite the popularity of using herbicides as desiccants for forcing maturity prior to mechanical harvest in Canada, limited documentation is available on the effects of herbicide aids on lentil milling parameters. Bruce (2008) found that under favourable harvest conditions, the pre-treatment use of desiccant with diquat had no effect on dehulling efficiency, football recovery and dehulling efficiency of lentil. However, under cool and wet harvest conditions, early desiccation with diquat reduced milling efficiency below 70% compared to swathing. Research information on how fungicides affect milling qualities of legumes are not published or documented. Limited research findings, particularly in rice (*Oryza sativa* L.) revealed that the application azoxystrobin at the boot stage resulting in significant reduction of head blight diseases and subsequently increased dehulled recovery yield compared to unsprayed controls (Groth, 2006). Published literature related to the impact of stemphylium blight (SB) in pulse crop on milling quality are not reported. Some studies have highlighted the impact of SB on seed yield and seed quality in lentil (Banniza et al., 2006). A late infection by SB of the upper and lower canopy resulted in reduction of seed quality by increasing the percentage of stained and wrinkled seeds (Caudillo-Ruiz, 2016).

2.5.2.7 Genetics and production environment

The effects of environment and genotype on milling quality have been studied in numbers of pulse crops. The dehulled yield varies widely among species of pulse crops, for example cowpea (47.8-90.2%), green gram (58.2-73.8%), pigeon pea (79.0-83.8%) and lentil (70-85%) (Ehiwe and Reichert, 1987; Wang, 2008; Bruce, 2008). The temperature, humidity, and rainfall at seed maturation and harvest can directly affect milling recovery yield (Cooper et al., 2006; Thompson and Mutters, 2006). While examining the performance of desi chickpea in Australia, Wood et al. (2008) found that environmental stress affected crop yield but had no apparent effect on seed coat removal during processing. Fasahat et al. (2014) noted that weather variability at crop maturity caused variation in percentage of milled rice among genotypes. They

also noticed under very high temperature, production of more immature seeds leads to more bran, and broken seeds due to the lower number of days during the grain filling period. Ehiwe (1985) noted temperature and rainfall variability at seed maturity caused differences in seed breakage among field pea genotypes. Hot and dry conditions at the pod maturation stage in lentil results in rapid pod desiccation, which favors efficient dehulling. Nevertheless, if the seeds are brittle, it may increase the amount of powder and broken seeds during processing (Bruce, 2008). In addition to the effect of genotype, growing season, location, and their interaction with genotype significantly affected the dehulling process in lentil, pigeon pea and chickpea (Erskine et al., 1985; Williams and Singh, 1987; Wood et al., 2008). In contrast to this, Erskine et al. (1991b) reported environment, particularly location, had the least effect and genotype had more impact on lentil dehulling.

2.6 Genetic basis of dehulling and milling quality of lentil

Milling quality characteristics of a given crop are complex traits and they are inherited quantitatively. They might be regulated by the interactions of many genes and by environment (Swamy et al., 2012). The seed coat and cotyledon chemistry, seed color, seed dimensions and morphology may have a significant association with milling quality parameters. Therefore, genes associated with these characteristics may influence the milling parameters in lentil. Vandenberg and Slinkard (1990) reported that two independent loci (*Ggc* and *Tgc*) determine the four-basic seed coat background colors in lentil; brown (*Ggc Tgc*), gray *(Ggc tgc)*, tan (*ggc Tgc*) and green (*ggc tgc*). A series of alleles at the *Scp* locus (Ladizinsky, 1979) determine seed coat patterns. The color genes *Ggc, Tgc* and pattern gene *Scp* were mapped in linkage groups 2, 3 and 6, respectively (Fedoruk et al., 2013). A single gene determines red or yellow cotyledon color gene *Yc,* with red color dominant yellow (Slinkard, 1978). A second gene (*I-yc)* causes cotyledons to be green and is epistatic to the *Yc* gene. The cotyledon color locus *Yc* was mapped in linkage group (LG) 1 (Fedoruk et al., 2013). These loci may have influence on milling traits.

Seed dimensions of lentil (diameter, thickness, and plumpness) and seed weight are found to be controlled by multiple genes (Fedoruk et al., 2013; Verma et al., 2015). The QTL associated with these traits are located on either LG1 or LG7 along with several SNP markers and the cotyledon color gene *Yc*. These loci can also be associated with milling traits. Erskine (1991a) found seed size had great impact on dehulling in lentil and Wood et al. (2012) reported

that a gene for round seed shape (*Rd/rd*) was significantly correlated with dehulling efficiency in chickpea. Examples of shape genes are available in cereal crops, for example, QTL analysis for milling efficiency in two hexaploid oat populations by Groh et al. (2000) found QTL of kernel width and length QTL were in same region with milling efficiency when tested 137 RIL using RFLP and AFLP markers.

2.7 Linkage mapping and genetic markers used in lentil

Linkage mapping is a molecular marker assembling process in which markers are arranged based on relative genetic distance and assigned as markers on linkage group based on recombination value (Jones et al., 1997). Recombination value is determined by the frequency of recombinant genotypes in a population. Constructing a detailed linkage map is crucial for identifying genes associated with phenotypic traits as a means of understanding the genetic background of crops (Eujayl et al., 1998). Production of mapping populations, identification of polymorphic markers and linkage analysis are the three main steps for linkage mapping. Double haploids, F_2 populations, RILs, backcross and near isogenic lines (NILs) are commonly used mapping populations (Kumar et al., 2015).

In lentil, both inter and intra specific mapping populations derived from crosses between *L. culinaris* and other *Lens* species were widely used for mapping (Durán et al., 2004). Population sizes of more than 100 individuals or higher are better for map construction to increase map resolution (Mohan et al., 1997). Several researchers reported lentil has a relatively low level of genetic variation compared to other plant species (Eujayl et al., 1998; Ferguson and Robertson, 1999; Ford et al., 2009). Genetic mapping in lentil was first conducted by Zamir and Ladizinsky (1984). The first maps using DNA-based markers were produced by Havey and Muehlbauer (1989). Rubeena et al. (2003) first developed an intraspecific linkage map which had 9 linkage groups with total length of 784.1 cM using 114 random amplified polymorphism (RAPD) DNA markers. With the development of single nucleotide polymorphism (SNP) based markers, several researchers published extensive linkage maps in lentil (Sharpe et al., 2013; Kumar et al., 2015; Verma, et al., 2015).

Morphological markers including cotyledon color (*Yc*), anthocyanin in stem (*Gs*), pod indehiscence (*Pi*), seed coat pattern (*Scp*), flower color (*W*), radiation frost tolerance locus (*Rf*), early flowering (*Sn*) and ground color of seed (*Gc*) were mapped using PCR based marker in

lentil (Eujayl et al., 1998; Hamwieh et al., 2009; Tullu et al., 2008; Sharpe et al., 2013). DNA based markers, for example, amplified fragment length polymorphic (AFLPs), restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPD) markers, simple sequence repeats (SSRs) and single nucleotide polymorphic (SNP) markers have been frequently used lentil for genetic map construction in lentil (Rubeena et al., 2003; Durán et al., 2004; Fratini et al., 2007; Ford et al., 2007; Liu et al., 2008).

2.7.1 Single nucleotide polymorphism (SNP) markers

SNP markers are the most abundant polymorphic markers and are now widely used for high-resolution genetic mapping of trait, and for association studies to identify candidate genes (Rafalski, 2002). The average SNP density in plant genomes is approximately one SNP per 200-500 bp, but is dependent on species (Weising et al., 2005). Discovery of large numbers of SNPs is possible in many crop species using recent sequencing technology. Re-sequencing PCR amplicon, electronic SNP discovery in shotgun genomic libraries or discovery in expressed sequence tag (EST) libraries are widely used methods for SNP discovery (Rafalski, 2002). These methods rely on sequence information. Thousands of SNPs can be genotyped in crops using high-throughput parallel multiplex assays, or chip technology. These SNP chips have been used in both cereals, such as barley and maize, and for pulse crops (Abbo et al., 2005; Close et al., 2009; Yan et al., 2009; Chagné et al., 2012; Fedoruk et al., 2013). Sharpe et al. (2013) developed a parallel allele-specific Illumina Golden Gate 1536 SNP assay using expressed sequence tags (EST) for SNP discovery from nine *L. culinaris* ssp. *culinaris* and two *L. ervoides* genotypes. This array is based on the Illumina Bead Chip™ technology, which has 1,536 bi-allelic SNPs that can be screened across mapping populations, association lentil diversity panels or any collection of lentil lines. Fedoruk et al. (2013) used these to identify SNP markers associated with lentil seed dimensions.

2.7.2 Achievements with quantitative trait loci (QTL) mapping in lentil

QTL mapping is the major tool for associating quantitatively inherited phenotypic traits of interest with molecular markers (Collard et al., 2005; Kumar et al., 2015). Single marker analysis, simple internal mapping, composite interval mapping (CIM) and multiple interval mapping (MIM) procedures have been used for QTL analysis (Liu, 2008; Kumar et al., 2015).

Molecular markers that are inherited with, or significantly associated with, a trait of interest can be useful for breeders to predict the phenotypic value.

Molecular markers are particularly important for breeders who wish to select and improve traits that have low heritability. QTLs for lentil were mapped for agronomic traits such as plant height, days to flower, winter hardiness, growth habit and yield, seed dimensions and seed micronutrient content (Durán et al., 2004; Fratini et al., 2007; Fedoruk et al., 2013; Verma et al., 2015). Durán et al. (2004) detected five QTL for flowering time, three for plant height, seven for pod dehiscence and one for shoot number and seed diameter in lentil. In intraspecific lentil populations, five and four QTLs were identified for winter survival and winter injury, respectively (Kahraman et al., 2004). Three QTL for ascochyta blight resistance at the seedling and podding stages that explained 34-61% of the phenotypic variation have been reported (Gupta et al., 2012). QTL conferring resistance to stemphylium blight and rust diseases using RIL populations were also detected in lentil (Saha et al., 2010).

Multiple QTL for seed dimensions (seed diameter, thickness, and plumpness) were detected in lentil using the intraspecific lentil recombinant inbred line (RIL) population LR-18 (Fedoruk et al., 2013; Fedoruk, 2013). QTL analysis was also used to study seed diameter and seed weight in an intraspecific lentil RIL population in India (Verma et al., 2015). So far, no published research has been reported for QTL and markers associated with milling quality traits in lentil.

2.8 Summary

Seed coat removal through dehulling is an important post harvest-processing step for the red lentil industry. Several factors affect the dehulling process, such as management of agronomic practices, environment, seed genetics, milling equipment and methods used for processing. The climate, agronomic management practices and genetic base that affect red lentils grown in northern temperate climates are unique and may result in poor milling recovery compared to lentil produced and milled in dry environments. Little research has been conducted to explore the effect of these variables on lentil milling efficiency in western Canada. Therefore, research is required to understand the effects of lentil agronomic management practices including pre-harvest treatments, disease management and lentil genetics on lentil milling and dehulling quality.

Prologue to Chapter 3

The following research chapter describes the effects of harvest aid herbicides alone on seed morphological, biological, and milling quality characteristics. In this study, action of both contact and systemic harvest aid desiccants with or without tank mixing of glyphosate were assessed under field conditions. This chapter was published in February 2017 as a manuscript in the journal Frontiers in Plant Sciences.

Subedi, M., Willenborg C.J., and Vandenberg, A. 2017. Influence of harvest aid herbicides on seed germination, seedling vigor and milling quality traits of red lentil (*Lens culinaris L.*). Front. Plant Sci. 8:311. doi: 10.3389/fpls.2017.00311

CHAPTER 3. EFFECT OF HARVEST AID HERBICIDES ON SEED AND MILLING QUALITY CHARACTERISTICS OF RED LENTIL (*LENS CULINARIS* MEDIKUS)

Abstract

Most red lentil produced worldwide is consumed in dehulled form, and post-harvest milling, and splitting qualities are major concerns in the secondary processing industry. Lentil producers in northern temperate regions usually apply pre-harvest desiccants as harvest aids to accelerate the drying process and facilitate harvesting operations of the lentil crop. This paper reports on field studies conducted at Scott and Saskatoon, Saskatchewan, Canada in the 2012 and 2013 cropping seasons to evaluate whether herbicides applied as harvest aids alone or tank mixed with glyphosate affect seed germination, seedling vigor, milling, and splitting qualities of red lentil. The site-year by desiccant treatment interaction for seed germination, vigor, and milling recovery yields was significant. Glyphosate applied alone or as tank mix with other herbicides (except diquat) reduced seed germination and seedling vigor at Saskatoon and Scott in 2012 only. Pyraflufen-ethyl (20 g ai ha^{-1}) applied with glyphosate as well as saflufenacil (36 g ai ha^{-1}) decreased dehulling efficiency, while saflufenacil and/or glufosinate with glyphosate reduced milling recovery and football recovery, although these effects were inconsistent. Application of diquat alone or in combination with glyphosate resulted in more consistent dehulling efficiency gains and increases in milling recovery yield. Significant but negative associations were observed between glyphosate residue in seeds and seed germination *($r=-0.84$, $p<0.001$),* seed vigor *($r=-0.62$, $p<0.001$),* dehulling efficiency *($r=-0.55$, $p<0.001$),* and milling recovery *($r=-0.62$, $p<0.001$).* These results indicate application of diquat alone or in combination with glyphosate may be a preferred option for lentil growers to improve milling recovery yield.

3.1 Introduction

Lentil (*Lens culinaris* Medikus.) is a valuable grain legume crop that is a good source of dietary protein, fiber, complex carbohydrates, and minerals (Jood et al., 1998; Xu and Chang, 2009; DellaValle et al., 2013). The western Canadian Prairies are the world's major lentil producing and exporting region, with a current production area of 2.3 million ha (Statistics Canada, 2016). The main destinations for red lentil are India, Turkey, the United Arab Emirates, and the European Union (Statistics Canada, 2016). About 90% of red lentils are consumed in dehulled form after removal of the seed coat through an abrasive dehulling process that improves the taste of cooked lentil by removing anti-nutritional factors, such as polyphenols and tannins, which are mostly retained in the seed coat fraction (Singh and Singh, 1992; Wang, 2008; DellaValle et al., 2013). Efficient loosening of the seed coat during dehulling process of red lentil is vital for the milling industry. The market value of red lentil largely depends on milling quality, which in turn depends on genetics but also on agronomic practices such as the application of pre-harvest desiccants and growing environment (Ramakrishnaiah and Kurien, 1983; Wang, 2008).

Lentils harvested under low humidity and high temperature conditions in Australia, Mediterranean and sub-tropical savannah climates are more efficiently dehulled than those harvested in a temperate climate (Brand, 2008). Northern temperate prairie environments experience much different climatic conditions compared to global competitors who tend to harvest during hot dry conditions. Indeed, red lentils produced in northern temperate prairie regions generally have a higher moisture content at harvest and different physical characteristics than those grown in Mediterranean and sub-tropical savannahs (Vandenberg, unpublished, 2016). The climate, genetic base, and agronomic practices for red lentil grown in northern temperate climates are unique and may result in poor milling recovery compared to lentil produced and milled in dry environments (Vandenberg, 2009).

In Western Canada, the environmental variation within fields and the indeterminate nature of crop growth means that lentil growers usually apply pre-harvest desiccants to optimize harvest conditions on the variable landscape. Lentils are considered sufficiently mature for desiccation with harvesting aids when 80% of the pods in the lower third of the canopy have turned from green to yellow or brown (Saskatchewan Pulse Growers, 2015). Desiccant chemistry and application timing are crucial as they may cause loss of yield and quality (Wilson and Smith,

2002; Bennett and Shaw, 2000a; Bennett and Shaw, 2000b; Zhang et al., 2016). In Western Canada, the herbicides registered as harvest aids for lentil and other pulses include diquat, glyphosate, saflufenacil, glufosinate, and flumioxazin (Risula, 2014). These desiccants have different modes of action and chemistry and, therefore, may differentially affect post-harvest seed quality.

Diquat is a quick acting contact herbicide that is traditionally used as a harvest aid for lentil. It rapidly and quickly dries plant tissues within few days of application and has no or low translocation in plants (Cobb and Reade, 2010; Zhang et al., 2016). However, pre-harvest application of diquat affects milling recovery yield and other post-harvest seed qualities in lentil when it is applied too early (Bruce, 2008). Glyphosate, a popular herbicide product, is used in lentil production to control late emerging annual weeds and acts as a desiccant. It is also used as a desiccant in common bean production in Canada (McNaughton et al., 2015). Seeds are a major photosynthate sink during maturation (Cakmak et al., 2009); glyphosate is translocated mainly through phloem and distributed throughout the plant, and seed germination and vigor may be reduced if seeds accumulate too much glyphosate residue (Clay and Griffin, 2000; Zhang et al., 2016).

Glufosinate and saflufenacil are newly registered as desiccants for lentil crops (Risula, 2014). Saflufenacil is a weak acid herbicide used for broadleaf weed control in soybean, corn, and other crops. It moves both acropetally and basipetally in plants and affects symptoms similar to other contact herbicides (Soltani et al., 2010). Glufosinate can translocate within plants but has rapid phytotoxicity that limits its mobility (Grossman et al., 2010b; Soltani et al., 2013). Pyraflufen-ethyl and flumioxazin are also potential harvest aids for lentil crops in Western Canada (Risula, 2014; Zhang et al., 2016) and are commonly used as desiccants in cotton (Griffin et al., 2010) and common bean (Soltani et al., 2013). Overall, minimal research has been conducted to assess the impact of the complete spectrum of harvest aids (e.g., diquat, pyraflufen, glufosinate ammonium, flumioxazin, saflufenacil) alone or in combination with glyphosate with respect to their effects on seed biology and post-harvest processing of lentil. This study was conducted to evaluate the effect of contact herbicides applied alone or as tank mixtures with glyphosate as harvest aids on milling recovery yield, seed germination, seedling vigor, and other seed quality attributes of red lentil.

3.2 Hypothesis

Harvest aids can provide adequate crop desiccation without affecting seed and milling quality. Secondly, increasing rate of herbicide do not have adverse effect on milling quality characteristics in lentil. In addition, glyphosate residues in seeds have adverse effects on post-harvest seed and milling quality characteristics in lentil.

3.3. Materials and methods

3.3.1 Field experiments and environmental conditions

Harvested seeds samples of red lentil (cv. CDC Maxim) were obtained from field experiments conducted by Zhang et al. (2016) in two Saskatchewan locations in 2012 and 2013. These locations (Saskatoon, 52°36' N, 108°84' W, altitude 659.6 m; Scott, 52°09' N, 106°33' W, altitude 505 m) are in the Dark Brown zone of Chernozemic soils (clay to sandy loam at Saskatoon and silt loam at Scott). The soil organic matter content and pH ranged from 2.4 to 4.5% and 7.5 to 7.9 at Saskatoon and 2.4 to 2.6% and 5.3 to 6.8 at Scott, respectively.
The experimental treatments (Table 3.1) were arranged in a randomized complete block design (RCBD) with four replicates. Each block consisted of 21 desiccants and an unsprayed control. The desiccants used in this study were pyraflufen-ethyl (10 and 20 g ai ha^{-1}), glufosinate (300 and 600 g ai ha^{-1}), flumioxazin (105 and 210 g ai ha^{-1}), saflufenacil (36 and 50 g ai ha^{-1}) and diquat (208 and 415 g ai ha^{-1}), with each desiccant applied alone or in combination with glyphosate (900 g ae ha^{-1}). The list of treatments is presented in Table 3.1. All desiccant treatments were applied with the recommended adjuvant, either Merge ® (50% surfactant; 50% petroleum hydrocarbons solvent) or Agral 90 ® (90% nonylphenoxy polyethoxy ethanol) with an air pressurized tractor mounted sprayer equipped with shielding (110-015 AirMix nozzles, 275 kPa, 45 cm spacing at Saskatoon and with a CO_2 pressurized bicycle sprayer (110-003 AirMix nozzles, 276 kPa, 25 cm) at Scott. Both sprayers were calibrated to deliver 200 L ha^{-1} of spray solution. Prior to crop harvest aid applications, the seed moisture content was determined by picking and bulking a few seeds from two border plots to create a composite sample, which was then dried at 90 °C for 24 h. Treatments were applied to the lentil crop at approximately 30% seed moisture content at the recommended stage (when lower seeds rattled, and pods started turning brown, and middle pods were yellow to brown). The crops were harvested 21 days after

desiccant application. Weather information related mean monthly temperature and precipitation during the growing season at each location in 2012 and 2013 are presented in Table 3.2.

Table 3.1. Desiccant (pre-harvest aids) treatments and their rate of application.

Desiccant /Herbicide	**Rate (g a.i./a.e. ha^{-1})**	**Surfactants**
Untreated check		-
Glyphosate	900	-
Pyraflufen	10	Merge (1% v/v)
	20	Merge (1% v/v)
Pyraflufen+Glyphosate	10+900	Merge (1% v/v)
	20+900	Merge (1% v/v)
Glufosinate	300	-
	600	-
Glufosinate +Glyphosate	300+900	-
	600+900	-
Flumioxazin	105	Agral 90 (0.25% v/v)
	210	Agral 90 (0.25% v/v)
Flumioxazin+Glyphosate	105+900	Agral 90 (0.25% v/v)
	210+900	Agral 90 (0.25% v/v)
Saflufenacil	36	Merge (1% v/v)
	50	Merge (1% v/v)
Saflufenacil+Glyphosate	36+900	Merge (0.5% v/v)
	50+900	Merge (0.5% v/v)
Diquat	208	Agral (0.1%v/v)
	415	Agral (0.1%v/v)
Diquat+Glyphosate	208+900	Agral (0.1%v/v)
	415+900	Agral (0.1%v/v)

The red cotyledon lentil cultivar CDC Maxim (40-45 mg seed weight) was chosen for the study because it is the most widely grown cultivar in the Canada. It is tolerant to imidazolinone herbicides. Details of field management are documented by Zhang et al. (2016). Prior to seeding, lentil seeds were inoculated with liquid Nodulator $^{@}$ inoculant (*Rhizobium leguminosarum biovar viceae* at a rate of 2.76 mL kg^{-1} in 2012, and with Tag Team$^{@}$ Granular (*Rhizobium leguminosarum and penicillium bilaii*) at a rate of 2.8 kg ha^{-1} in 2013. Seeds were treated with Apron Maxx RTA (0.73% fludioxonil; 1.10% metalaxyl-M and S-isomer) at a rate of 325 ml per 100 kg of seed before sowing in each site. After treatment, seeds were sown at 3 cm depth with seeding density of 130 seeds m^{-2} using a small plot drill equipped with hoe openers spaced at 22 cm between rows. Individual plots were 2.25 m wide and 6 m long at Saskatoon and 2 m wide and 5 m long at Scott and consisted of six rows at both sites. Sowing was performed in mid-May in each year at each location. A tank mixture of imazamox and imazethapyr (30 g a.i. ha^{-1}) was

sprayed between 5th and 6th lentil node stage at Saskatoon and quizalofop-p-ethyl (420 g a.i. ha^{-1}) was sprayed at 4th node stage of lentil for post emergence weed control. Hand weeding was carried out to maintain plots weed free. The fungicides triticonazole (166 g a.i. ha^{-1}) and boscalid (294 g a.i. ha^{-1}) were applied at Saskatoon and Scott, respectively, to control foliar diseases.

Table 3.2. Mean monthly temperature (°C) and total monthly precipitation (mm) during the growing season at Saskatoon and Scott, Saskatchewan, Canada, in 2012 and 2013.

	2012		2013	
Month	**Mean monthly temperature (°C)**	**Total precipitation (mm)**	**Mean monthly temperature (°C)**	**Total precipitation (mm)**
Saskatoon, Saskatchewan				
May	16.4	108.0	13.0	15.2
June	21.5	121.1	15.2	115.9
July	19.7	80.9	17.4	35.2
Aug.	17.3	48.5	18.9	14.7
Sep.	14.0	0.8	15.2	14.9
Scott, Saskatchewan				
May	16.4	50.6	19.9	38.9
June	21.2	164.6	20.0	113.5
July	24.3	56.4	22.7	26.1
Aug.	23.6	51.4	24.5	63.3
Sep.	20.2	24.4	22.1	0.0

Source: http://climate.weatheroffice.gc.ca/climateData/canada_e.html

3.3.2 Post-harvest seed measurements

Randomly selected 250-seed samples from individual plots were collected, counted using an electronic seed counter (ESC-1 Agricole Inc., Guelph, ON, Canada), and weighed to determine 1000-seed weight. Seed diameter and thickness were measured using round-hole and slotted-hole sieves (Hossain et al., 2010; Fedoruk et al., 2013). Seed diameter was measured by passing seed samples of approximately 100 g through a set of 10 round-hole sieves ranging from 5.95 mm (15/64"), 5.55 (14/64), 5.15(13/64), 4.76 (12/64), 4.36 (11/64), 3.96 (10/64) down to 3.57 mm (9/64") in 0.25 mm (1/64") increments. Seed thickness was measured by passing the same sample through a set of six slotted-hole sieves from 3.18 mm (8/64"), 2.98 (7.5/64), 2.78 (7/64), 2.58 (6.5/64), 2.38 (6/64), 2.18 (5.5/64) down to 1.98.0 mm (5/64") in 0.2 mm (0.5/64") increments. All samples were shaken for 1 min on a flatbed shaker (Lab Line Instruments, Melrose Park, Illinois, USA). The seed fractions remaining in each round and slotted-hole sieve were weighed. Seed diameter and thickness for each sample were calculated using the following formulas:

$$\textbf{Percentage on sieve} = \frac{weight\ of\ seed\ in\ each\ sieve\ (g)}{weight\ of\ total\ sample\ seed\ (g)} \times 100 \quad \text{.....(3.1)}$$

$$\textbf{Mean seed diameter} = \left[\Sigma \frac{\%\ of\ seed\ weight\ on\ round\ sieve}{100} \times\ sieve\ hole\ size\ (mm)\right] \text{.....(3.2)}$$

$$\textbf{Mean seed thickness} = \left[\Sigma \frac{\%\ of\ seed\ weight\ on\ slotted\ sieve}{100} \times\ sieve\ hole\ size\ (mm)\right] \text{.....(3.3)}$$

$$\textbf{Seed plumpness} = \frac{mean\ seed\ thickness}{mean\ seed\ diameter} \text{.....(3.4)}$$

3.3.3 Dehulling procedure and milling quality measurement

Prior to dehulling, initial moisture content of the seeds was determined using an oven dry method (AACC, 2000). For each whole seed sample, 16 g was dried at 130°C for 20 h, and the weight difference of each sample expressed as moisture percentage.

Lentil seed samples (30 g) that remained in the round (4.47 to 5.16 mm) and slotted (2.38-2.58 mm) sieves were tempered to 12.5% moisture (Wang, 2005) and then dehulled using a grain testing mill (TM05, Satake Engineering Co., Hiroshima, Japan) fitted with a 36-mesh abrasive wheel rotating at 1100 rpm for 38 s, as described by Wang (2005). After dehulling, milled samples were collected in a paper envelope and then the entire milled sample was weighed and separated into different fractions. For separation, the first sample was screened on Canada standard No. 14 (1.40 mm) and No. 35 mesh (850 µm) sieves. The powder was collected and weighed. The leftover fraction in the No. 14 sieve was passed through an aspiration unit to separate the hull portions. The sample seeds remaining in the aspiration column were further sieved to separate fractions. The remaining lentil seeds without powder and hulls were passed through a No. 6 (6/64") slotted sieve and No. 9 (9/64") round sieve. Whole lentils remaining over the slotted sieve were considered football, lentils remaining in the round sieve were considered split, and material in the pan was considered broken seed. Any whole and split lentils with adhering hulls were separated manually into their respective adhering hulled or dehulled classes. All fractions were weighed and then expressed as a proportion of the total original milled sample weight. Dehulling efficiency was defined as the percent of un-dehulled whole and split (%) seed relative to total initial sample weight (Wang, 2005; Bruce, 2008); and milling recovery indicated as the percent of dehulled splits and football fractions to total initial sample weight. Football recovery, milling recovery, and dehulling efficiency were calculated according to the following.

$$\textbf{Dehulling efficiency (DE, \%)} = \left[1 - \frac{(weight\ of\ undehulled\ whole\ seed\ (g) + undehulled\ split\ seed\ (g)}{Initial\ \ weight\ of\ sample\ seed\ (g)}\right] \times 100 \quad \text{.....(3.5)}$$

$$\textbf{Milling recovery (MR, \%)} = \frac{weight\ of\ milled\ seeds\ (g)}{Initial\ \ weight\ of\ sample\ seed\ (g)} \times 100 \quad \text{.....(3.6)}$$

$$\textbf{Football recovery (FR, \%)} = \frac{weight\ of\ football\ (un-split\ intact\ seed)(g)}{Initial\ \ weight\ of\ sample\ seed\ (g)} \times 100 \quad \text{.....(3.7)}$$

3.3.4 Germination and vigor tests

Seed germination tests were performed at Discovery Seed Labs Ltd. (Saskatoon, SK) using the rolled paper towel method and procedures recommended for lentil seed by the Canadian Food Inspection Agency (CFIA, 2012). Two hundred seeds from each plot in each replication were evenly spaced on two sheets of germination paper and then covered with a moistened paper. Four replications were used. The sheets of paper were rolled and placed in an upright position. The rolled paper sheets were moistened daily by adding water. The temperature was maintained at 20°C. After 7 d, the number of normal seedling and abnormal seedlings, such as number of un- germinated fresh, dormant, hard, and dead seed were counted. Then percentage of normal seedling were used to expresses germination percentage.

Seedling vigor was also determined by the standard method developed by Canadian Food Inspection Agency at Discovery Seed Labs Ltd., using 200-seed samples at 5°C for 7 d. The number of normal and vigorous seedlings were counted 7 d after emergence and expressed as a percentage.

3.3.5 Glyphosate residue content

The glyphosate residue data, reported by Zhang et al. (2016) using high performance liquid chromatography (HPLC) column switching and post-column derivation with fluorescence detection to determine glyphosate (at ALS Laboratories, Edmonton, AB, Canada), were used for correlation analysis among selected traits. Glyphosate residue content was analysed using both treated and un-treated seeds. Each 250-g seed sample was collected at 7 DAA from border rows, cleaned, placed into plastic bags, and kept in a freezer at -20^{o}C until all samples were collected. Samples were analysed by ALS Laboratories in Edmonton, AB, Canada, using standardized process provided by ALS Laboratories. High performance liquid chromatography (HPLC) using column switching and post-column derivatization with fluorescence detection was employed to

determine glyphosate and AMPA residue. Briefly, a mixture of 150 ml of 0.1 M hydrochloric acid and 50 ml of dichloromethane was added to ground samples. The solution was homogenized for 1 minute with a polytron and centrifuged at 5000 RPM for 10 minutes. The aqueous layer of this solution (100 ml) was decanted to a flask and diluted with deionized water to 350 ml and eluted through a Chelex 100 resin column at 2 drops per second. The wall of this column was then washed with 50 ml of deionized water and 100 ml of 0.2 M hydrochloric acid. All the eluent was discarded. Following this, 7 ml of 6 M hydrochloric acid was added to the column, and the eluent was discarded. 25 ml of 6 M hydrochloric acid was added again to the column, and with the eluent collected, mixed with 11 ml of concentrated hydrochloric acid, and applied to a AG1-X8 resin column to remove excess iron. After the eluent entered the AG1-X8 resin column, the column was rinsed with 10 ml of 6 M hydrochloric acid, and the eluent was concentrated on a rotary evaporator. The extract of glyphosate and AMPA was then determined with an HPLC equipped with a fluorescence detector. Differential retention time was used to distinguish between glyphosate and AMPA, with a limit of detection of 0.020 ppm for both compounds.

3.3.6 Data analyses

Normality and homogeneity tests were performed using residual data through the PROC UNIVARIATE procedure and Levene's test, respectively, prior to using a mixed model in SAS 9.3 (SAS Institute, Inc., Cary, NC, USA; SAS, 2015). Data were pooled and analyzed using the PROC MIXED procedure in a randomized complete block design. The pre-harvest aid (desiccant) treatment was considered a fixed factor, whereas environment (year × location (site year), environment × treatment interaction, and blocks were considered random factors. In the mixed model analysis, the significance of the fixed effect was tested using F-tests, whereas random effects were tested using a Z-test of the variance estimate. The REPEATED/GROUP statement was used to model heterogeneous variance for germination data from 2012 samples because these data did not meet the assumption to use ANOVA even after transformation. The covariance parameter estimation (COVTEST option of PROC MIXED) was used to determine whether data might be combined across site-years for analysis. The data were analyzed for each year and location separately for those variables that had significant interactions of site-year with desiccants. Fisher's least significant difference (LSD) was performed for mean separation with a

5% significance level. Additionally, all letter groupings for significance differences were established using PDMIX 800 in SAS (Saxton, 1998). Simple linear contrast estimate was used to compare differences in mean of groups. PROC CORR command in SAS 9.3 was used to analyze correlation among selected traits.

3.4 Results

3.4.1 Seed physical characteristics: diameter, thickness, and plumpness

Desiccation treatments had a marginal effect on 1000-seed weight and seed moisture content prior to conditioning; therefore, data for these parameters are not presented. Irrespective of year or location, glyphosate applied alone or as a tank mix with other herbicides had no significant effect on seed diameter, thickness, or seed plumpness (Table 3.3). Effects on seed physical dimensions were consistent across all site-years, as no significant interactions were observed between desiccant and site-year (Table 3.3). However, contrast analysis showed that the addition of contact herbicides to glyphosate increased seed diameter compared to glyphosate applied alone (Table 3. 4). Conversely, application of higher rates of contact herbicides significantly decreased seed diameter by 2%. In contrast, neither addition of contact herbicide with glyphosate nor glyphosate applied alone affected seed thickness or plumpness (Table 3.4). These results suggest that none of the contact herbicides considered, applied alone or in tank mixes with glyphosate, had any adverse effect on seed physical qualities.

Table 3.3. P values derived from combined analysis of variance using a mixed model for seed diameter (mm), seed thickness (mm), seed plumpness, seed germination (%), seed vigor (%), percent dehulling efficiency (DE), percent milling recovery (MR), and percent football recovery (FR) influenced by desiccation treatment at Saskatoon and Scott, Saskatchewan (SK) in 2012 and 2013.

Source of variation	Df	Seed physical and biological characteristics					Milling quality characteristics		
		Diameter	Thickness	Plumpness	Germination	Vigor	DE	MR	FR
Site-year (SY)	3	0.180	0.163	0.160	0.442	0.190	0.167	0.218	0.016*
Desiccant (D)	21	0.152	0.450	0.445	<0.001***	<0.001***	0.152	0.294	0.028*
SY × D	63	0.110	0.070	0.058	<0.000***	<0.000***	0.050*	0.037*	0.050*

*, **, and *** represent significant differences at *P<0.05, <0.01,* and *<0.001*, respectively, Df indicates degrees of freedom

Table 3.4. Mean seed thickness, seed diameter, and seed plumpness of lentil treated by desiccant treatments at Saskatoon and Scott, SK in 2012 and 2013. Contrast statements indicate differences in mean between treatments in seed diameter, seed thickness and seed plumpness.

Treatment	Rate (g a.i/a.e ha^{-1})	Seed diameter (mm) (d)		Seed thickness (mm) (b)		Seed plumpness (d/b)	
		Mean	SD	Mean	SD	Mean	SD
Untreated control	-	4.70	0.40	2.71	0.12	0.57	0.08
Glyphosate	900	4.68	0.43	2.69	0.10	0.57	0.07
Pyraflufen-ethyl	10	4.67	0.39	2.66	0.12	0.57	0.07
Pyraflufen-ethyl	20	4.68	0.29	2.62	0.11	0.56	0.06
Glufosinate	300	4.65	0.29	2.65	0.13	0.57	0.08
Glufosinate	600	4.66	0.41	2.67	0.13	0.57	0.08
Flumioxazin	105	4.67	0.41	2.68	0.13	0.57	0.08
Flumioxazin	210	4.68	0.48	2.76	0.11	0.59	0.09
Saflufenacil	36	4.66	0.40	2.67	0.13	0.57	0.07
Saflufenacil	50	4.69	0.38	2.68	0.12	0.57	0.07
Diquat	208	4.64	0.33	2.63	0.10	0.56	0.06
Diquat	415	4.66	0.32	2.62	0.13	0.56	0.06
Pyraflufen-ethyl	10+900	4.69	0.25	2.59	0.12	0.55	0.07
Pyraflufen-ethyl	20+900	4.68	0.39	2.64	0.11	0.56	0.08
Glufosinate + glyphosate	300+900	4.67	0.41	2.60	0.12	0.56	0.08
Glufosinate + glyphosate	600+900	4.64	0.44	2.68	0.11	0.57	0.08
Flumioxazin + glyphosate	105+900	4.66	0.45	2.69	0.12	0.58	0.08
Flumioxazin + glyphosate	210+900	4.67	0.34	2.63	0.09	0.56	0.07
Saflufenacil + glyphosate	36+900	4.66	0.40	2.68	0.13	0.57	0.07
Saflufenacil + glyphosate	50+900	4.68	0.29	2.62	0.12	0.56	0.05
Diquat + glyphosate	208+900	4.68	0.47	2.73	0.12	0.57	0.09
Diquat + glyphosate	415+900	4.65	0.39	2.67	0.14	0.57	0.07
LSD		ns		ns		ns	
Contrasts							
Untreated control vs. $TM^{\overline{T}}$ and glyphosate		0.03*		0.01		0.05	
Glyphosate vs.$TM^{\overline{T}}$ +glyphosate		-0.03*		0.01		0.06	
$TM^{\overline{T}}$ vs. $TM^{\overline{T}}$ +glyphosate		0.00		0.00		-0.02	
$TM^{\overline{T}}$ (low rate) vs.$TM^{\overline{T}}$ (high rate)		0.02**		0.00		0.00	

$TM^{\overline{T}}$: herbicides used as tank-mix; ns: non-significant at P<0.05. * and ** represent significant differences at *P<0.05* and *<0.01*, respectively. SD indicates standard deviation; LSD denotes Fisher's least significant differences value at 0.05 level of probability.

3.4.2 Seed biological characteristics: germination and seedling vigor

The impact of desiccant treatment on seed germination and seedling vigor varied with growing environment (Table 3.3) so these data were analyzed separately by site year (3.5). At Saskatoon in 2012, diquat, pyraflufen, glufosinate (300 g a.i. ha^{-1}), and flumioxazin did not affect lentil seed germination compared to the untreated control; in contrast, plots sprayed with glyphosate (900 g a.i. ha^{-1}) alone or with these desiccants as tank-mix with glyphosate except (diquat+glyphosate) resulted in a significant *(P<0.05)* reduction of seed germination compared to the untreated control (Table 3.6).

Table 3.5. F-values from analysis of variance (ANOVA) for seed germination, seed vigor, dehulling efficiency (DE), milling recovery (MR), and football recovery (FR) evaluated each site at Saskatoon and Scott, SK in 2012 and 2013.

Year	Location	Source	df	F-values				
				Seed germination	Seed vigor	DE	MR	FR
2012	Saskatoon	Desiccants	21	28.02**	4.07***	1.97*	1.82*	1.73*
	Scott	Desiccants	21	29.2***	7.68***	2.14**	1.84*	0.82^{ns}
2013	Saskatoon	Desiccants	21	1.22^{ns}	0.78^{ns}	1.23^{ns}	1.09^{ns}	3.47**
	Scott	Desiccants	21	1.31^{ns}	1.10^{ns}	0.92^{ns}	1.45^{ns}	1.91*

*, **, and *** represent significant differences at *P<0.05, <0.01, and <0.001*, respectively. ^{ns} represents non-significant. df denotes degree of freedom

Furthermore, adding other contact herbicides to glyphosate significantly increased seed germination (10.9%) over glyphosate applied alone as did application of lower rates of contact herbicide alone. Similar results were observed at Scott in 2012, where seeds from plots sprayed with glyphosate alone and in combination with pyraflufen (10 or 20 g a.i. ha^{-1}), glufosinate (300 g a.i. ha^{-1}), flumioxazin (105 or 210 g a.i. ha^{-1}), or saflufenacil (36 g a.i. ha^{-1}) had significantly reduced germination compared to the untreated control (Table 3.6). Adding glyphosate to contact herbicides as a tank mix significantly improved seed germination (9.9%) compared to glyphosate applied alone. In 2013, no adverse effect on seed germination was attributable to glyphosate treatment at either site (Table 3.6).

Table 3.6. Mean comparison of seed germination (%) of lentil influenced by desiccants at Saskatoon and Scott, SK in 2012 and 2013. Contrast statements indicate differences in mean between desiccant treatments in seed germination.

Treatment	Rate (g a.i /a.e ha^{-1})	Seed germination (%)							
		Saskatoon				Scott			
		2012		2013		2012		2013	
		Mean	SD	Mea	SD	Mean	SD	Mean	SD
Untreated control	-	90.0^{cd}	0.8	95.3	2.3	89.5^{a-d}	1.9	90.7	3.0
Glyphosate	900	66.3^{hi}	5.7	87.5	3.7	69.3^{l}	4.3	91.0	2.2
Pyraflufen-ethyl	10	92.3^{a-c}	1.7	94.8	1.3	91.3^{a-c}	3.8	89.5	4.4
Pyraflufen-ethyl	20	92.0^{bc}	1.8	94.3	1.5	90.3^{a-c}	2.1	92.2	3.4
Glufosinate	300	86.0^{a-f}	5.7	92.0	2.6	79.3^{g-j}	7.1	88.8	3.9
Glufosinate	600	75.8^{g}	2.5	90.8	2.6	78.5^{h-j}	1.9	89.7	3.3
Flumioxazin	105	95.3^{a}	1.0	96.8	1.2	91.3^{a-c}	1.3	92.5	2.4
Flumioxazin	210	92.8^{a-c}	1.5	94.5	2.9	93.5^{a}	2.4	87.5	6.0
Saflufenacil	36	87.0^{de}	3.2	95.5	1.7	88.0^{a-f}	3.6	91.7	2.5
Saflufenacil	50	87.5^{de}	2.6	95.3	2.6	88.8^{a-e}	3.9	87.2	2.6
Diquat	208	91.5^{a-e}	3.7	95.5	1.7	94.0^{a}	1.4	90.0	2.2
Diquat	415	92.5^{b}	1.3	94.0	2.9	93.0^{a-d}	2.4	90.5	2.5
Pyraflufen-ethyl +glyphosate	10+900	65.3^{hi}	5.9	93.0	3.1	71.0^{g-l}	8.2	90.5	3.1
Pyraflufen-ethyl +glyphosate	20+900	71.3^{g-i}	5.7	93.8	6.0	77.5^{f-i}	1.7	90.2	2.3
Glufosinate +glyphosate	300+900	73.5^{gh}	6.0	95.8	1.0	78.5^{e-j}	5.4	89.5	5.3
Glufosinate +glyphosate	600+900	73.8^{g-i}	6.1	94.5	1.7	80.0^{c-i}	7.2	88.5	3.4
Flumioxazin +glyphosate	105+900	64.0^{i}	3.0	91.3	7.8	69.3^{i}	6.9	90.7	2.5
Flumioxazin +glyphosate	210+900	66.8^{hi}	5.0	94.8	4.2	73.5^{g-i}	1.7	90.7	2.1
Saflufenacil +glyphosate	36+900	74.5^{f-i}	8.3	92.5	8.7	72.3^{hi}	8.5	91.5	4.6
Saflufenacil +glyphosate	50+900	78.8^{fg}	10.	92.8	3.0	76.3^{e-l}	9.7	89.2	2.4
Diquat +glyphosate	208+900	86.8^{d-f}	8.9	96.0	2.0	83.8^{a-g}	4.9	84.7	3.4
Diquat +glyphosate	415+900	86.5^{d-f}	1.3	95.8	3.5	82.5^{b-h}	2.4	89.5	3.8
LSD		6.70		ns		7.85		ns	
Contrasts									
Untreated control vs. TM$^{\overline{\tau}}$ and glyphosate		12.2***		2.1		10.9***		0.4	
Glyphosate vs. TM$^{\overline{\tau}}$ +glyphosate		-10.9***		1.9		-10.0***		0.50	
TM$^{\overline{\tau}}$ vs. TM$^{\overline{\tau}}$ +glyphosate		-2.9***		-1.3		-1.9		0.5	
TM$^{\overline{\tau}}$ (low rate) vs. TM$^{\overline{\tau}}$ (high rate)		5.1**		-0.4		4.0*		1.5	

Means followed by the same letter within a column are not significantly different *(P<0.05)*. TM$^{\overline{\tau}}$ represents herbicides used as tank-mix; ns denotes non-significant at P<0.05. *, **, and *** represent significant differences at P<*0.05, <0.01,* and *<0.001*, respectively, LSD denotes Fisher's least significant differences value at 0.05 level of probability.

Similar to seed germination results, glyphosate sprayed alone or tank mix with other contact herbicides, except pyraflufen ethyl (20 g air. ha^{-1}) and diquat (208 or 415 g air. ha^{-1}) plus glyphosate significantly reduced seedling vigor compared to the untreated control at Saskatoon in 2012 (Table 3.7). On average, the addition of glyphosate to other desiccants as a tank mixture partner significantly reduced seedling vigor compared to their sole application (Table 3.7). Likewise, the glyphosate, glufosinate (600 g a.i. ha^{-1}), saflufenacil (36 or 50 g a.i. ha^{-1}), pyraflufen (100 g a.i. ha^{-1}or20 g a.i. ha^{-1}) + glyphosate, glufosinate (300 g a.i. ha^{-1}) + glyphosate (900 g a.e. ha^{-1}), flumioxazin (105 or 210 g a.i. ha^{-1}) + glyphosate, and saflufenacil (36 or 50 g a.i. ha^{-1}) + glyphosate treatments resulted in a significant reduction of seedling vigor compared to the control at Scott in 2012. Overall, the addition of glyphosate to other desiccants as a tank mix significantly reduced seed vigor compared to desiccants applied alone. The high rates of sole application of tank mix herbicides also significantly reduced seedling vigor compared to the lower rate of these herbicides applied alone (Table 3.7).

Conversely, none of the desiccant treatments had a significant effect on seedling vigor in 2013 (Table 3.7). The lack of adverse effects and differences of treatments may have resulted from reduction of glyphosate translocation to lentil seed during desiccation and lower seed moisture content at the time of treatment application. Seed moisture at the time of application was 32 and 35% at Saskatoon and Scott in 2013, respectively, compared to 35 and 40% in 2012.

Table 3.7. Mean comparison of seed vigor (%) of lentil influenced by desiccants at Saskatoon and Scott, SK in 2012 and 2013. Contrast statements indicate differences in mean between lentil desiccant treatments in seed vigor.

Treatment	Rate (g ai /ae ha^{-1})	Seed vigor (%)							
		Saskatoon				Scott			
		2012		2013		2012		2013	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated control	-	78.0^{a-c}	4.1	89.5	2.9	87.7^{a-c}	2.5	83.0	2.5
Glyphosate	900	65.0^{d-h}	4.1	87.5	7.2	68.7^{hi}	2.3	82.2	5.6
Pyraflufen-ethyl	10	75.3^{a-d}	5.9	86.7	2.6	82.2^{b-e}	2.5	85.0	2.6
Pyraflufen-ethyl	20	75.3^{a-d}	2.2	89.5	2.1	86.2^{a-d}	2.9	82.0	2.6
Glufosinate	300	78.5^{ab}	5.5	80.5	2.1	80^{c-f}	1.8	82.0	5.7
Glufosinate	600	70.0^{b-f}	4.6	84.2	4.9	72.7^{f-i}	1.8	81.8	5.3
Flumioxazin	105	86.3^{a}	1.3	90.7	5.9	87.0^{a-c}	2.7	82.7	5.2
Flumioxazin	210	76.0^{a-d}	8.5	91.5	1.3	89.5^{ab}	2.2	80.7	3.4
Saflufenacil	36	70.3^{b-e}	2.1	90.0	5.0	73.0^{f-i}	2.0	78.0	3.7
Saflufenacil	50	61.8^{e-h}	3.6	87.2	2.8	76.5^{e-h}	2.8	84.7	0.5
Diquat	208	77.8^{a-c}	3.4	89.6	3.2	91.0^{a}	2.1	84.7	4.6
Diquat	415	78.3^{a-c}	1.7	88.0	6.6	89.0^{ab}	2.1	85.0	2.9
Pyraflufen-ethyl +glyphosate	10+900	61.5^{e-h}	2.1	86.8	3.3	74.7^{e-i}	4.3	85.3	4.2
Pyraflufen-ethyl +glyphosate	20+900	67.0^{c-g}	2.5	86.5	6.5	69.0^{hi}	3.5	78.7	9.8
Glufosinate +glyphosate	300+900	66.2^{a-d}	10	88.5	3.2	71.7^{g-i}	3.7	81.3	1.5
Glufosinate +glyphosate	600+900	66.3^{d-h}	5.9	88.7	6.1	75.2^{e-i}	2.7	82.5	4.0
Flumioxazin +glyphosate	105+900	55.5^{h}	1.0	86.5	7.9	69.5^{hi}	3.4	84.5	2.5
Flumioxazin +glyphosate	210+900	57.3^{gh}	2.6	83.5	0.9	68.5^{i}	1.8	80.5	6.0
Saflufenacil +glyphosate	36+900	65.0^{d-h}	4.9	86.5	5.6	70.5^{hi}	3.4	76.5	8.2
Saflufenacil +glyphosate	50+900	58.8^{f-h}	3.4	86.0	3.3	72.7^{f-i}	5.1	82.0	3.2
Diquat +glyphosate	208+900	72.5^{b-e}	4.3	83.5	6.6	82.5^{b-e}	1.5	82.5	6.1
Diquat +glyphosate	415+900	71.8^{b-e}	5.4	87.5	2.8	78.7^{d-g}	2.9	81.7	4.0
LSD		11.4		ns		7.8		ns	
Contrasts									
Untreated control vs. TM$^{\overline{\tau}}$ and glyphosate		8.3**		1.9		11.6***		0.5	
Glyphosate vs. TM$^{\overline{\tau}}$ +glyphosate		-6.9*		1.9		-10.9***		0.7	
TM$^{\overline{\tau}}$ vs.TM$^{\overline{\tau}}$ +glyphosate		2.2		0.1		-1.2		0.7	
TM$^{\overline{\tau}}$ (low rate) vs. TM$^{\overline{\tau}}$ (high rate)		1.4		0.9		6.2***		2.3	

Means followed by the same letter within a column are not significantly different (P<0.05). TM$^{\overline{\tau}}$ represents herbicides used as tank-mix; ns denotes non-significant at P<0.05. *, **, and *** represent significant differences at *P<0.05, <0.01,* and *<0.001,* respectively. LSD denotes Fisher's least significant differences value at 0.05 level of probability.

3.4.4 Milling quality characteristics

3.4.4.1 Dehulling efficiency (%)

The desiccant by site-year interaction was significant for dehulling efficiency, milling recovery, and football recovery % (Table 3.3), and thus these data were analyzed within site-years (Table 3.5). At Saskatoon in 2012, only application of pyraflufen (20 g a.i. ha^{-1}) with glyphosate (900 g a.e. ha^{-1}) significantly reduced dehulling efficiency compared to the control. Application of diquat (415 g a.i. ha^{-1}) increased dehulling efficiency by 5.6% over pyraflufen (20 g a.i. ha^{-1}) with glyphosate (Table 3.8). The contrast results show application of glyphosate in tank mixtures significantly lowered dehulling efficiency compared to sole application of all contact herbicides.

Table 3.8. Mean comparison of dehulling efficiency (%) of lentil treated with desiccants at Saskatoon, and Scott, SK in 2012 and 2013. Contrast statements indicate differences in mean between desiccant treatments in lentil in dehulling efficiency.

Treatment	Rate (g ai /ae ha^{-1})	Dehulling efficiency (%)							
		Saskatoon				Scott			
		2012		2013		2012		2013	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated control	-	92.9^{a-d}	1.0	97.5	0.8	94.1^{a-c}	0.6	96.7	0.8
Glyphosate	900	92.3^{a-e}	1.4	96.3	2.0	94.2^{a-c}	0.6	96.5	1.5
Pyraflufen-ethyl	10	93.0^{a-d}	1.0	97.7	0.6	93.7^{bc}	0.8	95.1	1.6
Pyraflufen-ethyl	20	91.8^{a-e}	1.0	98.2	0.5	93.6^{bc}	0.5	95.3	1.8
Glufosinate	300	92.9^{a-e}	1.7	97.6	0.3	93.7^{bc}	1.0	95.1	1.7
Glufosinate	600	92.2^{a-e}	0.8	97.6	1.1	94.8^{ab}	1.5	96.4	0.9
Flumioxazin	105	93.3^{a-c}	0.7	97.9	0.3	93.8^{a-c}	0.7	96.2	2.0
Flumioxazin	210	92.2^{a-e}	0.8	97.7	0.3	94.1^{a-c}	1.3	96.4	0.7
Saflufenacil	36	92.1^{a-e}	1.8	97.6	0.3	91.1^{d}	1.0	94.7	2.0
Saflufenacil	50	90.4^{d-f}	1.3	98.1	0.5	92.6^{cd}	2.0	96.5	1.1
Diquat	208	93.0^{a-c}	0.6	97.9	1.2	95.0^{ab}	0.6	95.6	1.3
Diquat	415	94.1^{a}	1.0	97.8	0.6	95.4^{a}	1.3	95.6	1.2
Pyraflufen-ethyl +glyphosate	10+900	90.7^{c-f}	1.4	97.1	0.3	94.2^{a-c}	1.0	95.6	0.9
Pyraflufen-ethyl +glyphosate	20+900	88.5^{f}	1.3	97.1	0.7	94.4^{ab}	1.9	96.6	0.9
Glufosinate +glyphosate	300+900	90.3^{ef}	1.2	97.5	0.4	94.5^{ab}	1.9	96.4	1.3
Glufosinate +glyphosate	600+900	91.2^{b-e}	1.3	98.0	0.7	94.4^{ab}	2.5	96.1	0.5
Flumioxazin +glyphosate	105+900	92.8^{a-e}	0.5	97.9	0.6	93.4^{bc}	1.0	96.6	0.6
Flumioxazin +glyphosate	210+900	92.9^{a-d}	0.5	97.9	0.5	93.9^{a-c}	0.6	96.1	1.0
Saflufenacil +glyphosate	36+900	91.6^{a-e}	1.0	97.5	0.9	93.7^{a-c}	0.6	95.2	1.0
Saflufenacil +glyphosate	50+900	92.8^{a-e}	0.7	98.1	0.8	94.1^{a-c}	1.0	96.2	0.7
Diquat +glyphosate	208+900	93.7^{ab}	0.3	97.5	0.9	95.0^{ab}	0.4	96.2	0.7
Diquat +glyphosate	415+900	92.4^{a-e}	0.7	98.2	0.7	94.5^{ab}	0.9	96.0	0.9
LSD		2.6		ns		1.7		ns	
Contrasts									
Untreated control vs. TM$^{\overline{\tau}}$ and glyphosate		1.4		-0.1		0.1		0.7	
Glyphosate vs. TM$^{\overline{\tau}}$ +glyphosate		1.5		-0.0		-0.0		0.8	
TM$^{\overline{\tau}}$ vs. TM$^{\overline{\tau}}$ +glyphosate		-1.1*		-0.4		0.5		0.0	
TM$^{\overline{\tau}}$ (low rate) vs. TM$^{\overline{\tau}}$ (high rate)		1.5		-0.2		-0.4		-0.3	

Means followed by the same letter within a column are not significantly different (*P<0.05*). TM$^{\overline{\tau}}$ represents herbicides used as tank-mix; ns denotes non-significant at *P<0.05*. *, **, and *** represent significant differences at *P<0.05, <0.01,* and *<0.001*, respectively, LSD denotes Fisher's least significant differences value at 0.05 level of probability.

At Scott in 2012, most desiccant treatments exhibited better or comparable dehulling efficiencies (%) compared to the untreated control; the only exception was treatment with saflufenacil (36 g a.i. ha^{-1}), which led to significantly reduced dehulling efficiency. Application of the high rate of diquat (415 g a.i. ha^{-1}) increased dehulling efficiency by 4.3% over application of saflufenacil (36 g a.i. ha^{-1}) alone. None of treatments applied at either rate had a significant impact on dehulling efficiency percentages at either Saskatoon or Scott in 2013 (Table 3.8).

3.4.4.2 Milling recovery (%)

At Saskatoon in 2012, most desiccant treatments had no significant effect on milling recovery. The glufosinate (300 g a.i. ha^{-1}) and pyraflufen (20 g a.i. ha^{-1}) + glyphosate (900 g a.e. ha^{-1}) treatment significantly reduced milling recovery compared to control. While diquat (415 g a.i. ha^{-1}) increased milling recovery by 5.0% compared to the pyraflufen (20 g a.i. ha^{-1}) with glyphosate treatment (Table 3.9). On average, adding glyphosate to desiccants reduced milling recovery by 1.2% compared to sole application of glyphosate. The low application rate of other contact herbicides increased milling recovery (1.2%) compared to the corresponding high rates. At Scott in 2012, only plots treated with saflufenacil (36 g a.i. ha^{-1}) had significantly lower milling recovery yield compared to the diquat treated plots. Application of the high rate of diquat (415 g a.i. ha^{-1}) increased milling recovery by 4.6% over the low rate of saflufenacil application. Contrast comparisons showed that neither sole application nor herbicide mixes with glyphosate significantly affected milling recovery. No differences in milling recovery were observed between high and low rates of herbicide application treatments (Table 3.9).

No desiccant treatments affected milling recovery (%) of lentil at Saskatoon or Scott in 2013 (Table 3.9). Milling recovery was also unaffected by either the addition of contact herbicides to glyphosate or application rate of these herbicides.

Table 3.9. Means comparison of milling recovery (%) of lentil treated with desiccants at Saskatoon and Scott, SK in 2012 and 2013. Contrast statements indicate differences in mean between desiccant treatments in milling recovery.

Treatment	Rate	Milling Recovery (%)							
		Saskatoon				Scott			
	(g ai /ae ha^{-1})	2012		2013		2012		2013	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated control	-	81.0^{a-d}	1.2	83.6	0.3	79.3^{ab}		84.9	1.3
Glyphosate	900	80.2^{a-e}	0.6	82.9	2.7	79.4^{ab}	1.2	84.3	1.4
Pyraflufen-ethyl	10	80.7^{a-e}	0.8	83.8	0.2	78.9^{ab}	0.5	83.9	0.5
Pyraflufen-ethyl	20	79.7^{a-f}	1.6	84.2	0.7	79.1^{ab}	1.2	83.2	1.0
Glufosinate	300	78.2^{ef}	0.6	83.3	0.9	79.2^{ab}	0.40	83.6	0.8
Glufosinate	600	80.6^{a-e}	0.6	83.7	0.7	79.6^{a}	1.36	84.5	0.6
Flumioxazin	105	81.3^{a-c}	1.2	84.0	2.9	79.5^{ab}	1.27	84.0	2.0
Flumioxazin	210	80.5^{a-e}	1.6	83.7	4.2	79.2^{ab}	0.77	82.3	3.8
Saflufenacil	36	79.7^{a-e}	0.8	83.2	0.7	76.2^{b}	0.55	82.8	1.5
Saflufenacil	50	79.5^{b-f}	0.4	83.6	1.2	78.4^{ab}	0.86	84.3	1.3
Diquat	208	80.9^{a-d}	1.0	84.5	1.2	80.1^{a}	2.4	83.5	1.7
Diquat	415	82.2^{a}	1.1	83.6	1.34	80.8^{a}	0.8	83.2	1.6
Pyraflufen-ethyl +glyphosate	10+900	78.8^{c-f}	1.1	82.9	0.8	78.7^{ab}	2.0	83.8	0.5
Pyraflufen-ethyl +glyphosate	20+900	77.2^{f}	1.1	83.4	1.6	79.1^{ab}	1.4	85.3	1.9
Glufosinate +glyphosate	300+900	78.5^{d-f}	1.2	82.5	1.2	79.5^{ab}	1.2	84.8	0.7
Glufosinate +glyphosate	600+900	79.3^{b-f}	0.4	83.5	0.5	79.4^{ab}	1.7	84.2	0.5
Flumioxazin +glyphosate	105+900	80.8^{a-e}	0.5	83.9	0.7	78.4^{ab}	1.9	86.0	3.0
Flumioxazin +glyphosate	210+900	81.0^{a-d}	0.8	83.4	0.4	79.4^{ab}	1.6	84.0	0.7
Saflufenacil +glyphosate	36+900	79.6^{b-f}	0.6	83.7	0.9	78.7^{ab}	1.2	82.9	1.4
Saflufenacil +glyphosate	50+900	80.4^{a-e}	0.4	83.3	0.3	79.2^{ab}	1.0	83.8	1.2
Diquat +glyphosate	208+900	81.8^{a}	1.3	83.2	0.9	79.8^{a}	0.8	83.8	1.2
Diquat +glyphosate	415+900	78.9^{c-f}	0.7	83.7	0.7	79.8^{a}	0.3	83.7	0.4
LSD		2.6		ns		1.8		ns	
Contrasts									
Untreated control vs. TM$^{\overline{\tau}}$ and glyphosate		1.4		0.4		0.1		0.6	
Glyphosate vs. TM$^{\overline{\tau}}$ +glyphosate		1.6		0.5		0.0		0.7	
TM$^{\overline{\tau}}$ vs. TM$^{\overline{\tau}}$ +glyphosate		1.6		-0.4		0.3		0.5	
TM$^{\overline{\tau}}$ (low rate) vs. TM$^{\overline{\tau}}$ (high rate)		-1.2**		0.2		-0.3		-0.4	

Means followed by the same letter within a column are not significantly different ($P<0.05$). TM$^{\overline{\tau}}$ represents herbicides used as tank-mix; ns denotes non-significant at $P<0.05$. *, **, and *** represent significant differences at $P<0.05$, <0.01, and <0.001, respectively, LSD denotes Fisher's least significant differences value at 0.05 level of probability.

3.4.4.3 Football recovery (%)

Football recovery yield was influenced by growing environment. At Saskatoon in 2012, most desiccant treatments had a marginal effect on football recovery (Table 3.10); the exception was significantly reduced recovery for the saflufenacil (50 g a.i. ha^{-1}) + glyphosate (900 g a.e. ha^{-1}) treatment compared to the control. Application of diquat (207 g a.i. ha^{-1}) increased football recovery by 9.5% compared to saflufenacil (50 g a.i. ha^{-1}) + glyphosate (900 g a.e. ha^{-1}). On average, adding glyphosate to other desiccants did not reduce football recovery compared to their sole application at either application rate. At Saskatoon in 2013, application of flumioxazin (210 g a.i. ha^{-1}), diquat (207 g a.i. ha^{-1}), and saflufenacil (36 g a.i. ha^{-1}) with glyphosate significantly improved football recovery compared to the untreated control. At Scott in 2012, none of desiccants applied alone or as a tank mixture with glyphosate significantly reduced football recovery compared to the control. At Scott in 2013, only application of glufosinate (300 g a.i. ha^{-1}) with glyphosate and saflufenacil (50 g a.i. ha^{-1}) with glyphosate significantly decreased the football recovery compared to the untreated control. Overall, glyphosate tank mixes with other contact herbicides or these herbicides applied in higher doses had no significant impact on football recovery in any site year (Table 3.10).

Table 3.10. Means comparison of football recovery (%) of lentil influenced by application of desiccants at Saskatoon and Scott, SK in 2012 and 2013. Contrast statements indicate mean differences between desiccant treatments in football recovery.

Treatment	Rate (g ai /ae ha^{-1})	Football recovery (%)							
		Saskatoon				Scott			
		2012		2013		2012		2013	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Untreated control	-	48.1^{a-d}	1.2	10.1^{c-e}	2.2	13.7	2.0	20.1^{a-c}	2.0
Glyphosate	900	46.5^{b-e}	1.9	9.7^{c-e}	2.3	12.2	3.9	17.9^{a-e}	4.5
Pyraflufen-ethyl	10	49.9^{ab}	1.8	10.9^{c-e}	2.0	12.4	4.4	20.6^{ab}	4.3
Pyraflufen-ethyl	20	47.2^{b-e}	0.5	11.5^{b-d}	3.0	14.6	2.6	16.9^{b-f}	4.2
Glufosinate	300	49.9^{ab}	2.2	10.9^{c-e}	0.3	12.7	2.3	21.4^{a}	2.3
Glufosinate	600	45.5^{b-e}	1.2	10.3^{c-e}	2.5	14.0	2.3	16.4^{c-f}	4.5
Flumioxazin	105	47.8^{a-d}	1.4	10.4^{c-e}	1.2	12.2	2.8	18.4^{a-d}	1.8
Flumioxazin	210	46.9^{b-e}	1.4	15.1^{a}	2.8	12.8	3.8	18.0^{a-e}	1.0
Saflufenacil	36	43.9^{de}	2.2	9.2^{de}	1.0	11.6	5.2	18.0^{a-e}	3.5
Saflufenacil	50	47.2^{b-e}	2.2	9.6^{c-e}	1.1	12.7	3.4	16.8^{b-f}	2.2
Diquat	208	52.2^{a}	0.9	13.4^{ab}	2.0	12.3	2.0	16.6^{c-f}	3.0
Diquat	415	50.1^{ab}	2.5	11.6^{bc}	1.8	15.0	2.5	16.2^{d-f}	3.7
Pyraflufen-ethyl +glyphosate	10+900	45.5^{b-e}	1.1	9.3^{c-e}	0.7	11.8	2.5	18.7^{ad}	3.0
Pyraflufen-ethyl +glyphosate	20+900	44.9^{c-e}	1.2	10.8^{c-e}	1.4	12.0	3.6	17.9^{a-e}	3.4
Glufosinate +glyphosate	300+90	49.6^{a-c}	0.9	9.0^{e}	1.0	12.4	3.2	13.4^{f}	2.6
Glufosinate +glyphosate	600+90	48.5^{a-d}	0.7	10.1^{c-e}	0.7	12.5	2.0	18.9^{a-d}	1.4
Flumioxazin	105+90	48.4^{a-d}	1.6	9.4^{c-e}	1.5	12.3	2.9	17.4^{b-e}	5.5
Flumioxazin	210+90	47.6^{a-d}	1.7	10.3^{c-e}	1.3	15.0	2.8	19.6^{a-d}	3.6
Saflufenacil	36+900	47.4^{b-e}	2.5	13.4^{ab}	3.0	13.6	3.4	19.1^{a-d}	4.2
Saflufenacil	50+900	42.7^{e}	2.4	9.7^{c-e}	1.5	11.2	2.1	14.5^{ef}	2.9
Diquat +glyphosate	208+90	48.6^{a-c}	1.0	9.5^{c-e}	0.6	12.0	2.0	16.6^{c-f}	2.2
Diquat +glyphosate	415+90	47.2^{b-e}	2.0	11.7^{bc}	1.3	14.4	5.1	17.5^{b-e}	2.9
LSD		4.7		2.4		ns		3.8	
Contrasts									
Untreated control vs. TM$^{\overline{\tau}}$ and glyphosate		0.5		-0.1		1.0		2.1	
Glyphosate vs. TM$^{\overline{\tau}}$ +glyphosate		0.6		0.2		1.0		2.1	
TM$^{\overline{\tau}}$ vs. TM$^{\overline{\tau}}$ +glyphosate		0.3		-0.7		0.1		0.7	
TM$^{\overline{\tau}}$ (low rate) vs. TM$^{\overline{\tau}}$ (high rate)		-0.2		0.1		0.3		1.2	

Means followed by the same letter within a column are not significantly different ($P<0.05$). TM$^{\overline{\tau}}$ represents herbicides used as tank-mix; ns denotes non-significant at $P<0.05$. *, **, and *** represent significant differences at $P<0.05$, <0.01, and <0.001, respectively LSD denotes Fisher's least significant differences value at 0.05 level of probability.

3.4.5 Correlation among lentil seed morphology traits, glyphosate residue, and milling characteristics

Pearson correlation coefficients (r) were calculated for glyphosate residue content in seeds with other parameters measured in this study. Data were averaged for three replications and combined over both site-years (Saskatoon and Scott) in 2012 and 2013 for correlation analysis. Positive and high correlation was observed between seed thickness and seed plumpness *(r=0.97, p<0.001*), seed diameter *(r=0.62, p<0.001),* and glyphosate residue content *(r=0.51, p<0.001).* Seed thickness was negatively correlated with seed germination, *(r=-0.46, p<0.001),* seedling vigor *(r=-0.26, p<0.01),* dehulling efficiency *(r=-0.33, p<0.01),* and milling recovery *(r=-0.63, p<0.001)* (Table 3.11). Seed diameter was positively and significantly correlated with seed plumpness *(r=0.51, p<0.001)* and glyphosate residue content *(r=0.53, p<0.001)* and negatively correlated with other biological traits (Table 3.11). Likewise, seed plumpness was only positively correlated with glyphosate residue content *(r =0.48, p<0.001).* Seed germination *(r=-0.84, p<0.001)* and seedling vigor *(r=-0.62, p<0.001)* were negatively and significantly correlated with glyphosate residues. Percent seed germination was positively correlated with seed vigor *(r=0.75, p<0.001),* dehulling efficiency *(r= 0.68, p<0.001),* and milling recovery *(r=-0.62, p<0.001).*

For milling characteristics, dehulling efficiency was significantly but negatively correlated with glyphosate residue *(r=-0.55, p<0.001)* and football recovery *(r=-0.64, p<0.001).* Milling recovery strongly but negatively correlated with glyphosate residues *(r=-0.62, p<0.001).* In contrast, football recovery was positively correlated with glyphosate resides *(r=0.30, p<0.01)* (Table 3.11).

Table 3.11. Correlation coefficients among lentil seed morphology traits, glyphosate residue, and milling characteristics of lentil.

Traits	STH	SD	SP	SG	SV	DE	FR	MR	GR
STH	-								
SD	0.62***	-							
SP	0.97***	0.51***	-						
SG	-0.46***	-0.46***	-0.42***	-					
SV	-0.26**	-0.39***	-0.23*	0.75***	-				
DE	-0.33**	-0.45***	-0.29***	0.68***	0.75***	-			
FR	-0.16ns	0.31***	-0.21*	-0.49***	0.68***	-0.64***	-		
MR	-0.63***	-0.68***	-0.57***	0.62***	0.49***	0.76***	-0.23*	-	
GR	0.51***	0.53***	0.48***	-0.84***	-0.62***	-0.55***	0.30**	-0.62***	-

STH=Seed thickness, SD=Seed diameter, SP=Seed plumpness, SG=Seed germination, SV=Seed vigor, DE=Dehulling efficiency, FR= Football recovery, MR=Milling recovery, GR=glyphosate residue. *, **, and *** indicate significance at *P<0.05*, *<0.01*, and *<0.001*, respectively; ns=non-significant.

3.5 Discussion

Uniform and early seed maturity is critical to produce high quality lentil on the Canadian Prairies. Extreme growing conditions combined with the heterogeneity of soil, precipitation patterns, and the indeterminate growth habit of lentil plants often result in uneven maturation of the crop. Crop desiccants are used as pre-harvest aids to rapidly dry vegetative and reproductive plant tissues, including seeds, without affecting seed yield and quality (Ratnayake and Shaw, 1992; Soltani et al., 2013). Most lentil growers in Western Canada use herbicidal desiccants to overcome challenges of heterogeneous maturity of the crop. However, some desiccants directly impact physiological aspects of different crop species, such as mean seed weight, seed germination, and dehulling efficiency (Darwent et al., 2000; Bruce, 2008).

The current study determined the impact of the use of contact herbicides as harvest aids as applied alone or in combination with glyphosate on selected physical, physiological, and processing characteristics of lentils. None of the desiccants applied alone or in tank mixes with glyphosate adversely affected seed physical qualities, including seed diameter, thickness, and plumpness. These results are similar to Ratnayake and Shaw (1992) who reported that pre-harvest use of glufosinate, glyphosate, or paraquat had no significant adverse effect on seed yield or quality in soybean when applied at the full maturity stage. Similarly, Zhang et al. (2016) and McNaughton et al. (2015) observed no reduction in yield or 1000-seed weight when desiccants were applied to lentil and dry bean, respectively. Wilson and Smith (2002) report that

glufosinate, paraquat, and diquat applied as desiccants to common bean accelerated seed maturity and desiccation. Glyphosate applied as a desiccant reduced pod length and seed weight when used as a harvest aid in cowpea (*Vigna unguiculata* L.) (Cedeira et al., 1985). Many studies reported reduced soybean yield and seed quality because of the application of desiccant prior to crop maturity (Azlin and McWhorter, 1981; Cerkauskas et al., 1982; Boudreaux and Griffin, 2011). On the other hand, Soltani et al. (2013) observed that the addition of diquat, glufosinate, carfentrazone, flumioxazin, or saflufenacil to glyphosate improved the drying of dry bean foliage and yield. The variability among results may be related to the timing of desiccant application. The application of desiccants before physiological maturity can inhibit photosynthesis or, because lentil is an indeterminate crop where bottom pods matured before upper canopy pods, may damage immature seeds.

In 2012, the application of glyphosate alone or as a tank mix with other herbicides (except diquat+glyphosate) significantly reduced germination percentage in lentil seeds compared to the untreated control. These results are similar to those reported by Yenish and Young (2000), who found that pre-harvest glyphosate-treated wheat had a 2-46 % lower seed germination than the control. Similarly, Hampton and Hebblethwaite (1982) found that pre-harvest application of glyphosate significantly lowered ryegrass (*Lolium perenne* L.) seed germination due to production of abnormal seedlings. They also reported that germination percentage of the glyphosate-treated seeds decreased with storage. Bennett and Shaw (2000a) report that sodium chlorate plus glyphosate or paraquat applied as a pre-harvest aid to sicklepod (*Senna obtusifolia* L) 14 days before harvest significantly reduced shoot growth and seed germination.

The reduced seed germination of glyphosate-treated plants may be caused by translocation of glyphosate to the maturing seeds and embryo, the major sink during the maturation process. Glyphosate inhibits the shikimate acid pathway for synthesis of branched aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Vivancos et al., 2011). Tryptophan is a direct precursor of indole-3-acetic acid (IAA), which affects coleoptile elongation and shoot and root initiation (Taiz and Zeiger, 1998). Clay and Griffin (2000) suggest that use of glyphosate as a desiccant during the plant maturation phase may affect the level of IAA, the main endogenous auxin, thereby causing inhibition of germination and growth. Unlike glyphosate, diquat applied alone or with glyphosate, pyraflufen, glufosinate (300 g a.i. ha^{-1}),

flumioxazin, and saflufenacil applied alone did not affect seed germination in the present study. Ratnayake and Shaw (1992) report similar results. Whigham and Stoller (1979) also observed paraquat applied as a harvest aid had no effect on germination percentage of soybean. These are contact herbicides with limited phloem mobility, and therefore low levels of these compounds would reach the seed.

Our study showed no adverse effect of glyphosate or any contact herbicides on seed germination of lentil at either site in 2013. This might be due to lower moisture content in seeds due to reduced rainfall at the time of application (Table 3.2). Low rainfall hastened the dry down process of lentil crops in 2013. Zhang et al. (2016) reported glyphosate residue in lentil seeds content < 2 ppm from Saskatoon and Scott when they had 32 and 35% moisture content in 2013 compared with 35 and 40% in 2012, respectively. Lower moisture content in seed harvested in 2013 might have reduced translocation of glyphosate in seeds. The application of glyphosate as a desiccant in lentil (Zhang et al., 2017) and common bean (McNaughton et al., 2015) prior to 30 % seed moisture content, increases its residue to an unacceptable level (> 2 ppm) causing reduced seed yield and mean seed weight. Higher translocation of glyphosate into seeds may also reduce seed germination and vigor as developing seeds are major photosynthesis sinks (Zhang et al., 2016). The significant and negative correlation between glyphosate residue and seed germination results support this explanation. Different countries do have different import policies in terms of the amount of glyphosate residue they will accept for import of lentil (Pratt, 2011). The current MRLs for glyphosate in lentil are 2 ppm, 4 ppm and 10 ppm for Canada, Japan, and the European Union (EU), respectively (Zhang et al., 2017).

A significant reduction in lentil seedling vigor was observed at Saskatoon and Scott in 2012 when glyphosate was sprayed alone or as a tank mix with other herbicides, but no such differences were observed in 2013. The 2012 results are comparable to those of Hampton and Hebblethwaite (1982), who show that seedling vigor of perennial rye grass (*Lolium perenne* L.) is reduced when glyphosate is applied as a pre-harvest aid. The pre-harvest application of glyphosate is also reported to cause poor seed germination and reduced seedling vigor when applied at >40% seed moisture content in field pea (Baig et al., 2003). Our study showed seedling vigor was highly negatively correlated with glyphosate residue in lentil seeds. Adverse effects of pre-harvest glyphosate application on seedling growth and vigor might be related to the reduction and physiological inactivation of mineral nutrients, such as Ca and Mn, due to

glyphosate in the seeds (Cakmak et al., 2009). Mineral nutrients can play an important role in seed viability and seedling vigor and establishment, particularly under adverse soil conditions (Welch, 1999).

The growing environment can have a significant effect on dehulling efficiency of lentil (Bruce, 2008). Results from the present study show the effects of desiccants on dehulling efficiency, milling recovery, and football recovery of lentils depend to a certain extent on growing environment and moisture content in seeds at the time of application. We observed that pyraflufen (20 g a.i. ha^{-1}) with glyphosate (900 g a.e. ha^{-1}) and saflufenacil (36 g a.i. ha^{-1}) reduced dehulling efficiency at Saskatoon and Scott in 2012, respectively. These results were concurrent with the studies on the effect of desiccation with saflufenacil in lentil (Zhang et al., 2017) and common bean (McNaughton et al., 2015) who found that dramatically reduced mean seed weight and seed yield if application of saflufenacil was made prior to 30% seed moisture content.

The saflufenacil residue in the seed was reported as 0.03 mg kg^{-1}. Adding saflufenacil to glyphosate did not reduced glyphosate residue in lentil compared to glyphosate applied alone, yet they found that tank mixture significantly reduced seed residue content of saflufenacil and improved crop desiccation. Saflufenacil residues present in harvested lentils may be a concern for lentil growers when it is used as a harvest aid at pre-harvest stages of crop because major lentil importing countries have also set MRLs for saflufenacil (Bryant Christie Inc., 2015). Reduction of dehulling efficiency due to saflufenacil applied alone or as a tank mix with pyraflufen-ethyl might have occurred because these herbicides belong to the uracil and phenyl pyrazole classes, respectively, which are protoporphyrinogen oxidase (PPO) inhibitors (Grossman et al., 2010b). PPO inhibitors bind sites of Protogen IX in the chloroplast, which causes peroxidation of foliar cell membrane lipids and subsequent rapid loss of membrane integrity and necrosis (Duke et al., 1991; Grossman et al., 2010b). Both saflufenacil and pyraflufen have limited mobility in phloem (Liebl et al., 2008). These herbicides translocate mainly through the xylem, and their slow mobility may result in accumulation in seeds, thereby interfering with the normal chemical composition and alignment of the bonding layer between the lentil seed coat and the cotyledon. Pre-harvest application of the low rate of pyraflufen with glyphosate or sole application of saflufenacil (36 g a.i. ha^{-1}) resulted in reduced milling recovery at Saskatoon and Scott, respectively, in 2012. Application of diquat with or without glyphosate

seemed to improve milling recovery in 2012. In contrast, Bruce (2008) reports that, during dry harvest conditions, no significant differences in milling recovery were evident between swathing and desiccation pre-harvest treatments.

In our study, differential effects of the pre-harvest treatments on football recovery percentage were observed in both years but only in Saskatoon. Like the dehulling efficiency, saflufenacil (50 g a.i. ha^{-1}) combined with glyphosate resulted in the lowest football recovery at Saskatoon in 2012. Different responses of crop harvest aids among years (2012 vs. 2013) and sites might have been the result of wet field conditions during the harvesting period and differences in moisture content and glyphosate residue in seeds. Our study shows that milling parameters, particularly dehulling efficiency and milling recovery, are inversely related to glyphosate residue in seeds. Zhang et al. (2016) report that seed moisture content in seeds at the time of application can strongly impact glyphosate translocation to seeds. They note that, irrespective of desiccation treatments, high moisture content in seeds at the time of application results in accumulation of high (>2.0 ppm) glyphosate residues compared to lower moisture in seeds while they desiccate lentil crops. In most cases (two exceptions), however, desiccant treatments had no significant impact on football recovery at Scott in either year. These results are comparable with those of Bruce (2008), who report no differences in football recovery between swathing and desiccation with diquat when treatments were applied at a later stage of plant maturity; however, early application of diquat decreased football recovery. He suggests that desiccation by diquat caused lentil seeds to separate at the cotyledons more easily compared to swathing followed by natural drying. Swathing allows biological processes related to cotyledon binding to continue for a given period, whereas desiccation may cease the processes rapidly and therefore make the seeds more brittle.

The inconsistency of some of the results in our study in relation to seed biological qualities and milling recovery over environment indicate that further research over many environments may be required to determine the consistency of treatment effects and their economic impact. Moisture content of seeds is a key cause of translocation of glyphosate residue to seeds, which can result in adverse effects on both seed biological and milling qualities. Therefore, glyphosate is recommended for use as a desiccant in lentil once the seed moisture is 30 % or less (Saskatchewan Ministry of Agriculture, 2016). Future research could focus on the relationship between moisture content of seeds and post- harvest qualities of lentil.

Environmental variation and the nature of herbicide sensitivity of lentil crops may result in different impacts on seed quality; this has been demonstrated in differences of sensitivity among crops species to flumioxazin, saflufenacil, and pyraflufen (Ivany 2005; Soltani et al., 2010) in dry bean.

3.6. Conclusions

Use of glyphosate alone or in tank mix with other contact herbicides as pre-harvest aids in lentil production adversely affected seed germination and seedling vigor particularly if glyphosate is translocated to the seeds. Consistent improvements in milling recovery and dehulling efficiency of lentil were only observed when diquat was used alone or in tank mix with glyphosate, suggesting that lentil growers should consider these desiccant treatments to optimize dry-down of lentil without harming seed quality if they wish to gain premium prices for red lentil based on milling efficiency.

Prologue to Chapter 4

In this chapter of the thesis, the effects of fungicides, which are used to reduce foliar diseases in the canopy are investigated from the standpoint of the potential effects of this agronomic treatment on milling quality of lentils. This chapter has two sub-sections. The first examined the effects of three foliar fungicides on lentil seed yield and milling quality characteristics. A second small experiment was subsequently conducted to test the hypothesis that infection by stemphylium blight within the lentil canopy will decrease milling efficiency as determined by percent dehulling efficiency (DE), percent milling recovery (MR) and percent football recovery (FR). Results of the second experiment are presented in Appendix A2.

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CHAPTER 4. EFFECT OF FOLIAR FUNGICIDES ON SEED AND MILLING QUALITY CHARACTERISTICS OF RED LENTIL (*LENS CULINARIS* MEDIKUS)

Abstract

Fungicides are commonly used to manage foliar diseases, and to improve seed yield and quality of lentil. Field studies were conducted at two locations in 2013 and 2014 to determine the effect of foliar fungicides on seed yield, seed quality and milling qualities of lentil. Pyraclostrobin (250 g L^{-1}), azoxystrobin, (250 g L^{-1}) and chlorothalonil (500 g L^{-1}) were applied at the mid-flowering stage to lentil cultivars CDC Maxim, and CDC Dazil. Site year and site-year × cultivar interaction effects differed significantly for seed yield while only site-year significantly differed for disease severity of stemphylium blight (SB) caused by *Stemphylium botryosum*. Linear analysis showed that only the SB infected site (SPG 2014) had significantly higher SB disease infection (31% increase) and seed yield was reduced significantly (by -1443 kg ha^{-1}) compared to the other three site-years. Azoxystrobin significantly ($P<0.05$) reduced SB disease severity compared to the control at the SPG 2014 site but was not completely effective. Fungicide applications had no effect on days to maturity, seed weight or seed yield compared to the controls in any site-years. Pyraclostrobin treatment resulted in significantly reduced seed diameter and thickness without reducing seed yield or milling quality. Results showed that site-year, and site-year by fungicide or cultivar interaction had significant effects on percent dehulling efficiency (DE), milling recovery (MR) and football recovery (FR). At the SPG 2013 sites, plots treated with pyraclostrobin fungicide produced seeds with significantly higher DE (3.5%) compared to the control. None of the fungicides significantly improved MR nor FR percentages in any site-year. Linear contrast analysis showed that the SPG 2014 site which was infected severely by SB in the latter half of the season had significantly reduced DE (-29%,) MR (-28%) and FR (-16%) compared to the three sites that had no SB infection. A single application of strobilurin or chlorothalonil fungicide prior to mid-flowering may not be effective to control of polycyclic fungal diseases like *Stemphylium botryosum* that have the potential to greatly reduce DE, MR, and FR quality of lentil.

4.1 Introduction

Red lentil is consumed predominantly in the dehulled form. Adherence of the seed coat to cotyledons after dehulling is visually unacceptable to consumers and results in lower product quality and value. Achieving high seed yield with maximum milling efficiency are challenges for both lentil growers and processers. Yield and milling quality of lentil is primarily dependent on the production environment and genetics (Erskine et al., 1991a; Erskine et al., 1991b), but it also largely influenced by cultural practices of lentil production (Bruce, 2008; Subedi et al., 2017). The commercial foliar fungicides chlorothalonil, azoxystrobin and pyraclostrobin are often applied to lentil crops to control fungal diseases such as ascochyta blight caused by *Ascochyta lentis* and anthracnose caused by *Colletotrichum lentis* in Western Canada (Saskatchewan Ministry of Agriculture, 2016). Chlorothalonil is a broad spectrum protective fungicide with limited translocation in plants. Azoxystrobin and pyraclostrobin are more readily translocated within plants, resulting in protective and preventive effects (Mahoney et al., 2014; Butzen et al., 2005; Gillard et al., 2012b; Saskatchewan Ministry of Agriculture, 2016).

Fungicides also have a wide range of physiological effects (Petit et al., 2012; Dias, 2012). For example, strobilurin fungicides can elicit non-fungicidal plant health effects, the so-called 'greening effect' (Bartlett et al., 2002; Venancio et al., 2003; Fagan, et al., 2010).This is associated with the ability of strobilurins to maintain photosynthetically active green leaves for a longer period, thereby maximizing the grain/pod filling period (Morrison et al., 1999; Kumudini et al., 2001). Prolongation of the pod filling period resulting from a strobilurin application has been reported to increase both seed yield and seed quality in a number of cereal and grain legume species such as common bean (*Phaseolus vulgaris* L.*)* (Bradley and Sweets, 2008; Mahoney et al., 2014; Mahoney and Gillard, 2014) possibly due to production of antioxidant enzymes, hormonal balance, chlorophyll conservation (Wu and Tiedemann, 2002; Zhang et al., 2010; Grossmann and Retzlaff, 1997), and reduction of stomatal aperture to conserve water (Ko¨hle et al., 1997; Ko¨hle et al., 2002; Grossmann et al., 1999; Nason et al., 2007). Delayed senescence of older leaves following application of strobilurin under water scarcity may be economically detrimental in lentil if maturity delay prevents timely application of desiccants under terminal drought conditions in regions like the Canadian Prairies.

Azoxystrobin application at the booting stage of rice (*Oryza sativa* L.) reduced head blight diseases, and subsequently increased milling recovery yield (Groth, 2006). Disease control

in lentil by fungicide application may differ at the top and bottom of the plant canopy. The lentil crop has an indeterminate growth habit, consequently seeds can be matured at different periods on the plant, and therefore, fungicides may have differential effects on seed coat removal. We assume that fungal infection of the canopy may also influence seed development, including an effect on the post-harvest milling quality of lentil.

No published information is available regarding the effect of strobilurin fungicides on seed yield, post-harvest seed and milling quality in lentil. Research was required to determine the effectiveness of strobilurin fungicides for control of fungal infection, and, also to determine if these fungicides could improve both seed yield and post-harvest seed and milling quality. The objectives of this study were to determine the effect of fungicides on seed yield, thousand seed weight, seed dimension, and milling quality parameters in lentil as determined by dehulling efficiency, milling and football recovery percentages.

4.2 Hypothesis

The hypothesis of this study was that application of foliar fungicides and their subsequent influence on disease management during seed formation would influence the post-harvest seed and milling quality characteristics as measured by the percentages of dehulling efficiency, milling recovery and football recovery.

4.3 Materials and methods

4.3.1 Plant materials

Seed of commercial lentil cultivars CDC Maxim and CDC Dazil were obtained from the Crop Development Centre (CDC), University of Saskatchewan (U of S). CDC Maxim is a small-seeded (40 g/1000 seeds) red lentil cultivar. CDC Dazil is a red lentil cultivar with slightly smaller seeds (35 g/1000 seeds). Both cultivars are imidazolinone tolerant (Clearfield®), are resistant to ascochyta blight, have gray seed coats, and have early to medium maturity. They exhibit similar yield potential, and in recent years have been the most widely grown red lentil cultivars in North America.

4.3.2 Site description, experimental design, and environmental conditions

Field experiments were conducted at the U of S experimental site at Preston Avenue in Saskatoon (Preston - 52° 07' 35.5" N, 106° 37' 19.6" W) and at the Saskatchewan Pulse Growers farm (SPG - 52.05° N, 106.41° W) near Saskatoon, Saskatchewan in 2013 and 2014. Soil organic matter content was 3.7 and 4.0 %; soil pH was 7.4 and 7.6; soil texture was silt loam and loam, respectively. The study included two factors (fungicide and lentil cultivar) and treatments were arranged as factorial combinations in a randomized complete block design with four replicates at each site.

Three fungicide treatments consisting of two systemic strobilurins: pyraclostrobin 250 g L^{-1} (BASF Canada Inc.; and azoxystrobin 250 g L^{-1} (Syngenta Crop Protection Canada Inc.), both of which are systemic. The contact fungicide chlorothalonil- 500 g L^{-1} (Syngenta Crop Protection Canada Inc.) was the third fungicide treatment and water-sprayed plots served as controls. Total 2013 seasonal precipitation was lower than average at 210 mm and 211 mm at the Preston and SPG sites, respectively. In 2014, precipitation was higher than average at 290 mm and 301 mm, respectively, at the Preston and the SPG sites (Table 4.1). The higher rainfall in 2014 created canopy conditions that were conducive to stemphylium blight disease development.

Table 4.1. Mean monthly temperature (°C) and total monthly precipitation (mm) during the growing season at SPG and Preston experimental plots, SK in 2013 and 2014.

		Temperature (°C)					
Location	Year	May	June	July	August	September	Mean
Preston Ave.	2013	13.0	15.5	17.4	18.9	15.2	16.0
	2014	11.0	14.2	20.3	17.9	13.5	17.2
SPG	2013	13.3	15.6	17.7	18.6	15.3	16.9
	2014	11.1	14.1	18.3	17.9	12.4	14.8
Normal¥		11.2	15.8	18.5	17.6	11.4	14.9
		Precipitation (mm)					Total
Preston Ave.	2013	13.5	125.0	41.5	13.8	15.9	209.7
	2014	60.0	119.0	57.5	33.5	17.0	298.0
SPG	2013	12.5	127.5	37.5	14.0	19.5	211.0
	2014	69.0	121.0	64.5	37.5	20.0	301.0
Normal¥		43.0	65.8	60.3	62.6	35.4	267.1

Source: Normal¥ 1981 – 2010 Canadian Climate normal obtained from Environment Canada (2016)

4.3.3 Experimental procedures

Plot size was approximately 2 m × 6 m for all experiments, with six rows per plot at 130 seeds m^{-2} using 30-cm row spacing. Plots were sown on May 28, 2014 and May 12, 2014 at Preston, and on May 29, 2013 and June 2, 2014 at SPG. Late sowing of lentil at SPG was due to wetter soil conditions prior to mid-May. Seed was placed at 2.5-cm depth using a small plot hoe drill. Prior to sowing, seeds were treated with Apron Maxx RTA (0.73% fludioxonil; 1.1% metalaxyl-M and S-isomer) at 325 mL per 100 kg of seed and inoculated with liquid Nodulator ® inoculant (*Rhizobium leguminosarum* biovar *viceae*) at a rate of 2.76 mL kg^{-1}. Edge® (ethalfuralin) herbicide was applied pre-seeding at the recommended rate (17.3 kg ha^{-1}), and the post emergent herbicides Axial® (pinoxaden 0.096 L ha^{-1} and Pursuit (imazethapyr 96 g ai ha^{-1})) were applied at SPG to control weeds. At the Preston experimental site, a pre-seeding application of Edge® at the recommended rate was applied and hand weeding was done as required. Fungicide treatments (pyraclostrobin 250 g L^{-1}, azoxystrobin 250 g L^{-1} and chlorothalonil- 500 g L^{-1}) were applied at the mid-bloom stage using an air pressurized tractor mounted sprayer equipped with shielding (110-015 AirMix nozzles, 275 kPa, 45 cm spacing). Sprayers were calibrated to deliver 100 L ha^{-1} of spray solution. Application timing, application dates and environmental conditions are presented in Table 4.2. The detail of agronomic management practices is presented in Appendix 4.1.

Table 4.2. Crop growth stage, date of application, temperature, relative humidity, and wind speed at the time of fungicides application at Preston and SPG sites, SK in 2013-2014.

Site	Year	Crop growth stage	Application date	Environmental condition		
				Temperature (°C)	Relative humidity (%)	Wind speed (km/hr)
Preston	2013	Mid-bloom	July 17	24.2	40	15
	2014	Mid-bloom	July 15	23.8	47	8
SPG	2013	Mid-bloom	July 17	25.1	42	12
	2014	Mid-bloom	July 27	20.0	60	11

4.3.4 Data collection

At all site years, no signs of ascochyta blight or anthracnose diseases were observed. However, lentil experimental plots at SPG 2014 were affected by stemphylium blight (SB) caused by *Stemphylium botryosum* prior to maturity. Final disease scores for SB were used to

analyze data in this study. Visual disease ratings for SB were collected based on the increment of disease lesions in plant tissue (Banniza et al., 2006) and recorded using a 1- 10 scale representing 10% increments of aggregate leaf area covered by disease lesions. Scores were transformed to percentage values using the class midpoints. Visual disease ratings were recorded from three randomly selected plants in each of the four inner rows of the plots. Days to maturity (DTM) was recorded when 90% of the lower pods of plants had started to turn yellow to brown in color (Saskatchewan Pulse Growers, 2015). The border rows of each plot were separated and trampled prior to harvest, and the four interior rows of each plot were harvested using a combine harvester. Seed samples from each plot were placed in a forced air drier at 39 to 42^{o}C for 4-5 days to reduce seed moisture to approximately 13%. Seeds were cleaned using an air blower to remove immature seeds and other residue in the harvest samples. The clean seeds were used to determine seed yield, seed weight and all post-harvest quality parameters. 1000-seed weight was estimated by weighing 250 randomly selected seeds and multiplied by 4. Seed dimensions (seed diameter, thickness, and plumpness) were determined by passing 100 g seed samples through round-hole and slotted-hole sieves. All milling quality characteristics (dehulling efficiency, milling and football recovery percentages) were determined using methods described in Chapter 3 (Subedi et al., 2017).

4.3.5 Data analyses

Statistical analyses were performed using SAS V9.4 (SAS Institute Inc., 2016). Prior to analysis, residuals were assessed for normality and homogeneity of variances with PROC UNIVARIATE and Levene's test, respectively. Heterogeneous variance structures were modeled using repeated/group statement with mixed models where residuals did not meet assumption of ANOVA. All data were analyzed using the MIXED procedure in SAS 9.4. For combined analysis, fungicides treatments and cultivars were treated as fixed effects. Growing environment (site-years) and blocks nested within site-year and the interaction between fixed and environmental effects were treated as random effects. The significance of random effects (site-years), their interactions with fixed effects, and scatterplots were used to determine whether data could be combined for analysis. Wherever data could not be combined, they were analyzed within site-years. For single environment analysis, the effects of fungicides, cultivars and interaction between fungicides and cultivars were considered fixed and replication was

considered as random. Least squared means were computed for fixed effects using the LSMEANS statement. Significance of variances were declared at 5%. Means were separated using Fisher's least significant difference (LSD). Additionally, all letter groupings for significance differences were established using PDMIX 800 in SAS (Saxton, 1998). Single degree of freedom contrasts was performed to make specific comparisons of interest. Graphs were plotted using SigmaPlot V2. (Sigma Plot, 2010).

4.4 Results

4.4.1 Disease severity

Analysis of variance indicated site-year was significant ($P<0.05$) for final SB disease severity (4.3). Therefore, data were analysed separately for each site-year (Table 4.4). Except for SPG in 2014, disease severity was minimal (2.4% - 13.5%) in the other three site-years where treatments were not significant (Table 4.4). At the SPG site in 2014, both cultivar and fungicide effects were significant ($P<0.05$) for disease severity (Table 4.3). Among fungicides, azoxystrobin significantly ($P<0.05$) reduced disease severity compared to the unsprayed control (Figure 4.1). However, these fungicides applied at mid-flowering did not control SB effectively. The results of linear contrast analysis showed that SPG 2014 plots had significantly higher disease level (31.5% more) compared to the other three other site-years ($P<0.001$). No differences in disease severity were observed between strobilurin and non-strobilurin fungicides (Table 4.5).

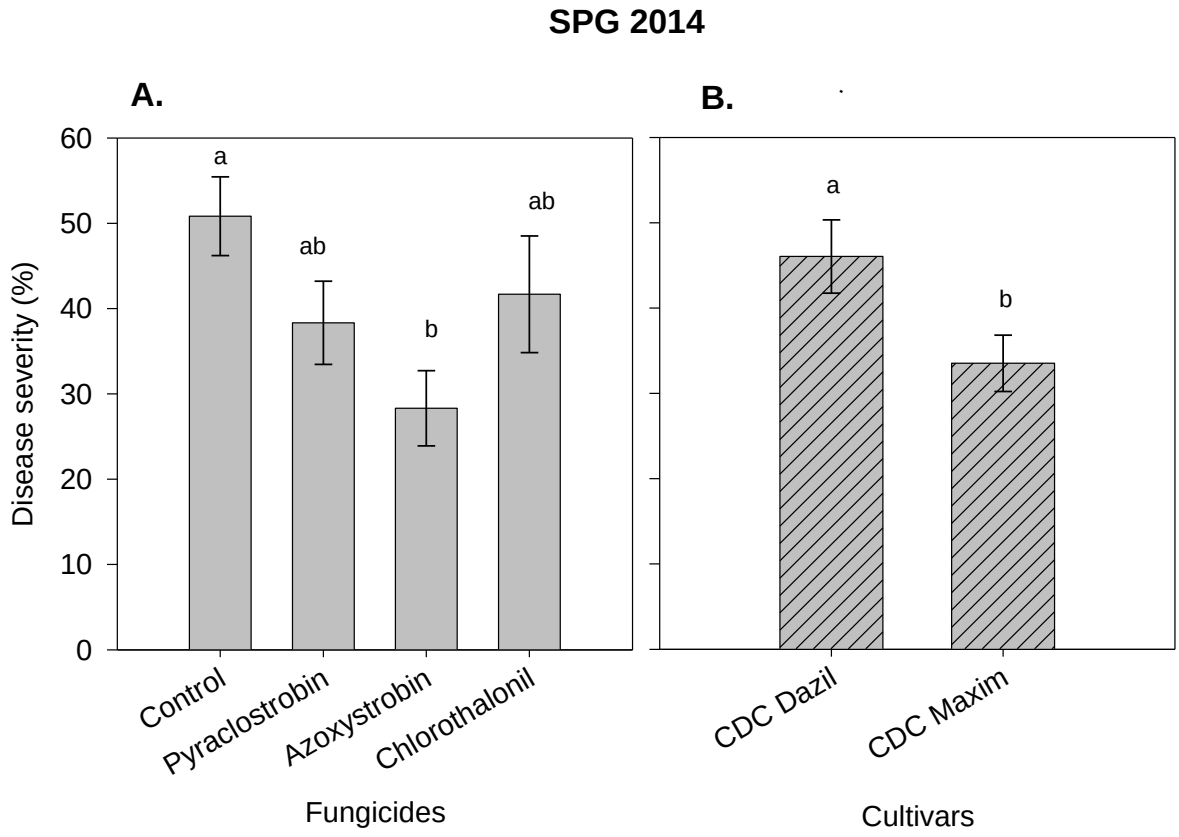
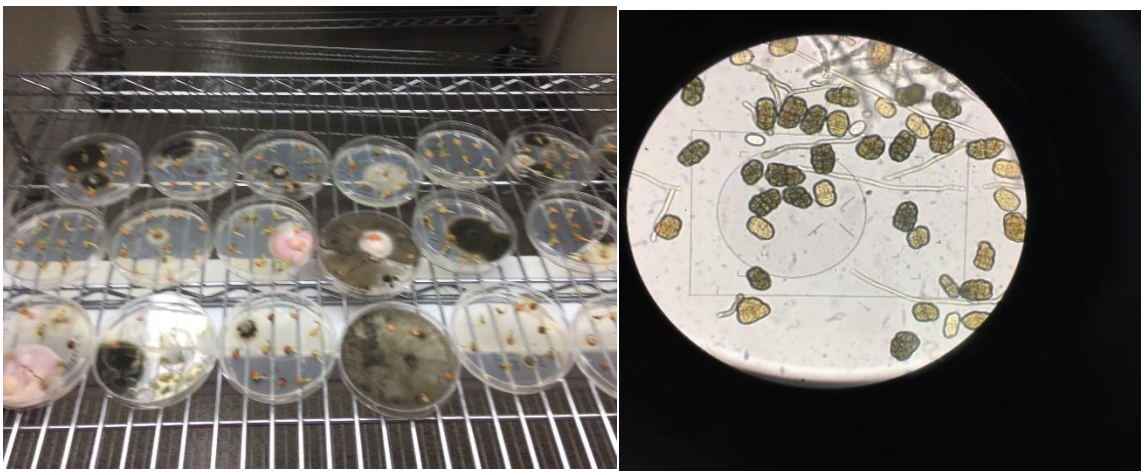


Figure 4.1. Mean values of stemphylium disease severity level (%) for fungicide treatments (Figure A) and between cultivars (Figure B) at SPG 2014. Error bars denote standard errors of treatment means. Comparisons made between treatments with similar lower-case letters are not significantly different between fungicides at $LSD_{0.05}$ =13.8 and between cultivars at $LSD_{0.05}$ = 9.0. LSD denotes Fisher's least significant difference.



A B

Figure 4.2. Growth of *Stemphylium botryosum* on infected seeds harvested at SPG 2014 (A) site in growth media and conidia of infected seeds under electron microscope (B).

4.4.2 Days to maturity, seed yield and 1000-seed weight

Neither fungicides nor fungicide × cultivar interaction had significant (*P<0.05*) effects on seed yield, days to maturity or 1000-seed weight (Table 4.3). Site-year × cultivar interaction for seed yield was significant *(P=0.001)* and thus, individual site-year ANOVA analysis was performed (Table 4.4). Cultivars differed significantly (*P<0.001*) for seed yield except at the Preston 2013 site. In both years at SPG, CDC Maxim had significantly higher seed yield than CDC Dazil (*P<0.05).* CDC Dazil produced significantly higher seed yield at the 2014 Preston site (Figure 3.3). Linear contrast analysis showed SB disease affected SPG 2014 site had significantly lowered seed yield (1443 kg ha^{-1}) compared to the other three site-years (Table 4.5).

Table 4.3. P values from combined analysis of variance of mixed model for the effect of fungicides, cultivars and site-years and their interactions with fixed effects on disease severity, days to maturity, 1000-seed weight, seed yield, seed diameter, seed thickness and seed plumpness, dehulling efficiency (DE), milling recovery (MR) and football recovery (FR) at two locations, SK over two years.

Source of variation	Df	Disease severity	Days to maturity	1000-seed weight	Seed yield	Seed diameter	Seed thickness	Seed plumpness	DE	MR	FR
						P values					
Site-years (SY)	3	0.024*	0.144	0.269	0.052*	0.131	0.217	0.159	0.000***	0.000***	0.004**
Cultivars (C)	1	0.995	0.269	0.021*	0.512	0.003**	0.119	0.094	0.463	0.476	0.707
Fungicides (F)	3	0.234	0.438	0.430	0.060	0.037*	0.027*	0.444	0.511	0.503	0.202
C ×F	3	0.954	0.949	0.213	0.325	0.937	0.492	0.102	0.816	0.606	0.140
C ×SY	3	0.534	0.323	0.128	0.000***	0.164	0.121	0.126	0.000***	0.001**	0.003*
F ×SY	9	0.270	0.466	NA	0.527	NA	NA	NA	0.007**	0.094	0.371
C ×F×SY	9	0.638	0.373	NA	0.738	0.352	NA	NA	0.982	0.931	0.928

Df – degrees of freedom, NA: not applicable, *, ** and *** significant difference at *P<0.05, 0.01* and *0.001*, respectively.

Table 4.4. Analysis of variance (P values, mean and standard error of mean) for disease severity (%), seed yield (kg ha^{-1}), dehulling efficiency (DE) (%), milling recovery (MR) (%) and football recovery (%) (FR) associated to fungicides treatments evaluated at each site year in Preston and SPG sites, SK in 2013 to 2014.

Site	Year	Source of variation	Df	Disease severity	Seed yield	DE	MR	FR
					P values			
Preston	2013	Cultivar (C)	1	0.345	0.659	0.000***	<0.000***	0.155
		Fungicides (F)	3	0.513	0.738	0.786	0.819	0.748
		C×F	3	0.613	0.856	0.879	0.529	0.281
Preston	2014	Cultivar (C)	1	0.214	0.003**	0.058	0.0475**	0.059
		Fungicides (F)	3	0.910	0.565	0.489	0.4936	0.551
		C×F	3	0.602	0.508	0.977	0.911	0.870
SPG	2013	Cultivar (C)	1	0.598	0.001**	0.743	0.546	0.038*
		Fungicides (F)	3	0.328	0.139	0.042*	0.211	0.444
		C×F	3	0.779	0.435	0.932	0.747	0.590
SPG	2014	Cultivar (C)	1	0.014*	<.000***	0.116	0.124	0.681
		Fungicides(F)	3	0.023*	0.320	0.199	0.397	0.961
		C×F	3	0.820	0.244	0.800	0.750	0.705
					Mean			
Preston	2013			13.2±2.2	2610.2±95.2	94.2±0.6	81.80±0.5	34.8±0.7
	2014			2.7±0.9	3511.4±98.1	86.8±0.6	74.29±0.6	20.8±1.0
SPG	2013			10.2±2.4	3184.0±114.3	91.3±0.6	80.19±0.5	53.6±1.1
	2014			48.2±2.8	1658.0±106.25	61.5±0.9	50.6±0.8	20.4±0.6

Df: degrees of freedom; *, ** and *** significant difference at *P<0.05, 0.01* and *0.001*, respectively; values after ± indicate standard error of mean.

Table 4.5. Mean differences of disease severity, seed yield, 1000-seed weight (TSW), seed diameter, thickness, and plumpness, percent dehulling efficiency (DE), milling recovery (MR) and football recovery (FR) between SB affected disease site (SPG 2014) vs. no-disease (three other site-years), fungicides treated vs. no-treated, and strobilurin vs. no strobilurin fungicides treatment from contrast analysis at Preston and SPG sites, SK from 2013 to 2014.

Contrasts			Seed characteristics				Milling quality characteristics		
	Disease severity (%)	Yield (kg ha^{-1})	TSW (g)	Diameter (mm)	Thickness (mm)	Plumpness	DE (%)	MR (%)	FR (%)
Disease vs. no disease	31.02***	-1443.40*	0.14^{ns}	0.06^{ns}	-0.06^{ns}	-0.02^{ns}	-29.20***	-28.40***	-16.08**
Control vs. fungicides	-2.19	-18.20	0.26	-0.09*	0.01	0.00	0.82	0.55	0.02
Strobilurins vs. chlorothalonil	3.81	7.70	0.30	0.03*	0.01	0.00	0.83	0.61	-0.05

*, ** and *** significant difference at *P<0.05, 0.01* and *0.001*, respectively; ^{ns} represent non-significant.

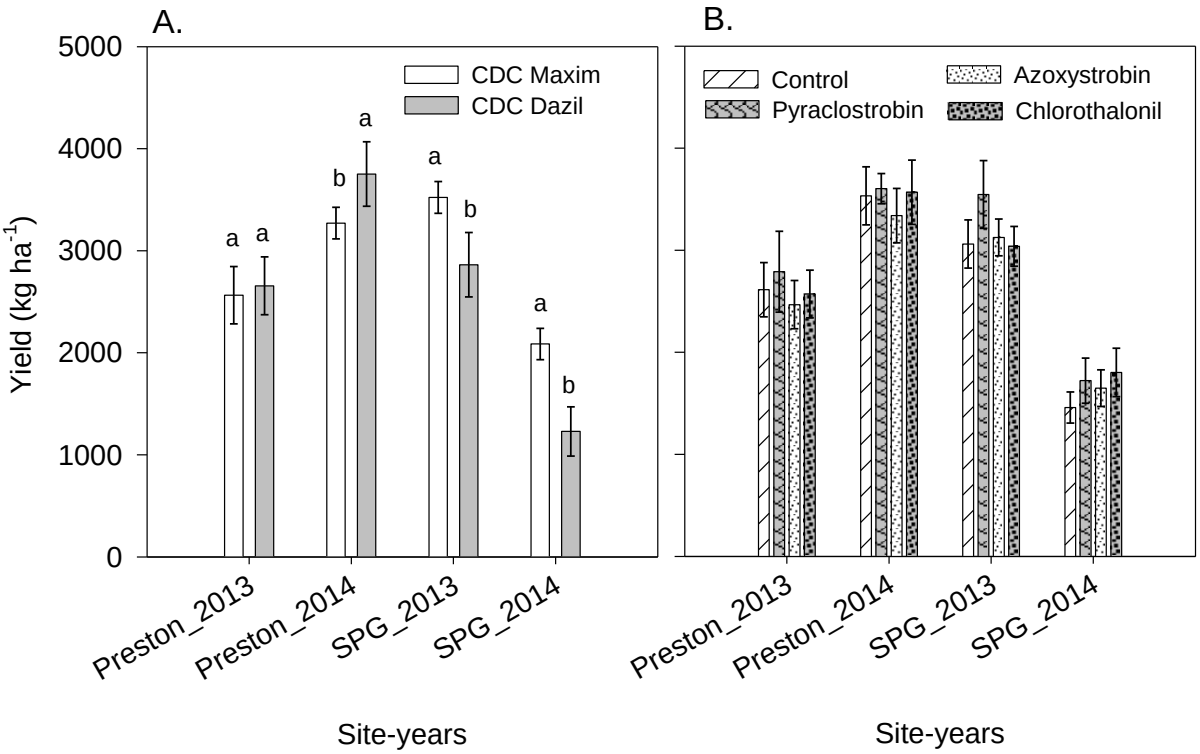


Figure 4.3. Mean seed yield (kg ha^{-1}) of lentil cultivars (Figure A) and fungicide treatments (Figure B) in four site-years at Saskatoon, SK in 2013 and 2014. Error bars represent standard errors of treatment means. Comparisons made between treatments with similar lower-case letters indicate no significant difference between lentil cultivars at $LSD_{0.05}$ at Preston 2014 = 294, $LSD_{0.05}$ at SPG 2013 = 511 $LSD_{0.05}$ at SPG 2014 =275. LSD denotes Fisher's least significant difference (LSD).

4.4.3 Seed morphological characteristics: seed diameter, thickness, and plumpness

Combined ANOVA results revealed that fungicide treatments had a uniform effect on seed dimension across growing environments. Therefore, the data were pooled and analyzed (Table 4.3). Overall, none of the fungicide × cultivar interactions had significant effects on seed dimension characteristics (Table 4.3). However, fungicide and cultivar effects were significant *($P<0.05$*) for seed diameter. CDC Maxim produced seeds with significantly greater diameter than CDC Dazil, Azoxystrobin and chlorothalonil application resulted in thicker seeds in comparison to those produced on pyraclostrobin treated plants but were not different from the control (Table 4.6). No cultivar difference for seed thickness was observed in any site-year. Seed plumpness

did not differ between cultivars or fungicides in any site-year. Linear contrast analysis indicated that fungicide treated plots had significantly larger seeds - 0.09 mm greater in diameter compared to control plots. Similarly, strobilurin-sprayed plots produced lentil seeds with 0.03 mm larger diameter compared to those produced in chlorothalonil-sprayed plots (Table 4.6), but there were no differences in seed dimensions or weight between SB disease-affected vs. no affected sites.

Table 4.6. Mean seed diameter, thickness, and plumpness of two red lentil cultivars subjected to fungicides treatments at four site-years in SK, 2013 and 2014.

Treatment	Seed diameter (mm)			Seed thickness (mm)			Seed plumpness		
	CDC Dazil	CDC Maxim	Mean	CDC Dazil	CDC Maxim	Mean	CDC Dazil	CDC Maxim	Mean
Control	4.38	4.59	4.48^{ab}	2.32	2.37	2.35^{ab}	0.53	0.51	0.52
Pyraclostrobin	4.37	4.60	4.47^{b}	2.31	2.37	2.34^{b}	0.52	0.51	0.52
Azoxystrobin	4.43	4.60	4.49^{ab}	2.33	2.39	2.36^{a}	0.53	0.52	0.53
Chlorothalonil	4.39	4.61	4.51^{a}	2.34	2.40	2.36^{a}	0.53	0.52	0.52
Mean	4.39^{b}	4.59^{a}		2.34	2.38		0.53	0.52	0.52
LSD (0.05)	0.09		0.04			0.028			
CV (%)	2.94			2.48			2.90		

Means followed by same letters within rows and columns are not significantly different at *P<0.05,* LSD denotes Fisher's least significant difference (LSD). CV denotes coefficient of variation.

4.4.4 Milling characteristics: dehulling efficiency, milling recovery and football recovery

Milling quality traits exhibited a significant site-year × cultivar interaction ($P \leq 0.05$) and significantly differed among growing environments (Table 4.3). Data were therefore analyzed within site-year. None of the fungicide × cultivar interactions had a significant effect on milling parameters in all site years (Table 4.4). Irrespective of treatments, seed produced at SPG in 2014 had significantly lowered DE values. Results of linear contrast analysis showed that on average SPG 2014 site (SB affected) significantly reduced DE by 29% compared to site years that had no SB infection (Table 4.5).

At Preston 2013, CDC Dazil had significantly higher DE compared to CDC Maxim (*P<0.001*) but there were similar DE values for both cultivars in other site-years (Figure 4.4). At SPG 2013, plots treated with pyraclostrobin produced seeds with significantly higher DE compared to the other fungicide treatments (Figure 4.4). Fungicide treatments applied prior to mid flowering had no effect on either MR or FR in all site-years (Table 4.5). CDC Dazil had significantly higher *(P=0.0001)* MR (4%) than CDC Maxim in 2013 whereas CDC Maxim produced significantly higher (*(P=0.047)* MR (2.5% times more) in 2014 at the Preston site (Table 4.7). At SPG 2013 CDC Dazil had significantly higher (*P=0.038)* FR by 6.3% more than CDC Maxim (Table 4.7). Linear contrast analysis showed that the SPG 2014 site, which had affected by SB, had significantly lower MR (reduced by -28%) FR (reduced by -16%) compared to the than the other site-years (Table 4.5).

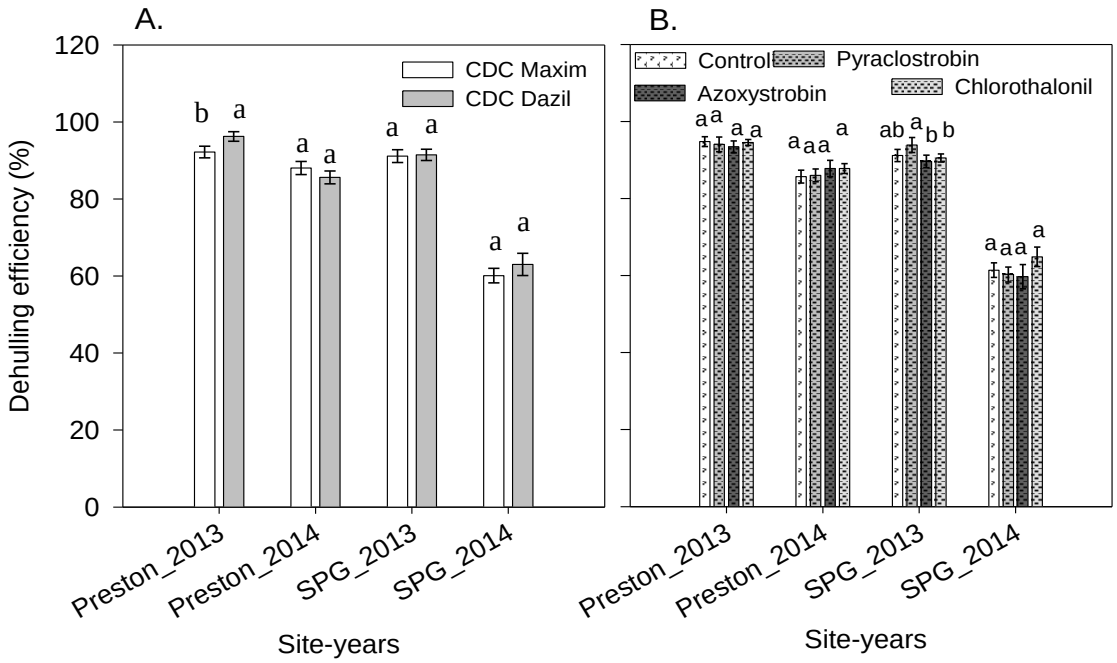


Figure 4.4. Mean dehulling efficiency (%) of lentil cultivars (Figure A) and fungicide treatments (Figure B) in four site-years at Saskatoon, SK in 2013 and 2014. Error bars represent standard errors of treatment means. In Figure B, comparisons made between treatments with similar lower-case letters indicate no significant difference between lentil cultivars at $LSD_{0.05}$ at Preston 2013 = 2.1 and similar lower-case letters indicated no significant difference between fungicide treatments at $LSD_{0.05}$ at SPG 2013 = 3.0. LSD denotes Fisher's least significant difference (LSD).

Table 4.7. Mean milling recovery (%) and football recovery (%) for two red lentil cultivars receiving fungicide applications at mid-flowering at four site-years.

Site-year	Fungicide	Milling recovery (%)			Football recovery (%)		
		CDC Maxim	CDC Dazil	Mean	CDC Maxim	CDC Dazil	Mean
Preston 2013	Control	79.46	83.35	81.40	36.40	34.83	35.61
	Pyraclostrobin	78.90	83.35	81.12	31.61	35.87	33.74
	Azoxystrobin	79.53	81.76	80.64	34.36	34.64	34.50
	Chlorothalonil	78.95	84.22	81.59	32.92	37.74	35.33
	Mean	79.20[b]	83.17[a]	81.19	33.82	35.77	34.79
	LSD (0.05)	1.54					
	CV (%)	3.56			11.85		
Preston 2014	Control	73.77	72.54	73.16	20.35	16.67	18.51
	Pyraclostrobin	74.80	72.32	73.56	22.57	18.32	20.45
	Azoxystrobin	77.07	73.38	75.22	26.27	19.32	22.80
	Chlorothalonil	76.62	73.88	75.25	22.41	20.44	21.42
	Mean	75.57[a]	73.03[b]	74.30	22.90	18.69	20.79
	LSD (0.05)	2.50					
	CV (%)	4.78			28.83		
SPG 2013	Control	80.67	80.06	80.37	51.77	55.15	53.46
	Pyraclostrobin	81.90	81.75	81.82	50.72	57.41	54.06
	Azoxystrobin	78.52	79.48	79.00	52.35	59.70	56.02
	Chlorothalonil	78.54	80.67	79.61	50.19	58.16	54.17
	Mean	79.91	80.49	80.20	51.26[b]	57.60[a]	54.43
	LSD (0.05)				4.51		
	CV (%)	3.65			11.94		
SPG 2014	Control	49.56	51.04	50.30	20.67	20.94	20.81
	Pyraclostrobin	48.98	49.40	49.19	21.03	19.36	20.19
	Azoxystrobin	45.97	51.06	48.52	20.23	20.98	20.60
	Chlorothalonil	50.63	53.91	52.27	18.36	21.32	19.84
	Mean	48.78	51.35	50.07	20.07	20.65	20.36
	LSD (0.05)						
	CV (%)	9.08			17.5		

Different letters within rows significant difference based on $LSD_{0.05}$; CV denotes coefficient of variation.

4.5 Discussion

The results clearly demonstrated that a single application of any of the three fungicides prior to mid-flowering had minimal impact on effectively controlling of stemphylium blight (SB) disease caused by *Stemphylium botryosum* that appeared later in the growing season in SPG 2014. Stemphylium blight disease is polycyclic in nature and more difficult to control than other fungal diseases (Banniza, et al, 2006). Compared to other sites, SB infected SPG 2014 site significantly reduced seed yield (-1443 kg ha^{-1}). Previous research also reported a significant reduction of seed yield due to SB infection in lentil (Morrall et al., 2006; Hosen et al., 2009). Fungicide applications had no effect on crop maturity, seed weight, or seed yield relative to the control at any site year. Strobilurin fungicides are mainly recommended to control anthracnose diseases in lentil and, however very limited research results were available for efficacy of mid-season applied strobilurin or chlorothalonil on seed yield or yield components in lentil or other related pulse crops such as chickpea or pea. Therefore, we could not compare these results with previous findings. Similar observations were, however, reported in common bean (*Phaseolus vulgaris).* Mahoney and Gillard (2014) illustrated that application of azoxystrobin and pyraclostrobin did not increase seed yield and delayed crop maturity when applied at the flowering stage of common bean. Contrary to our results, some studies on strobilurin applications have shown increased seed yield and weight in dry bean (*Phaseolus vulgaris*) (Pynenburg et al., 2011) and yield of soybean (*Glycine max*) (Mahoney et al., 2015). However, effects of strobilurin are inconsistent across crops, years, and environmental conditions. Lentil is a cool season indeterminate pulse crop, and it may be that the positive effects of a single application of fungicide on the assimilation process may not be expressed for lentil growing under the temperate conditions of the Canadian Prairies.

Pyraclostrobin-treated lentil plots produced seeds that were significantly smaller in diameter and in thickness without decreasing seed yield relative to the control or other fungicides (Table 4.3). This result indicates that pyraclostrobin may have a negative effect on seed morphological qualities in lentil under conditions of moderate disease pressure. Apart from DE, our results revealed that football and milling recovery percentages did not differ in response to fungicide application in any site year. At SPG in 2013 pyraclostrobin treated plots produced significantly *($P<0.05$)* higher lentil DE compared to chlorothalonil or azoxystrobin treatments.

Inconsistent DE in this study might be due to the weather variability during growing seasons. In 2013, the Preston and SPG sites received 51 mm and 55 mm precipitation, respectively, during the second half of the growing season (July and August) while 91 mm and 102 mm were received, respectively, in 2014. Higher DE of lentils from plants treated with pyraclostrobin in SPG 2013 might be due to the production of higher percentage of uniform shaped seeds. Previous research also highlighted that more uniform shaped lentil seeds led to a greater dehulling efficiency compared to those thinner and less plump lentil seeds (Erskine et al., 1991a; Wang, 2008).

These observations lend some support to the hypothesis that strobilurins can have positive impacts on DE, but not on MR or FR. Our results suggest that fungicide response and effect are not two sides of the same coin as suggested by Groth (2006). For example, application of azoxystrobin at the booting stage in rice, resulted in significant reduction of head blight diseases and subsequently increased rice yield compared to unsprayed plots. However, results cannot be directly compared because growth pattern, yield development and seed chemistry are completely different in lentil and cereals. The consistent results for MR and FR values over site years of this study indicated that response of strobilurin fungicides or chlorothalonil application on respective traits had negligible effects.

Cultivar differences for milling quality were observed, but results were not consistent across cultivars and site-years. This may be due to differences in flowering time and expression of seed coat color between the two lentil cultivars. For instance, CDC Dazil seeds have lighter seed coat color than CDC Maxim. We observed significant infection by SB at the SPG 2014 site, and a large reduction in milling efficiency such as DE (-29%), MR (-28%), and FR (-16%) percentages (Figure 4.4, and Table 4.4). This onset of SB was likely enhanced by late planting coupled with higher precipitation, combined with more humid conditions due to lower night temperatures in the later part of the growing season (Table 4.1). Stemphylium blight is polycyclic (Mwakutuya and Banniza, 2010) and is favoured by high humidity in the canopy. A single fungicide application is not sufficient to control a polycyclic disease since this disease can produce higher amounts of discolored and deformed mature lentil seeds **(**Caudillo-Ruiz, 2016). Higher amounts of discolored or misshapen seeds impart problems of loosening of seed coats during the dehulling process. High levels of natural SB infection of plots could be a major underlying cause of reduction of milling quality that may be unique to lentil production at higher

latitudes. Therefore, a small follow up field experiment was conducted to test the hypothesis that the efficacy of high levels of stemphylium disease infection can severely impacted milling qualities in lentil. The results are presented in Appendix A2.

In summary, results indicated that high infection of SB due to high humidity prior to harvest of late-planted lentil resulted in significant losses in yield and milling quality. If SB is not managed under these environmental conditions, lentil growers may be at risk of a double loss due to a reduction of both yield and of milling quality. Therefore, under environmental conditions that amplify the likelihood of experiencing additional cycles of SB infection in the plant canopy, it may be justifiable to develop appropriate agronomic strategies that would help minimize direct disease effects and maximize post-harvest milling quality of red lentil. This could include developing methods to improve timing and frequency of strobilurin fungicide application to control stemphylium blight infection cycles. Further consideration could be given to appropriate crop rotations, and potentially to an investigation of how row spacing, and plant density might be adjusted to improve milling quality. The results also imply that early sowing can mitigate the risk of higher moisture and the potential for SB disease infection, during seed filling prior to harvest, the main factors which may reduce overall milling quality.

4.6. Conclusions

One of the main conclusions of this research is that late season infection of the crop canopy by stemphylium has a major effect on milling efficiency of red lentil. A single application of pyraclostrobin, azoxystrobin or chlorothalonil prior to mid-flowering had no effect on late-season control of polycyclic SB disease infection, or on maturity, seed yield, 1000 seed weight, milling recovery (MR) or football recovery (FR) percentages. Pyraclostrobin reduced seed size with no effect on yield or quality. A modest positive effect of pyraclostrobin on DE and on other milling quality parameters suggests that lentil growers may maximize their economic benefit if they spray this fungicide when disease pressure is expected to increase in the second half of the growing season. Control of late season SB infections using fungicides and developing effective resistance breeding methods would help to increase the overall value of Canadian red lentils.

Prologue to Chapter 5

In chapters 3 and 4, it was demonstrated that typical cultural practices such as harvest aids and common fungicides used in lentil production in Western Canada can impact post-harvest seed and milling quality of lentil. In chapter 5 the aim was to determine the effect of seed coat ground color on milling quality characteristics of red lentil genotypes which were visually assessed under field condition.

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Subedi, M., Tabil L.G., and Vandenberg, A. 2017. Influence of seed coat color on milling quality characteristics of red lentil (*Lens culinaris* Medikus).

CHAPTER 5. INFLUENCE OF SEED COAT COLOR ON MILLING QUALITY CHARACTERISTICS OF RED LENTIL (*LENS CULINARIS* MEDIKUS)

Abstract

Efficient milling characteristics are key economic traits for the red lentil industry. Various factors including seed coat color and seed coat chemistry can influence milling characteristics. Four basic seed coat ground colors (green, gray, tan, and brown) of 16 red lentil genotypes from a common genetic background were compared to determine the effect of seed coat ground color on three milling quality traits: dehulling efficiency (DE), milling recovery (MR), and football recovery (FR). These genotypes were grown at two locations in Saskatchewan, Canada for two years. DE, MR, and FR results varied depending on the seed coat color conferred by specific genotypes. Green seed coat genotypes (homozygous recessive *ggc tgc*) had significantly higher DE in all site-years compared to brown seed coat genotypes (homozygous dominant *Tgc Ggc)* while gray seed coat (genotype *Ggc tgc*) had significantly higher DE only in three site years, respectively compared to brown seed coat genotypes. Except one-site year, green or gray seed coat color genotypes had significantly higher MR compared to the brown seed coat genotypes. Seeds with brown or tan seed coats (homozygous dominant *Tgc* allele) had significantly higher FR percentages in two-site years. Milling red cotyledon lentils having uniform shape with green or gray seed coat color might be more profitable for millers who wish to maximize DE and MR of red lentil, but brown seed coat color might be preferable in terms of FR.

5.1 Introduction

Dhal yield is an important post-harvest processing trait that lentil processers aim to optimize. Reduction in dhal yield and value is attributed to seeds that are not successfully dehulled, and to losses caused by chipping and abrasion. This reduction can vary according to whether genotypes are difficult or easy to dehull (Bruce, 2008; Wood et al., 2012). Significant variation with respect to dehulling has been observed among lentil genotypes (Erskine et al. 1991; Wang 2008; Bruce 2008) as well as other pulses (Singh et al., 1992; Black et al., 1998; Wood et al., 2017). Seeds of specific pulse crop genotypes can vary in shape, size, chemistry, and composition of the seed coat, including the adhesive or cohesive mechanism occurring at the interface of seed tissues. All of these factors play an important role in the efficiency of the dehulling process (Kurien, 1984; Erskine et al., 1991; Wood and Malcolmson, 2011). Variation in percent dehulling efficiency (DE), milling recovery (MR), and football recovery (FR) has been reported among lentil cultivars, and attributed to differences in seed shape and seed coat content (Bruce 2008; Shahin et al., 2012). An inverse relationship between amount of seed coat and milling recovery has been reported in studies of pigeon pea, lentil, and chickpea (Wang 2008; Wood et al., 2008; Wood et al., 2017).

The thickness of seed coats of seeds can also vary depending on seed coat color and chemistry. Seeds of zero tannin lentil genotypes (homozygous *tan*), which have greatly reduced polyphenol content and diversity (Mirali et al., 2016b) have seed coats that are about 20% thinner and about 20% less by weight than lentil genotypes with normal seed coats (Vaillancourt et al., 1986). Normal lentil genotypes (*Tan*) also vary with respect to seed coat color and pattern (Vandenberg and Slinkard, 1990). Two independent genes (*Ggc* and *Tgc*) determine seed coat background color in lentil. Dominant and recessive combinations of the two alleles at each locus determine the four-basic seed coat ground colors: brown (*Ggc Tgc*), gray (*Ggc tgc*), tan (*ggc Tgc*), and green (*ggc tgc*). Differences in biochemistry, specifically the amount and types of polyphenolic compounds in the seed coat, determine the color of the seed coat in lentil. Gray and green seed coats have higher levels of flavan-3-ols, proanthocyanidins, and some flavones. The colourless proanthocyanidins can be oxidized by polyphenol oxidase, resulting in colour and structural changes (Mirali et al., 2017). The concentration of the different polyphenolic compounds can influence the dehulling process, resulting in variation in the efficiency of loosening of the seed coats from the cotyledons during milling. However, no information is

currently available regarding the relationship between lentil seed coat color genes and their association with milling quality characteristics. This study compared red cotyledon lentil genotypes with green, gray, tan, and brown seed coat ground colors in terms of milling quality characteristics.

5.2 Hypothesis

Specific genetic combinations of the alleles of the *Ggc* and *Tgc* loci determine the green, gray, tan, and brown seed coat color phenotypes. This will result in differences in milling qualities as measured by dehulling efficiency (DE), milling recovery (MR) and football recovery (FR).

5.3 Materials and methods

5.3.1 Plant material

Sixteen recombinant inbred lines (RILs) of red cotyledon genotypes that differ in seed coat color (4 each of green, gray, brown, and tan phenotypes) were randomly selected from the 147 LR-18 lentil RIL population (Fedoruk et al. 2013 (Table 5.1; Figure 5.1) and used to determine milling quality parameters. These lines were homogeneous in diameter and plumpness, and had no seed coat pattern (Mirali et al., 2017). The parents of LR-18 are CDC Robin (small-seeded, brown seed coat with red cotyledons) and 964a-46 (large-seeded, pale green seed coat with yellow cotyledons). The LR-18 population segregates for seed coat color independently for both alleles of the *Ggc* and *Tgc* genes.

Table 5.1. Homozygous genotypes and phenotypes of 16 red lentil RILs selected for analysis of milling quality.

RIL group	No. of RILs	Genotype at *Ggc* locus	Genotype at *Tgc* locus	Seed coat color phenotype	Genotype at *Yc* locus	Cotyledon color phenotype
1	4	*Ggc Ggc*	*TgcTgc*	Brown	*Yc*	Red
2	4	*Ggc Ggc*	*tgc tgc*	Gray	*Yc*	Red
3	4	*ggc ggc*	*Tgc Tgc*	Tan	*Yc*	Red
4	4	*ggc ggc*	*tgc tgc*	Green	*Yc*	Red

Green (*ggc tgc*) Gray (*Ggc tgc*) Tan (*ggc Tgc*) Brown (G*gc Tgc*)

Figure 5.1. Representative samples of the four-basic seed coat ground color phenotypes and genotypes of lentil.

5.3.2 Field experiments and environmental conditions

The 16 selected lines were grown in three replicates in a randomized complete block design at two locations in Saskatchewan —the Saskatchewan Pulse Growers farm (SPG; 52.05° N, 106.41° W) and the University of Saskatchewan experimental farm in Sutherland (52.14° N, 106.61° W)—in both 2013 and 2014. The plots were sown in the May 13 and May 14 at Sutherland and May 28 and June 3 at SPG respectively in 2013 and 2014 at a rate of 130 seeds/m^2 in 1 m^2 microplots. Individual microplots were hand harvested when 95% of the lower canopy pods had turned yellow to brown in color. Complete site details and cultivation practices are described in Appendix 5.1. Mean temperature and total monthly precipitation data for each month of the growing season at Saskatoon, Saskatchewan for both years were obtained from Environment Canada, 2016 (Table 5.2).

Table 5.2. Mean daily temperature (°C) and total monthly precipitation (mm) for the 2013 and 2014 growing seasons at Saskatoon, Saskatchewan, Canada.

Climate details	May		June		July		Aug		Sep	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
Temperature (°C)	13.0	10.1	15.5	14.1	17.4	18.3	18.9	17.9	15.2	12.4
Precipitation (mm)	15.9	61.1	117.7	94.8	35.6	44.5	14.9	18.5	15.4	10.7

Source: http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID=47707.

5.3.3 Data collection

5.3.3.1 Measurement of milling quality characteristics

The milling quality traits for DE, MR and FR of the seed samples were determined using the procedures described in detail in Chapter 3 (Subedi et al., 2017).

5.3.4 Data analyses

The location and year of the field trials were treated as environments (site-years). Analysis of variance (ANOVA) was performed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., 2016). Homogeneity and normality were checked before subjecting data to ANOVA. To determine the best fit model in the combined analysis, genotype and site year were considered fixed, and replications nested within site-years were considered random effects. Heterogeneous variance structures were modeled using the repeated/group statement with mixed models wherever required. Table 5.3 shows the *P*-values from the mixed model ANOVA F-test for the response variables. Site-year had a significant effect ($P=0.001$) on DE, MR, and FR and therefore, these data were analysed separately for each site-year. Least squared means were computed for fixed effects using the LSMEANS statement. Significance of variance was declared at 5%. Means were separated using Fisher's least significant difference (LSD) at $P<0.05$. Additionally, all letter groupings for significance differences were established using PDMIX 800 in SAS (Saxton, 1998). Graphs were plotted using a Sigma Plot V2. (Sigma Plot, 2010).

5.4 Results

5.4.1 Effect of seed coat color on dehulling efficiency

Results from all environments showed significant differences *(P < 0.001*) for DE values among lentil genotypes with different seed coat color phenotypes (Table 5.4). Figure 5.2 shows mean comparisons for DE for individual site-years. Except for SPG 2014, lentil genotypes with green and gray seed coat colors had significantly higher (*P< 0.001*) (8-10% higher) DE compared to those with brown seed coat color. At SPG 2014, the green seed coat color genotypes had significantly (*P<0.001*) higher DE compared to those with brown seed coats (Figure 5.2).

Table 5.3. P-values from mixed model ANOVA F-test for the effect of seed coat color, site year, seed coat color by site year interaction on dehulling efficiency, milling recovery, and football recovery at SPG and Sutherland, Saskatoon, SK in 2013 and 2014.

Source of variation	Degrees of freedom	Dehulling efficiency	Milling Recovery	Football recovery
			P values	
Seed coat color	3	<.000***	0.010**	0.001***
Site-year	3	<.000***	<.000***	<.000***
Seed coat color ×site-year	9	0.066	0.070	0.006**

*, ** and *** denote significant at *P<0.05, 0.01* and *0.001*, respectively.

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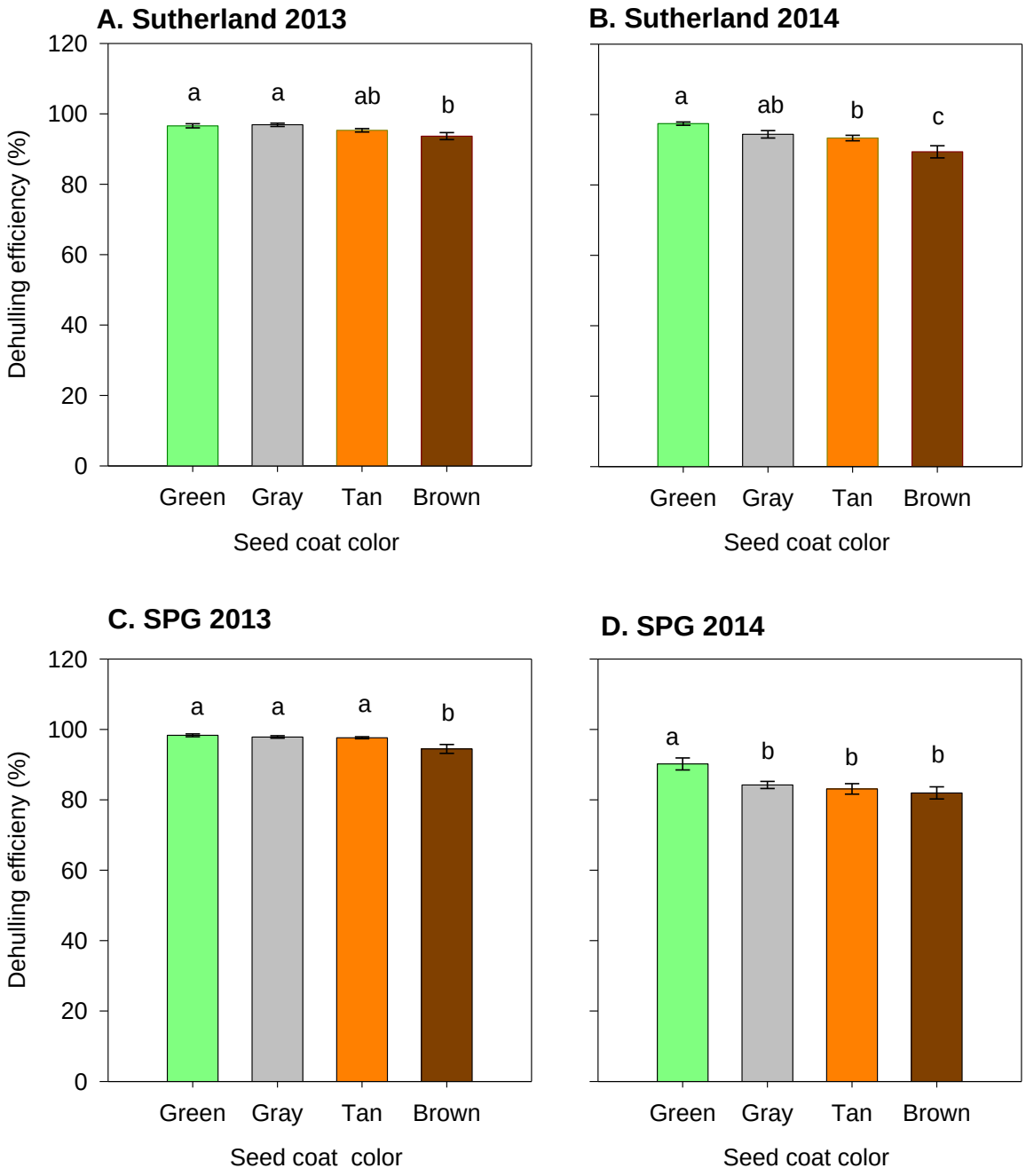

Figure 5.2. Mean dehulling efficiency (%) of four lentil seed coat color phenotypes (green, gray, tan, and brown) at Sutherland (A and B) and SPG (C and D) sites, SK in 2013 (A and C) and 2014 (B and D). Error bars are standard error of replicates in each site-year. Means with similar letters above bar indicate no significant difference between seed coat color groups at $LSD_{0.05}$ (Sutherland 2013=1.9; Sutherland 2014=3.2; SPG 2013=1.89; SPG 2014=4.3).

Table 5.4. P-values from mixed model ANOVA F-test for the fixed effect of seed coat color on milling quality traits for four site-years (Sutherland and SPG, SK in 2013 and 2014).

Site-year	Source of variation	Degrees of freedom	Dehulling Efficiency	Milling Recovery	Football Recovery
Sutherland 2013	Seed coat color	3	0.006**	0.007**	0.005**
SPG 2013	Seed coat color	3	0.001***	0.000***	0.001**
Sutherland 2014	Seed coat color	3	0.000***	0.000***	0.886
SPG 2014	Seed coat color	3	0.002**	0.003**	0.656

*, ** and ***, significant at *P<0.05, 0.01* and *0.001*, respectively.

5.4.2 Effect of seed coat color on milling recovery

Results for MR showed significant differences (*P<0.001*) among lentil genotypes with different seed coat colors at all locations and years (Table 5.4). The brown seed coat color genotypes had significantly lower *(P<0.001*) MR (-5 to -8% lower) values compared to green, gray, and tan color genotypes in three site-years. In SPG 2014, brown seed coat genotypes displayed significantly (*P<0.05*) lower MR values than did green seed coat color genotypes but were not different than gray or tan genotypes (Figure 5.3).

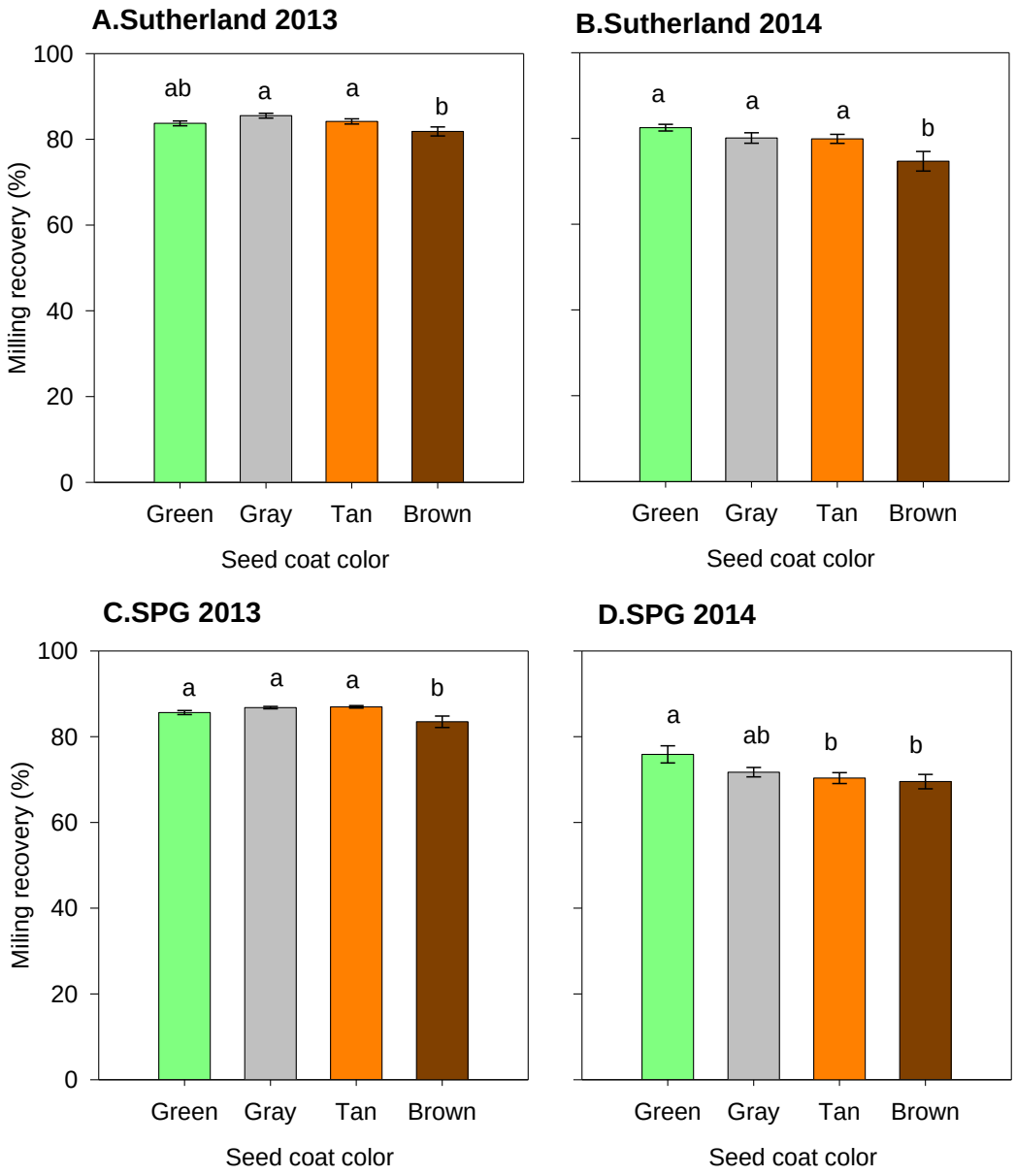

Figure 5.3. Mean milling recovery (%) of four lentil seed coat color phenotypes (green, gray, tan, and brown) at Sutherland (A and B) and SPG (C and D) sites, SK in 2013 (A and C) and 2014 (B and D). Error bar are standard errors for three replicates of each types in each site-year. Means with similar letters above bar indicate no significant differences between phenotype at LSD $_{0.05}$ (for Sutherland 2013=2.00; Sutherland 2014=4.2; SPG 2013=2.1; SPG 2014=4.5). LSD denotes Fisher's least significant difference.

5.4.3 Effect of seed coat color on football recovery

Genotypes with different seed coat colors had significantly *(P<0.001)* different FR at both sites in 2013 but not in 2014 (Table 5.4). Tan (*ggc Tgc*) and brown seed coat (*Ggc Tgc*) color genotypes had significantly higher (15-20% higher) *(P<0.001)* FR than green seed coat color

genotypes at both sites in 2013 (Figure 5.4). Gray color (*Ggc tgc*) (Figure 5.3). Gray color (*Ggc tgc*) genotypes had significantly *(P<0.05)* lower FR than the tan and brown genotypes in SPG 2013 but, had similar FR to green seed coat genotypes in both site-years (Figure 5.4).

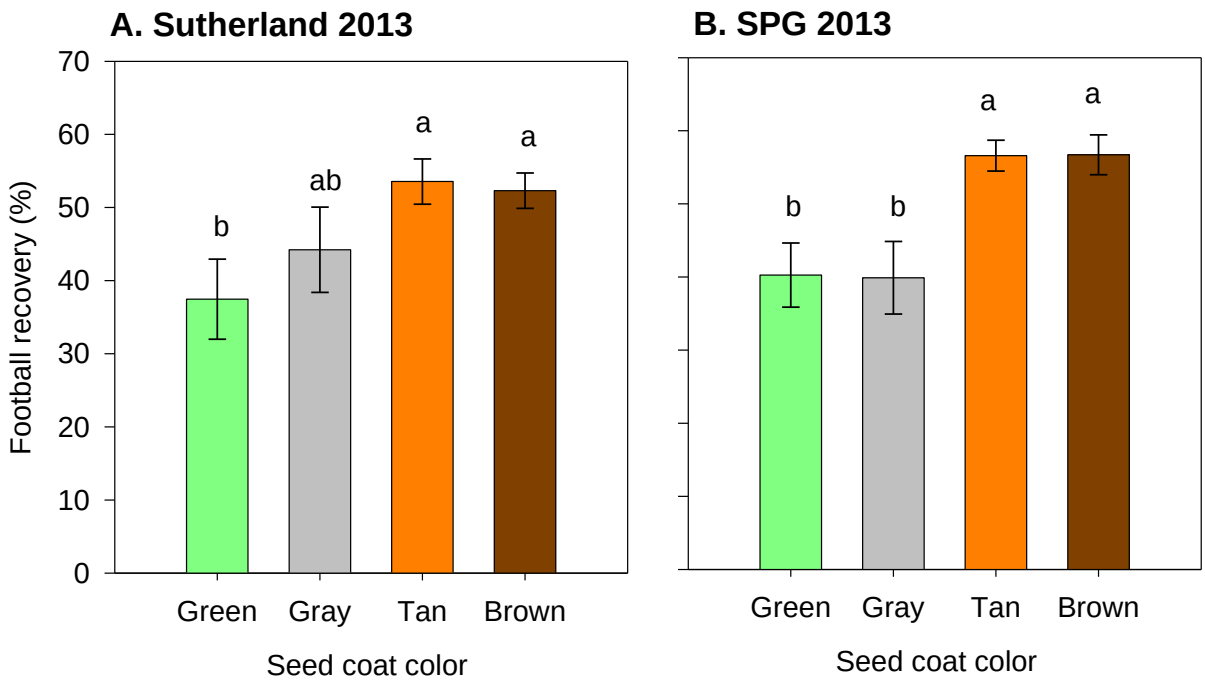

Figure 5.4. Mean football recovery (%) of four lentil seed coat color phenotypes (green, gray, tan, and brown) at Sutherland (A) and SPG (B) sites, SK in 2013. Error bar are standard errors for three replicates of each types in each site-year. Means with similar letters above bar indicate no significant differences between phenotype group at $LSD_{0.05}$ (Sutherland 2013=12.7; SPG 2013=11.4).

5.5 Discussion

These results show that both green (*ggc tgc*) and gray (*Ggc tgc*) genotypes had significantly greater DE (8-10% higher) and MR (5-8% higher) than brown (*Ggc Tgc*) seed coat color genotypes depending on the site-year. Gray and green seed coats are homozygous for the recessive *tgc* allele, whereas the brown seed coat is homozygous for the dominant allele (*Tgc*). This indicates that DE and MR percentages are highly influenced by the presence or absence of the *Tgc* allele. The higher MR and DE for green and gray seed coat phenotypes could be the

result of differences in seed coat color chemistry. LR-18 RILs with green or gray seed coats, i.e., which express the homozygous *tgc* allele, have higher amounts of flavan-3-ols, proanthocyanins, and some flavanol groups compared to other seed coat phenotypes (Mirali et al., 2017). The proanthocyanin group of phenolic compounds are basically colorless (Lepiniec et al., 2006; Marles et al., 2003); and might make gray and green seed coats more transparent compared to brown seed coats in lentil (Mirali et al., 2017). These transparent seed coats might be thinner than the opaque types, possibly affecting their ability to absorb and release moisture. Therefore, lentil with green or gray seed coats might be easier to loosen from the cotyledons during abrasive dehulling. We did not specifically collect data related to thickness of seed coat of these genotypes, but several researchers (Kurien, 1984; Wang, 2008; Wood et al., 2008) have observed an inverse relationship between the amount of seed coat and efficient milling recovery in other pulses. Other studies have noted that white color seed coats are thinner than black seed coats in lima bean (*Phaseolus lunatus* L.) due to reduced cell size in the palisade layer (Kannenberg and Allard, 1964); white seeded common bean genotypes are deficient in polyphenols (Akond et al., 2011); and differences in the thickness of seed coat palisade cell layers caused different capacities for water imbibition in two lima bean lines (Agbo et al., 1986).

Rapid and smooth imbibition of seeds during conditioning is essential for easy dehulling in lentil (Wang, 2005). The lower DE and MR of brown seed coat genotypes noted in our study is consistent with a milling efficiency study in which brown seed coat lentil cultivars (CDC Robin and CDC Imperial) produced lower DE and MR values (Bruce, 2008). The genotypes used in our study had CDC Robin as a parent. We observed significant reduction of DE and MR values of all seed coat color groups at both sites in 2014 compared to 2013. This could be attributable to weather variability between the two growing years, particularly more seasonal moisture (157.8 mm) in the second half of the 2014 growing season (Table 5.2), because wet environments cause lentil cultivars to have reduced DE and MR (Bruce, 2008). Moreover, the 2014 plots were affected by stemphylium blight (SB) caused by *Stemphylium botryosum*. This disease might also have lowered DE and MR values because disease-infected seeds become misshapen and have stained seed coats (Caudillo-Ruiz, 2016). In the current study, the magnitude of the reduction of DE and MR percentages was high for brown seed compared to other seed coat colors, indicating that lentils with brown seed coats might be more susceptible to damage due to wet conditions. This is supported by reports that seed coat color has a large

impact on weathering ability of lentil seeds and that gray seed coat lentil genotypes are able to better withstand unfavourable wet weather relative to brown seed coat lentil genotypes (Bruce, 2008). Seed coat color is associated with physical and chemical characteristics that affect the weathering ability of common bean (*Phaseolus vulgaris* L.) (Beninger et al., 1998).

The presence of dense polyphenolic compounds can make seeds harder and more difficult to split during milling processes, as noted for pigeon pea (*Cajanus cajan*) and kidney bean (Singh et al., 1992; Reichert et al., 1984). In this study, only lentils with brown and tan seed coat color phenotypes had significantly greater FR (15-20%) compared to green or gray seed coat colors in the 2013 season. This might be due to both brown and tan genotypes having more opaque seed coats than green or gray due to the presence of dense and oxidized polyphenolic compounds (Mirali et al., 2017). Lentil genotypes with brown or tan seed coats might also absorb more moisture through the seed coat into cotyledon tissues, resulting in increased adherence between the cotyledons that results in less splitting during the milling process. Green or gray seed coat genotypes could also have different hygroscopic properties that influence moisture absorption and transmission through cotyledonary tissues. This is supported by the finding that brown lentil cultivars produce a significantly higher FR portion if harvested in wet weather conditions (Bruce, 2008)

Inconsistency of FR values among genotypes across site-years in our results might also be an indication that FR is more sensitive to the growing environment compared to other milling traits. Significant differences in FR among seed coat color groups were only observed in dry and optimal weather, aligning with previous milling efficiency results in lentil (Bruce, 2008). This suggests that, given the typical optimal harvest weather in Saskatchewan, lentil millers might develop a preference for specific red lentil cultivars with brown or tan seed coats if they wish to maximize football recovery. At the commercial scale, stone separation, roller speed, and other milling configurations could be adjusted based on seed coat surface, texture, or seed thickness; investigating these aspects was not possible at the lab scale using our Satake dehuller. Genotypes with brown or tan seed coat colors and harvested under optimal conditions produced higher FR values for the techniques and instruments used in this study.

Overall, the findings from this study suggest that the red lentil milling industry in northern temperate climatic zones might be able to increase DE and MR recovery by using red lentils that have green or gray seed coat color. Green and gray seed coats contain beneficial

polyphenolic compounds such as proanthocyanin oligomers and some flavanols (Mirali et al. 2017). These compounds have higher antioxidant capacity (Dueñas et al., 2006) and additional economic value could be achievable from use of the seed coats. One tonne of milled lentil can have 80-110 kg of seed coats (Dueñas et al., 2002); if 9-22% seed coat-methanol-water extract can be extracted (i.e., 7-24 kg extract) from the seed coats (Ronzio et al. 1998), this represents 0.7-2.4 kg of phenolic compounds that can be obtained. Therefore, lentil seed coat extract could have potential as a source of plant-based antioxidants. Uses for this source of natural antioxidants from lentil seed coats could be developed for fortified foods and nutraceuticals.

In contrast, if millers wished to maximize FR, they could choose red lentils with either brown or tan seed coats. Therefore, future red lentil breeding program objectives could focus on the development of red cotyledon varieties with green or gray background color to achieve higher MR and DE. For specific niche markets requiring higher FR, cultivars with brown or tan seed coats might be more suitable. The study findings also suggest that higher seasonal precipitation during the seed filling period might result in the development of seed coat characteristics that are less desirable in terms of milling quality.

5.6 Conclusions

Analysis of the effects of the four basic seed coat background colors of red lentil on milling characteristics indicated that seed coat color influences DE, MR, and FR. Genotypes that express the homozygous recessive *tgc* allele (green and gray seed coats) produced the highest values of DE and MR. This suggests that milling lentils with green or gray seed coats may more profitable for millers who wish to maximize total milling recovery yield for lentils. For maximizing FR, brown or tan seed coat color genotypes might be preferable under optimal weather conditions; however, inconsistent results of FR analysis suggest the need for further research to confirm this finding under a wider range of conditions.

Prologue to Chapter 6

In Chapter 5, I evaluated how seed coat color impacts milling quality characteristics of red lentil genotypes. It was demonstrated that genotypes with green or gray types seed coat (homozygous recessive for the *tgc* allele determining seed coat color, had greater milling efficiency by producing significant higher DE and MR values while brown or dark colored genotypes had greater amount of football recovery. The study in chapter 6 is a continuation of the theme of Chapter 5 with a specific focus on genetic mapping (QTL mapping) for post-harvest seed and milling quality parameters in all lines developed from LR-18 RIL populations which segregates for the four-seed coat background colours, red and yellow cotyledon colours, and seed dimensions.

Chapter 6 was accepted as a manuscript for the publication in the Plant Genome journal on January 17,2018.

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CHAPTER 6. GENETIC MAPPING OF MILLING QUALITY TRAITS IN LENTIL (*LENS CULINARIS MEDIKUS*)

Abstract

Milling qualities are key traits for the red lentil industry because the price is largely determined by *dhal* recovery yield. Genetic control of milling traits of lentil is poorly understood. The objectives of the study were to determine the heritability of the milling traits – dehulling efficiency (DE), milling recovery (MR), and football recovery (FR), and to identify the genomic regions controlling them. We used the lentil recombinant inbred population from a cross CDC Robin × 946a-46 that have contrasting seed dimensions, cotyledon, and seed coat color. The mapping population consists of 127 F_7 derived lentil recombinant inbred lines that were phenotyped for milling quality parameters from four site-years in Saskatchewan, Canada. A total of 534 single nucleotide polymorphisms markers, seven simple sequence repeat markers, and four morphological markers were used for quantitative trait locus (QTL) mapping. A significant variation was exhibited by the RILs for seed quality and milling quality parameters, dehulling efficiency, milling recovery, and football recovery. The broad sense heritability was high for seed weight and seed shape while moderate for DE (0.68) and MR (0.52), and low for FR (0.41). Milling quality traits were significantly correlated with seed shape (seed diameter and seed plumpness). Multiple QTLs for milling traits were detected in six of seven linkage groups (LGs). The most stable QTLs governing DE and MR were clustered on LGs 1, 2, 3 and 7 whereas FR QTLs were clustered on LGs 4, 5, 6 and 7. The molecular markers identified for these traits could be used for improving milling quality in lentil breeding programs.

6.1 Introduction

Red lentils are a source of stable protein and nutritious food option in many parts of Indian sub-continent and Mediterranean regions such as Turkey, Syria, Egypt where they are primarily consumed in dehulled form as split cotyledons (Vandenberg, 2009). Milling involves removal of the seed coat, a process that results in the seed being split to produce two separated cotyledons (splits) or unsplit cotyledons (footballs), both products are referred to as '*dhal'* (Wang, 2008; Wood et al., 2012). The milling characteristics of the red lentils are important quality parameters that determine their price and quality. Seed characteristics, including seed coat thickness, seed coat components, seed size, and seed dimensions, are important traits, influenced by both genetics and environment that have been reported to influence milling performances of lentil and other legume crops (Ramakrishnaiah and Kurien 1983, Kurien, 1984, Wang, 2008; Wood and Malcolmson, 2011; Wood et al., 2012).

Efficient dehulling of lentil requires genotypes with uniformly sized seeds and a plump shape since thin seeds are inclined to have greater damage during processing leading to decreased dehulled yields (Erskine et al., 1991a; Wang, 2008; Shahin et al., 2012). Larger seeded lentils tend to have lower percentage of loss during decortication because the proportion of hull to seed mass is lower compare to small seeds (Vandenberg, 2009). Erskine et al. (1991a) found that lentil with a mean seed diameter of 4 mm lost about 8.2% of their weight during dehulling compare with losses from lentil seeds with 3 mm in diameter which averaged 9.8%.

A number of seed-related morphological traits have been genetically mapped in lentil (Fratini et al., 2007; Fedoruk et al. 2013; Saha et al., 2013; Khazaei et al., 2018). Three major quantitative trait loci (QTL) governing seed diameter were mapped in lentil using RAPD (random amplified polymorphic DNA) markers by Fratini et al. (2007). Verma et al. (2015) detected QTLs related to seed weight and size of lentil that were co-localized using SSR (simple sequence repeat) markers. Multiple QTLs for lentil seed diameter, thickness and plumpness were mapped in lentil using single nucleotide polymorphism (SNP) markers (Fedoruk et al., 2013). The most stable and significant QTLs for seed diameter and plumpness were detected near the cotyledon color locus (*Yc*) which explained 60% and 50% of the phenotypic variation for these traits, respectively, in that population (Fedoruk et al., 2013). Recently these genomic regions were validated using a cultivated lentil association mapping panel (Khazaei et al., 2018).

Limited studies in genetic control for milling traits in cereal crops revealed that milling quality traits were control by multi genes with small effects (Groh et al., 2000; Kepiro et al., 2008; Swamy et al., 2012). Seed morphological characteristics have tremendous impact on milling performance, QTL for these traits could be associated with milling quality traits in lentil. Genetic control of milling traits is poorly understood in lentil, and in pulse crops generally. To date, no published research describes how genotype and environment influence milling traits, and there are no reports identifying molecular markers for specific regions of the genome that contain genes that control milling quality in lentil. Therefore, an experiment was conducted to detect these regions in an advanced lentil recombinant inbred line (RIL) population using extensive phenotyping in multiple site-years and molecular markers spanning the genome.

6.2 Hypothesis

Major genes determining seed coat color and/or seed dimension affect milling quality characteristics and genetic markers associated with milling quality characteristics can be identified in the LR-18 RIL lentil population.

6.3 Materials and methods

6.3.1 Plant material

The LR-18 RIL population was developed from a cross between CDC Robin and 964a-46 (Tar'an et al., 2003). CDC Robin has a small and relatively plump seed, and red cotyledons with brown seed coat color (Vandenberg et al., 2002). Line 964a-46 has larger seed and relatively flat seed shape, yellow cotyledons, and a pale green seed coat. Seed from single F_7 plants were bulked to develop advanced generations (Sharpe et al., 2013). A total of 127 F_7-derived RILs of the LR-18 population were used for this study.

6.3.2 Field experiments and environment conditions

Field experiments were conducted near Saskatoon SK in 2013 and 2014 to produce seeds of the 127 RILs and both parental lines at two locations -Saskatchewan Pulse Growers farm (SPG, 52.05° N, 106.41° W) and University of Saskatchewan experimental farm in Sutherland (52.14° N, 106.61° W). Site details are described in Appendix 5.1. One m^2 microplots were sown at 130 seeds/m^2 in a randomized complete block design with three replicates. Individual micro-

plots were hand harvested when 95% of the lower canopy pods had turned yellow to brown in color. The local mean temperature and total monthly precipitation for each month of growing season at Saskatoon, Saskatchewan in both years were obtained from Environment Canada, 2016 (Table 5.1., *Source* http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID=47707).

6.3.3 Data collection

6.3.3.1 Phenotyping

6.3.3.1.1 Seed weight and seed dimension

Hand harvested plants were air-dried at 21^{o}C for 4 days, then threshed. The threshed seeds were cleaned, and seed weight was determined from samples of 250 randomly selected seeds. Seed diameter, thickness, and plumpness were estimated by passing a 100 g seed sample through round-hole and slotted-hole sieves described in chapter 3, and then calculating seed diameter, thickness and plumpness based on equations described in Fedoruk et al. (2013).

6.3.3.1.2 Measurement of milling quality traits

Milling quality traits were determined using a procedure described in chapter 3 (Subedi et al., 2017). Briefly, 30 g of uniformly dried seed samples were tempered overnight to 12.5% moisture and then the tempered seeds were dehulled using a grain testing mill (TM05, Satake Engineering Co., Hiroshima, Japan) fitted with a 36-mesh abrasive wheel rotating at 1100 rpm for 38 s. (Wang, 2005). After dehulling, milled seed samples were passed through a series of slotted and round sieves to separate into football and split fractions. All fractions were weighed and expressed as a proportion of the total original milled sample and determine percent dehulling efficiency, milling and football recovery using the formulae illustrated in chapter 3.

6.3.4 Phenotypic data analyses

The location and year of the field trials were treated as environments (site-years). Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., 2015). Homogeneity and normality were checked before subjecting data to ANOVA. Genotypes were considered fixed, whereas site year and replications nested within site-years were considered random effects. Genotype by site-year was significant (Table 6.1 and

6.2) so data were analysed separately. In single environment, genotypes were considered as fixed and replications were considered random. Parental data were removed from the dataset prior to estimating variance components of the RILs to omit confounding effects. The VAR COMP procedure was used to determine variance components where genotypes, locations, years, replicates, and their interaction were treated as random effects to determine the genetic variability.

Phenotypic variance (σ^2_p) was measured and then used to estimate genotype variance as reported by Falconer and Mackay (1996): $\sigma^2_p=\sigma^2_g + \sigma^2_{gy}/y + \sigma^2_{gl}/l + \sigma^2_{gly}/ly + \sigma^2_e/lyr$, where σ^2_g is the estimated genotypic variance, σ^2_{gy} is the genotype × year interaction variance; σ^2_{gl} is the genotype × location interaction variance and σ^2_{gyl} is the genotype × year × location interaction variance. σ^2e is the error variance. y indicates the number of tested years, l is the number of locations, r is the number replicates each location and year. Broad sense heritability (H^2) of the traits was estimated as: (σ^2_g / σ^2_p).

Correlations between milling quality parameters and seed morphological traits were determined using the means of the three replications in each site-year (PROC CORR procedure in SAS). Box and whisker plots were drawn using boxplot. command in R v.1.0.136 (R Core Team, 2014). Frequency distribution figures were drawn using the histogram command in Sigma Plot V2. (Sigma Plot, 2010).

6.3.5 Linkage map construction and QTL analysis

The genetic linkage map constructed by Fedoruk et al. (2013) was used in this study. The map was originally generated using a 1536-SNP Illumina Golden Gate assay as described by Sharpe et al. (2013). The final map was comprised of 534 SNP markers, 7 SSR markers and four morphological markers: cotyledon color (*Yc*), seed coat ground color (*Ggc* and *Tgc* are independent genes that determine seed coat background color in lentil (Vandenberg and Slinkard, 1990) and seed coat pattern (*Scp*). The map spanned 697 cM at an average marker distance of 1.2 cM between markers (Fedoruk et al., 2013). Genotyping information, including the contig sequences of the SNP markers, can be found through the KnowPulse database accessible at:
http://knowpulse.usask.ca/portal/search/markers?genus%5B%5D=Lens&species%5B%5D=All&type%5B%5D=Illumina+Golden+Gate+Assay&uniquename=&name=&project_id=

QTL analysis was performed using the QTL Cartographer V2.5 software package (Wang et al., 2012). Interval mapping was used to select markers with high logarithm of odds (LOD) values that were then used as co-factors for composite interval mapping using the CIM procedure. The CIM analysis employed forward and backward stepwise regression, a window size of 10 cM, and a step size of 2 cM. One thousand permutation tests were run to determine the threshold of minimum value of LOD at a 0.01 probability. The threshold values were used to declare the significant QTL for each trait individually in each site-year. Adjacent QTLs on the same chromosome for the same trait were considered as different if intervals did not overlap. MapChart (Voorrips, 2002) was used to draw the linkage map and QTL positions. The proportions of phenotypic variance (R^2) accounting for each trait were used to define the variance explained by each QTL. An increase in phenotypic value of the specified traits contributed by the alleles from CDC Robin and 964a-46 are shown in Table 6.6 with a positive and negative additive effect values, respectively.

6.4 Results

6.4.1 Seed morphological characteristics: seed weight, diameter, thickness, and plumpness

Genotypes differed significantly for 1000-seed weight, seed diameter, thickness, and plumpness ($P \leq 0.001$) (Table 6.1, Appendix 6.1). Both site-year and genotype by site-year (G×E) interaction had a significant impact on all seed characteristics (Table 6.1). The individual RILs also exhibited a variation for these traits as indicated by their mean values. The mean 1000-seed weight, seed diameter and thickness of the RIL population were significantly higher at the Sutherland 2013 site compared to other site years (Figure 6.1). In contrast, SPG 2014 had the greatest seed plumpness compared to other site years. On thousand seed weight, seed diameter, thickness, and seed plumpness for the RIL population ranged from 27.7- 70.7 g, 4.0-5.7 mm; 2.3-2.8 mm and 0.5-0.6, respectively (Figure 6.1; Appendix 6.2). The distributions of seed traits, such as 1000-seed weight, seed diameter and thickness were continuous and were skewed to the large-seeded parent 964a-46, whereas the seed plumpness values were skewed towards the small-seeded parent, CDC Robin (Figure 6.1).

Table 6.1. Analysis of variance results with P values and level of significance for 1000-seed weight, seed diameter, thickness, and plumpness of 127 recombinant inbred lines of the LR-18 lentil population and parents grown at two SK locations (Sutherland and Saskatchewan Pulse Growers farm sites) in 2013 and 2014.

Source of variation	Degrees of freedom	Seed physical characteristics			
		1000-seed weight	Diameter	Thickness	Plumpness
		P values			
Genotype (G)	128	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***
Site-year (E)	3	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***
G ×E	384	<.0001 ***	<.0001 ***	<.0001 ***	<.0001 ***
CV%		19.67	8.01	3.79	7.34

** and *** significant at $P<0.05$, significant at $P< 0.01$, and $P< 0.001$, respectively.

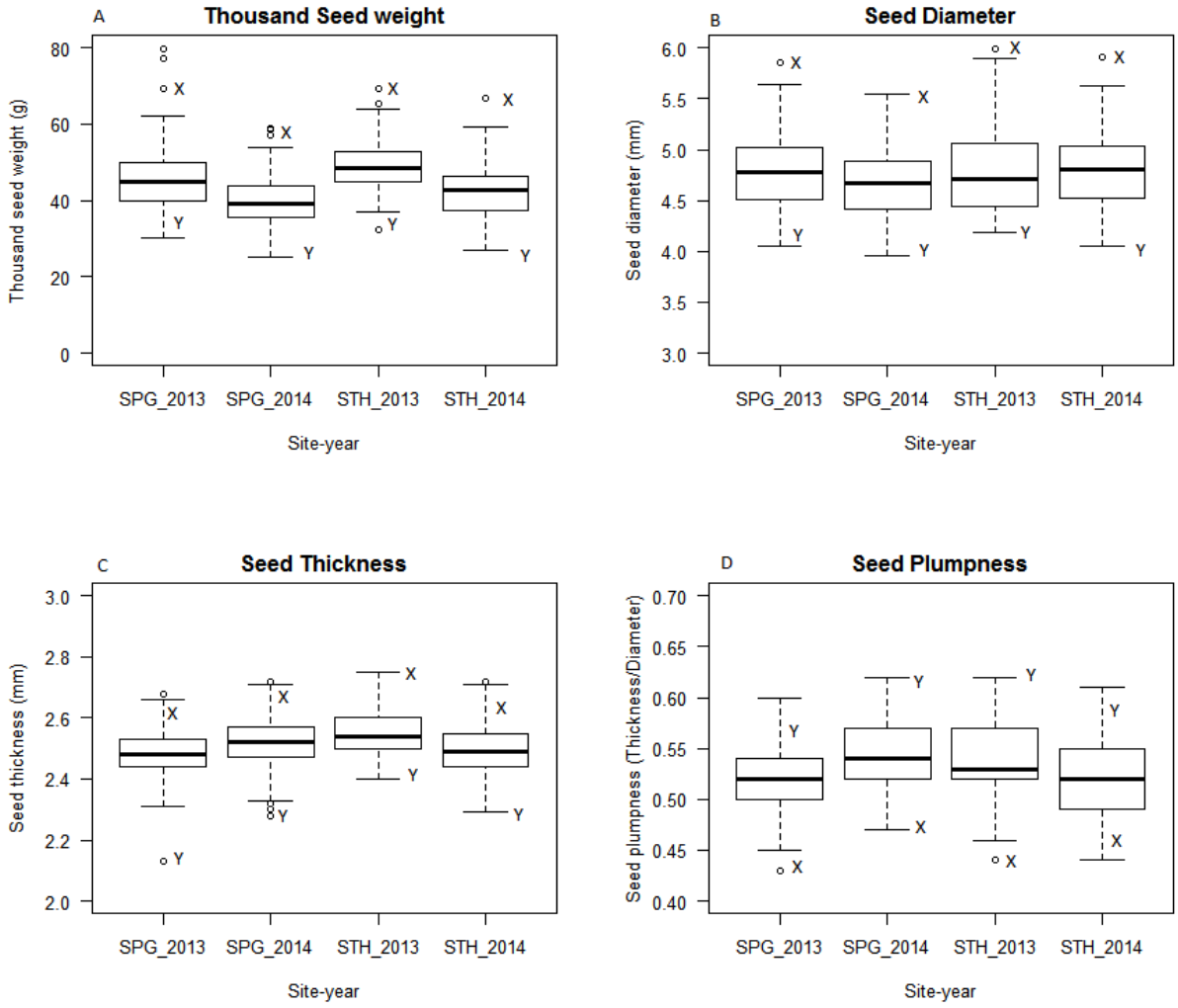

Figure 6.1. Box and whisker plots of the distribution of 1000-seed weight (Figure A), seed diameter (Figure B), seed thickness (Figure C) and seed plumpness (Figure D) in the 127 F_7 derived LR-18 lentil inbred population (CDC Robin × 964a-96) grown at two locations [Sutherland (STH) and Saskatchewan Pulse Growers (SPG) sites], SK, in 2013 and 2014. Letters X and Y denote mean value of 964a-46 and CDC Robin RIL parents, respectively.

6.4.2 Milling quality traits: dehulling efficiency, milling recovery and football recovery

The genotype, site-year, and genotype × site-year interactions effects were significant ($P \leq 0.001$) for the percentages of DE, MR, and FR (Table 6.2, Appendix 6.1). The mean DE, MR, and FR values were significantly greater in the 2013 growing season than in 2014 (Table 6.3; Figure 6.3). The DE, MR, and FR values of the RIL population over the two years ranged

from 80.0 - 99.1%, 66.5 - 87.5% and 7.8 - 59.3%, respectively (Table 6.2). Frequency distributions showed continuous variation for milling traits (Figures 1 and 2). DE and MR percentages were slightly skewed toward the large-seeded parent 964a-46, while FR percentage was skewed toward the small-seeded parent CDC Robin. The mean value of MR, and FR of some individual RILs were higher than both parental values indicating that some RILs exhibited transgressive segregation for milling quality traits (Figure 6.2).

Table 6.2. Analysis of variance result with P values and level of significance for dehulling efficiency, milling recovery and football recovery of lentil seeds for the 127-recombinant inbred line of LR-18 lentil population and their parents grown at two locations (Sutherland and Saskatchewan Pulse Growers test sites) SK in 2013 and 2014.

Source of variation	Degrees of freedom	Dehulling efficiency	Milling recovery	Football recovery
Genotype	128	<.0001 ***	<.0001 ***	<.0001 ***
Site-year	3	<.0001 ***	<.0001 ***	<.0001 ***
Genotype × site-year	384	<.0001 ***	<.0001 ***	<.0001 ***
CV (%)		6.80	8.74	5.66

*** significant at $P< 0.001$; CV (%) indicates coefficient of variation.

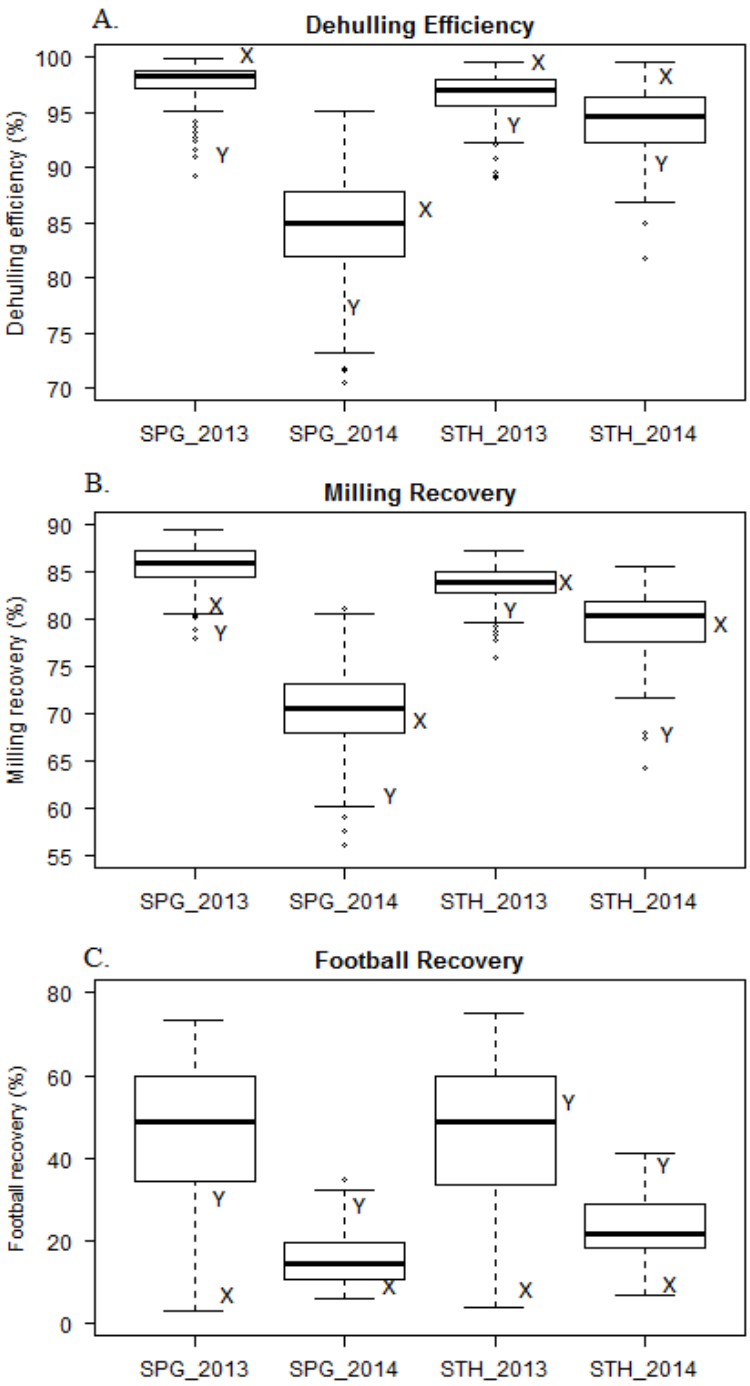

Figure 6.2. Box and whisker plots for the distribution of milling traits [dehulling efficiency (A). milling recovery (B), and football recovery (C)] in the 127 F_7 derived LR-18 lentil inbred population grown at two locations [Sutherland (STH) and Saskatchewan Pulse Growers (SPG) sites], Saskatchewan, in 2013 and 2014. Letters X and Y represent value of 964a-46 and CDC Robin, respectively.

Table 6.3. Mean, minimum, maximum, and standard deviation (SD) for percent dehulling efficiency (DE), percent milling recovery (MR) and percent football recovery (FR) for LR-18 lentil RIL population (CDC Robin × 964a-46) and means of parent cultivars grown over two site-years, in SK in 2013 and 2014.

Year	Site	Traits	Parental lines		RILs			
			CDC Robin	964a-46	mean	min	max	SD
2013	Sutherland	DE	94.3	99.6	96.5	86.6	99.7	2.3
		MR	80.3	84.2	83.8	75.6	88.8	2.4
		FR	53.9	3.8	45.7	7.4	77.5	17.0
	SPG	DE	92.7	98.9	97.8	88.0	100.0	1.8
		MR	79.0	80.4	85.8	77.4	91.0	2.2
		FR	16.9	3.0	47.5	13.7	75.2	14.5
2014	Sutherland	DE	90.2	98.8	94.0	78.0	99.8	3.8
		MR	67.5	77.4	79.6	59.8	86.7	4.0
		FR	38.5	6.9	23.3	5.9	48.4	8.4
	SPG	DE	74.9	86.8	84.6	67.4	96.9	5.9
		MR	60.1	67.3	70.4	53.2	83.3	5.5
		FR	20.9	8.2	15.2	4.2	35.9	6.3
Overall mean		DE	88.0	96.0	93.2	80.0	99.1	3.4
		MR	71.9	77.3	79.9	66.5	87.5	3.5
		FR	32.5	5.5	32.9	7.8	59.3	11.6

SD, standard deviation

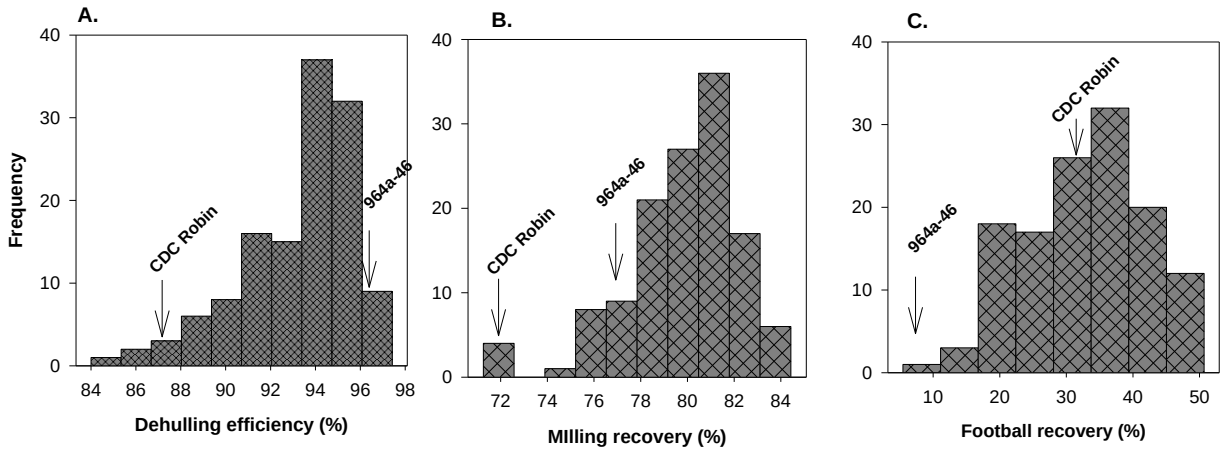

Figure 6.3. Frequency distribution of the average phenotypic values of percent dehulling efficiency (Figure A), milling recovery (Figure B), and football recovery (Figure C) over two years and two locations for the 127 F_7 LR-18 lentil recombinant inbred population from a cross of CDC Robin and 964a-46. Arrows indicate parental mean values.

6.4.3 Estimation of variance components and heritability

Broad sense heritability for seed related parameters (1000-seed weight, seed diameter, thickness, and plumpness) were high whereas heritability was moderate for DE and MR (0.68 and 0.52), and low for FR with a value of 0.41 (Table 6.4).

Table 6.4. Estimates of variance components and broad sense heritability for seed morphological and milling quality traits in a lentil recombinant inbred population (LR-18) grown at two locations (Sutherland and Saskatchewan Pulse Growers test sites), Saskatoon, SK over two years.

Variance component	1000-seed weight	Seed diameter	Seed thickness	Seed plumpness	Dehulling efficiency	Milling recovery	Football recovery
σ^2_g	32.31	0.1205	0.00418	0.00121	4.42	2.90	31.62
σ^2_{gl}	0.38	0.0004	0.00020	0.00001	0.07	0.26	0.19
σ^2_{gy}	0.35	0.0009	0.00029	0.00002	1.07	1.30	34.47
σ^2_{gly}	2.34	0.0010	0.00013	0.00001	0.44	0.51	8.80
σ^2_e	1.27	0.0002	0.00015	0.00001	0.53	0.56	1.65
σ^2_P	36.63	0.1230	0.00495	0.00125	6.54	5.52	76.74
H^2	0.88	0.98	0.85	0.96	0.68	0.52	0.41

σ^2_g, genotypic variance; σ^2_{gy}, genotype × year interaction variance; σ^2_{gl}, genotype × location interaction variance; σ^2_{gly}, genotype × location × year interaction variance; σ^2_e, error variance; σ^2_p, phenotypic variance; H^2, broad sense heritability.

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6.4.4 Correlations between seed characteristics and milling traits

Seed weight had a positive significant correlation with DE, and a negative correlation with FR (Table 6.5). Seed diameter was significantly positively correlated with DE in all site-years. Two of four site-years had significant correlations between seed diameter and FR. Seed thickness was positively correlated with DE but had a negative association with FR in two site-years. Seed plumpness was negatively correlated with DE but positively correlated with FR. Similarly, DE was positively correlated with MR but negatively correlated with FR in all site-years (Table 6.5)

Table 6.5. Pearson's correlation coefficients among thousand seed weight, seed dimensions and milling quality parameters of the LR-18 RIL population grown at Sutherland (STH) and Saskatchewan Pulse GGrowers site (SPG), SK in 2013 and 2014 (n=129).

Site-year	Traits	TSW	SD	TH	PLU	DE	MR	FR	Site-year
	TSW	-	0.96***	0.48***	-0.78***	0.52***	0.42***	-0.20*	STH 2014
		-	0.93***	0.61***	-0.74***	0.32***	0.02^{ns}	-0.15^{ns}	SPG 2014
STH 2013	SD	0.60***	-	0.34***	-0.90***	0.49***	-0.39***	-0.17^{ns}	STH 2014
SPG 2013		0.80***	-	0.51***	-0.88***	0.35***	0.06^{ns}	-0.03^{ns}	SPG 2014
STH 2013	TH	0.37***	0.34***	-	0.10^{ns}	0.18*	0.19*	-0.23**	STH 2014
SPG 2013		0.39***	0.42***	-	-0.05^{ns}	0.15^{ns}	0.01^{ns}	-0.29***	SPG 2014
STH 2013	PLU	-0.48***	-0.93***	0.01^{ns}	-	-0.46***	-0.37***	0.07^{ns}	STH 2014
SPG 2013		-0.68***	-0.90***	-0.01^{ns}	-	-0.33***	-0.07^{ns}	-0.12^{ns}	SPG 2014
STH 2013	DE	0.41***	0.62***	0.20*	-0.57***	-	0.88***	-0.18*	STH 2014
SPG 2013		0.36***	0.45***	0.28***	-0.39***	-	0.92***	-0.03^{ns}	SPG 2014
STH 2013	MR	0.00^{ns}	-0.01^{ns}	0.07^{ns}	0.03^{ns}	0.60***	-	0.06^{ns}	STH 2014
SPG 2013		-0.14^{ns}	-0.17^{ns}	0.09^{ns}	0.20*	0.65***	-	0.09^{ns}	SPG 2014
STH 2013	FR	-0.36***	-0.55***	-0.15^{ns}	0.51***	-0.37***	0.35***	-	-
SPG 2013		-0.43**	-0.46***	0.05^{ns}	0.52***	-0.04^{ns}	0.60***	-	-

Data above and below the diagonal are correlation coefficients at Sutherland and SPG in 2013 and 2014, respectively. *, ** and *** denote significant at *P<0.05, 0.01* and *0.001*, respectively, ns=non-significant; TSW= 1000-seed weight, SD=Seed diameter, TH=Seed thickness, PLU=Seed plumpness, DE=Dehulling efficiency, MR=Milling recovery and FR=football recovery

6.4.5 Quantitative trait locus (QTL) analysis for milling quality traits

Quantitative trait loci for DE were located on four (LG1, LG2, LG3, and LG7) of seven linkage groups (Table 6.6; Figure 6.4). Ten significant QTLs specific to each site-year were identified for DE. The most stable QTL was associated with SNP marker LcC4611p576 on LG7 and was present in all site-years. This QTL explained an average of 15.5% of the phenotypic variation for DE across site-years. Three additional QTLs on LG7 were associated with DE; one was detected in two site-years and two other at both sites in 2014 and explained 26.8% (combined for two-site years), 19.5% and 20.4% phenotypic variation for DE, respectively (Table 6.6). Additional QTLs, explaining an average of 10% phenotypic variation for DE, were also located on LG1, LG2, and LG3, but these QTLs were specific to each site years (Table 6.6, Figure 6.4). The QTL linked with marker locus LcC04945p251 on LG1 appeared for two sites in 2013 and explained 21.1% and 10.3% phenotypic variation for DE, respectively at each site. The additive effect results showed that alleles contributing to the QTL for higher DE came from 964a-46, the parent with green seed coats, larger diameter, and higher DE (Table 6.6).

There were multiple and significant QTLs for MR located on all LGs except LG5 and LG6 (Table 6.6; Figure 6.4). These QTLs were not stable and were specific to individual site-years (Table 6.6). The QTL for MR associated with SNP marker LcC03047p221 on LG2 and another with SNP marker LcC13668p162 on LG4 were present only for SPG 2013 and SPG 2014, but explained over 16% phenotypic variation in MR. Additional MR QTLs linked with multiple SNPs were coincident with QTL for DE on LG1, LG3 and LG7, and it explained an average of 10% of the phenotypic variation for MR (Table 6.7, Figure 6.4.). One QTL, only found in SPG 2014, was associated with same region as the *Yc* locus, and explained over 11% of the phenotypic variation for MR. The alleles contributing to increased MR came from the 964a-46 parent (Table 6.6).

There were ten significant QTLs for FR present on LG4, LG5, LG6, and LG7 (Table 6.6, Figure 6.4). The QTL that explained the most variation on FR was linked to multiple SNPs in a similar region on LG7. This was coincident with the QTL for DE on LG7 across three site-years. These QTL explained 20-35% of the phenotypic variation for FR across three site-years. Five additional FR QTL associated with SNP markers LcC00537p593 (LG4), LcC01056p334 (LG5),

LcC00149p593 (LG5), and LcC06259p289 (LG6) and SSR marker SSR156 (LG5), were identified across two site years, and each explained over 10% of the variation. The alleles contributing to increased FR came from the plump, brown-seeded parent, CDC Robin (Table 6.6)

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Table 6.6. Quantitative trait loci identified for milling quality characteristics dehulling efficiency (DE), milling recovery (MR) and football recovery (FR) in the LR-18 RIL population derived from a cross between CDC Robin and 964a-46 and evaluated over four site-years in SK.

Traits	Markers	Linkage group	Site-year	Position (cM)	Intervals	LOD ŦScore	% Exp	LOD[a] at $P<0.01$	Additive effects €
DE	LcC04945p251	1	STH 2013	43.81	38.2-47.8	10.20	21.1	3.45	-1.01
			SPG 2013	44.81	36.8-48.1	4.37	10.3	3.13	-0.56
	LcC07044p718	1	SPG 2014	79.11	70.7-81.5	4.97	10.5	3.61	-1.60
	LcC09885p369	1	STH 2014	78.11	75.5-81.7	4.09	6.6	3.72	-0.88
	LcC03047p221	2	STH 2014	20.91	17-26.5	4.83	7.9	3.72	-0.97
	LcC11339p472	3	STH 2014	133.81	128.1-135.8	5.88	9.5	3.72	-1.06
	LcC01331p146	3	STH 2014	142.01	141-147.7	3.77	6.3	3.72	-0.89
		3	SPG 2014	142.01	141-147.7	3.39	7.8	3.13	-1.37
	LcC17753P341	7	STH 2013	1.01	0-10.9	6.48	14.1	3.45	-0.79
		7	STH 2014	0.01	0-10.9	5.38	12.8	3.72	-1.76
	LcC04611p576	7	STH 2013	21.91	20.3-24.5	9.57	17.9	3.45	-0.88
			SPG 2013	21.91	20.3-24.7	3.74	8.6	3.13	-0.49
			STH 2014	21.91	20.3-24.6	9.84	18.0	3.72	-1.44
			SPG 2014	21.91	20.3-24.8	7.86	17.4	3.61	-2.07
	LcC00890p1387	7	STH 2014	27.01	26.5-28.6	10.90	19.5	3.72	-1.55
	LcC20366p221	7	SPG 2014	28.01	26.5-30.6	8.97	20.4	3.61	-2.26
MR	LcC10111p524	1	STH 2013	48.31	50.5-51.3	3.61	9.70	3.56	-0.66
	Yc	1	SPG 2014	33.01	30.1-33.8	4.64	11.33	3.50	-1.60
	LcC07044p718	1	SPG 2014	79.11	68.8-81.5	4.44	10.36	3.50	-1.49
	LcC03047p221	2	STH 2014	19.01	12.8-23.1	7.65	16.61	3.33	-1.51
	LcC11339p472	3	STH 2014	133.81	128-135.4	3.58	6.84	3.33	-0.95
	LcC01331p146	3	SPG 2014	142.01	141.4-147.6	4.32	11.29	3.50	-1.50
	LcC13668p162	4	SPG 2013	117.21	116.8-119.8	6.47	16.37	3.44	-0.87
	LcC05757p414	4	STH 2014	135.71	123.5-155.6	3.38	14.28	3.33	-1.38
	LcC17753P341	7	STH 2014	0.01	8.2-12.0	4.72	9.52	3.33	-1.12
	LcC04611p576	7	STH 2014	21.91	15.1-24.5	4.80	9.52	3.33	-1.12
	LcC000890p1387	7	STH 2014	27.01	24.5-30	4.27	8.54	3.33	-1.10
FR	LcC00537p593	4	SPG 2014	51.61	45.8-60.4	5.10	12.71	3.54	2.23

LcC01056p334	5	SPG 2014	54.71	44.6-60.2	4.69	12.19	3.54	2.37
SSR156	5	SPG 2013	86.51	78.4-89.6	4.09	10.59	3.54	4.68
LcC00149p593	5	SPG 2014	63.81	60.2-66.5	3.82	9.65	3.54	2.39
LcC06259p289	6	SPG 2014	42.81	21.0-50.1	4.29	12.60	3.54	2.17
	6	STH 2013	47.81	38.7-56.0	3.62	9.36	3.61	5.04
LcC22183p646	7	SPG 2013	21.01	18.7-21.9	8.13	19.01	3.54	6.20
LcC10086p103	7	STH 2013	22.81	26.3-30.0	10.24	27.19	3.61	8.69
LcC20366p221	7	STH 2013	28.01	21.0-33.7	13.70	34.74	3.61	9.92
LcC03203p214	7	SPG 2013	26.51	24.5-29.0	9.05	20.93	3.54	6.60
LcC10025p199	7	STH 2014	31.21	30.2-33.0	7.15	19.53	3.61	3.41
	7	SPG 2013	31.71	31.2-34.0	7.11	17.06	3.61	5.89

DE: dehulling efficiency (%); MR: milling recovery (%); FR: football recovery (%); STH University experimental plot in Sutherland, Saskatoon; SPG, Saskatchewan Pulse Growers land;

LOD[₸] logarithm of the odds;

LOD [a] Significant LOD threshold from 1000 permutation test at *P<0.01* level of significance.

% Exp., percent variability explained by the locus; [$]Additive. effects[€], additive effect attributed to the locus. Negative values represent alleles originating from 964a-46; positive values represent allele originating from CDC Robin

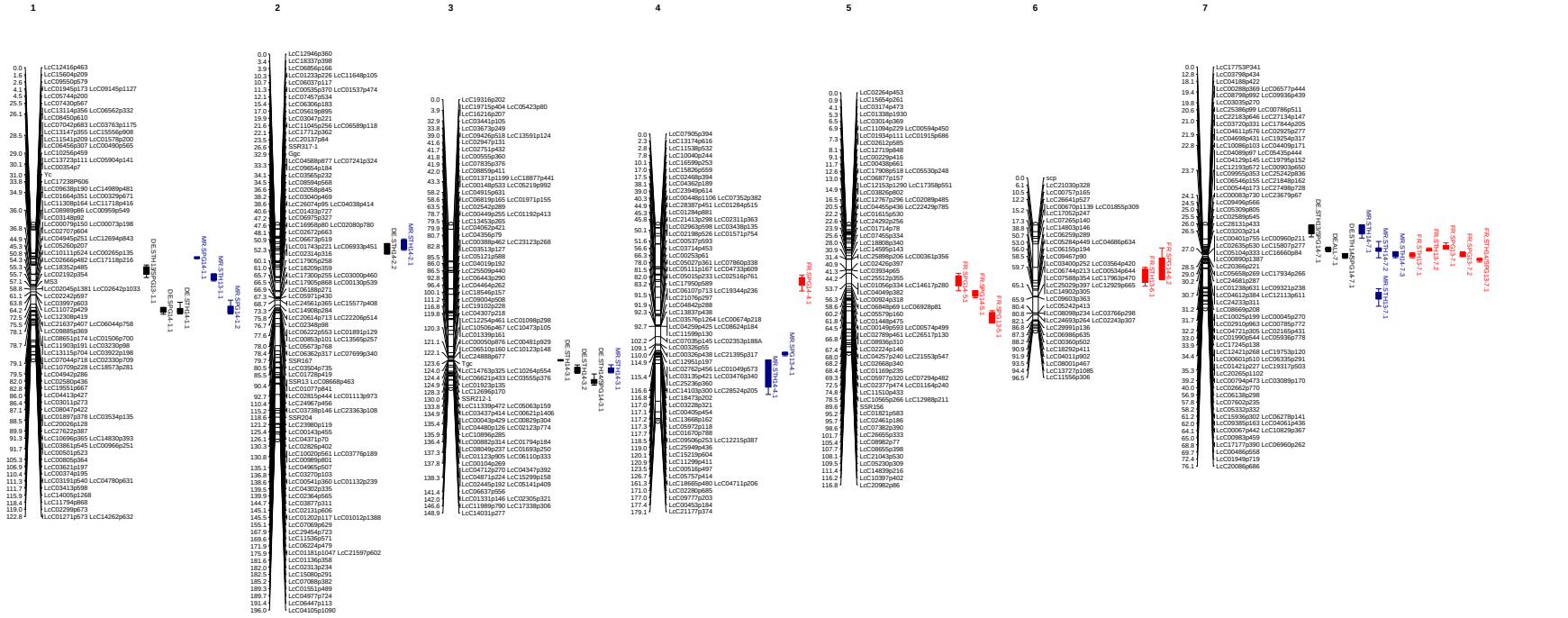

Figure: 6.4. Quantative trait loci for dehulling efficiency (%), milling recovery (%) and football recovery (%) from LR-18 lentil inbred population derived from a cross CDC Robin × 964a-46. DE=dehulling efficiency (%), MR=milling recovery (%) and FR= football recovery (%). QTL with all respective variables means QTL presented all site-years. QTLs followed with STH and SPG denote QTLs detected at experimental plot at Sutherland and Saskatchewan Pulse Grower sites in 2013 and 2014, respectively. Legend with black color denote DE QTL, Legend with blue color denote MR QTL and Legend with red color denote FR QTL.

6.5 Discussion

The phenotypic and genotypic diversity of lentil for milling quality traits is poorly understood yet forms the basis for profitability in the dehulling industry. I phenotyped milling quality traits (DE, MR, and FR) in an inbred lentil mapping population and then searched for the genomic regions controlling these traits. To my knowledge, this is the first report of genetic mapping for milling quality parameters in lentil. Evaluation of the RILs under diverse field conditions indicated considerable variation among the lines for milling traits. Genotyping was done with relatively high numbers of genetic markers as described by Fedoruk et al. (2013). Here we report the genomic regions governing DE, MR, and FR in cultivated lentil.

Results showed a significant effect of genotype, site-year, and genotype by site-year interaction on seed quality traits (1000-seed weight, seed diameter, thickness, and plumpness). Results of significant genotype and environment effects on seed weight and shape are similar with Cobos et al. (2007), Fedoruk et al. (2013) and Verma et al. (2015) who reported that seed weight and shape are complex attributes under polygenic influence and environmental effects. Our results confirmed that there is a considerable genotypic diversity among the RILs for milling quality traits (DE, MR, and FR). Site-year and genotype by site-year interaction had a significant impact on these traits, mainly caused by the difference in precipitation in the latter part of the growing seasons of the tested years (2013 and 2014). In 2014, the crop received higher moisture (157.8 mm in July-August) during the latter part of the growing season, whereas in 2013 the moisture level was optimal (Table 5.2).

Variability in seasonal moisture levels would have caused differences in DE, MR, and FR as high moisture prior to harvest significantly affects the dehulling process (Chapter 3).). Complex traits are strongly influenced by the interaction the genotype × environment interaction, which leads to phenotypic variation beyond that caused by the genotype (Purcell, 2002; Long et al., 2007). Our results agree with the results obtained in chickpea (*Cicer arietinum* L.) by Wood et al. (2008), who highlighted that milling efficiency in pulses is strongly influenced by growing environment and genotype, although the chickpea seed anatomy and its growing environments are different from those of lentils. Erskine et al. (1991b) noted that genotype had greater impact on DE than locations when they evaluated 23 diverse microsperma red lentil cultivars in three locations in Lebanon and Syria. Their estimation methodology for DE was quite different from ours, as they determined DE value by summing of split dehulled seed, whole dehulled seed, and

whole hulled seed. Most importantly, these experimental trials were conducted in Mediterranean environments where the lentil harvest coincides with high temperature, low humidity, and increasing day length. Lentil crops grown in northern temperate regions typically mature during a period when days are becoming shorter and night temperatures are cooler, resulting in high humidity in the canopy overnight.

The study results revealed significantly higher percentage of DE, MR, and FR in 2013 than in 2014 at both sites. Differences in DE, MR or FR may be caused by progressive variations in seed dimensions and seed weight within the indeterminate crop canopy. In the northern temperate region, flowering of lentil plants typically begins in late June / early July and continues until soil moisture becomes depleted in the root zone. Flowering and seed development occurs simultaneously and acropetally from multiple growing points on primary, secondary, and tertiary branches in lentil over a period which would have been extended in 2014. In addition, because of high late-season humidity, the lentil canopy at 2014 test sites were also severely affected by stemphylium blight, which is caused by *Stemphylium botryosum*. In lentil, late-season canopy infection of lentil by this disease can result in higher percentages of stained and wrinkled seeds (Caudillo-Ruiz, 2016) and such seeds are more difficult to dehull or split.

Differences in seed dimensions may have an impact on milling characteristics and could possibly be affected by the shape of the dehulling surface of the mill stone. The correlation analysis showed that seed diameter, seed thickness, and mean seed weight were positively associated with DE and MR. Seed plumpness was negatively correlated with DE, but positively correlated with FR. These relationships indicate that seed shape characteristics have a direct impact on DE and FR in lentil. Previous research has also highlighted the importance of seed shape for higher milling efficiency, for example, plumper lentil seeds exhibited greater DE than thinner ones (Erskine, 1991b; Wang, 2008; Shanin et al., 2012) but, thin seeds with sharp edges broke easily during the dehulling process. Wood et al. (2012) found that in near isogenic lines of desi chickpea, genetic modification of seed shape had profound effects on milling quality.

The level of genotypic and environmental variation for each milling trait was also reflected in the heritability estimates, as our results revealed that lentil seed shape and weight had high heritability, while milling traits had moderate to low heritability for DE, MR, and FR. The contribution of genetic factors to total variability for DE and MR were higher than location, year, and their interaction (Table 6.4). The contribution of environmental variability was higher for

FR, possibly, because FR was positively correlated with seed plumpness and thickness rather than seed diameter and these are sensitive to the environmental conditions during seed filling. In legume seed development, the expansion phase is the most sensitive phase to environmental variability (Le et al., 2007). This has also been demonstrated in studies of pea (*Pisum sativum* L., Domoney et al., 2006). Erskine (1991b) reported medium to high heritability for DE in lentil in a Mediterranean climate where seed set is during a hot, dry period so not typically affected by wet weather as experienced in temperate regions.

I identified several QTLs with additive effects for all milling traits in lentil. This confirms that these traits primarily have quantitative inheritance with polygenic control and small individual effects. Most significant QTLs detected for DE and MR were clustered together in the same regions of LG1 and LG7; however for FR, the most significant and stable QTLs were clustered only in LG7 in the same region where QTL for seed shape, particularly seed diameter, seed weight and seed plumpness, were detected by Fedoruk et al., (2013) (Appendix Figure 6A.1.1 and 6A.1.1 2). This suggests that seed shape and milling traits, particularly DE and FR, are inherited together through either linkage or pleiotropy, making it difficult to select each trait separately, since they are dependent. No consistent QTL was detected for MR, probably because of the significant genotype × environment interaction. We observed one QTL for DE and MR only in STH 2014 on LG2 (Figure 6.4) but on same genomic region on LG2 Fedoruk et al. (2013) found a more stable QTLs that explained seed shape for all site-years. Stable QTLs for seed diameter and seed weight also appeared in the present study (Appendix Figure 6A1.1 2).). This might have been caused by significant but moderate correlation between seed diameter or seed thickness and DE or MR (Table 6.5).

Seed coat chemistry may also influence DE or MR. In some site-years we observed QTL for DE and MR near the gray ground color gene (*Ggc)* on LG2 and near the tan ground color gene (*Tgc*) on LG3. This could be caused by variation of specific polyphenolic compounds present in seed coats of lentil. These compounds are also influenced by environment (Mirali et al., 2017). Seed coats of genotypes with the homozygous recessive *tgc* allele (green = *ggc ggc tgc tgc;* gray = *Ggc Ggc tgc tgc*) both had higher amounts of flavan-3-ols, proanthocyanidins, and some flavonols in comparison to the brown or tan seed coats which express the dominant *Tgc* allele (Mirali et al., 2017). The absence of expression of *Tgc* may make seed coats more transparent and therefore, possibly easier to dehull as indicated in chapter 5. There is also

evidence that the polyphenol profile changes over time, for example, storage of seeds with green seed coats, results in biochemical changes (Mirali et al., 2016b) that make the seed coats more brittle.

Other possibilities that may cause the differences among pulse crops for DE are the influences of the biochemical composition of starchy compounds or proteins (Vishwakarma et al., 2017; Wood et al., 2017). Wang (2008) reported that higher amounts of protein on the adjoining surface of seed coat and cotyledon significantly affected the DE of red lentil. This type of analysis would require a different set of experimental procedures that focused on the possible protein and starch interactions, which may also be influenced by seed coat color chemistry.

Molecular markers linked with milling traits in this study could be useful for marker assisted selection (MAS) in lentil breeding programs. Phenotyping for these traits is a time-consuming, laborious, and expensive endeavour and the heritability is moderate to low. As a result, breeding material cannot be selected for milling quality traits in early generations. In contrast, MAS would allow breeders to select for at least some of the important regions of the genome in early generations, thus increasing the likelihood of developing superior varieties. The markers for milling traits were detected only in bi-parental population with four site-years of phenotyping. To validate these results and possibly identify additional regions of importance, evaluation of additional populations and germplasm would be beneficial.

6.6 Conclusions

I identified multiple QTLs related to milling quality in a bi-parental lentil population. The markers associated with QTLs for milling traits could be a valuable resource for eventual marker assisted selection and could be exploited for improving the processing quality of lentil in breeding programs. The results suggest that by using molecular markers, lentil breeding programs could consider focusing on plump seeds, greater diameter and higher seed weight in cases where this does not interfere with market preference to increase milling efficiency and profitability.

CHAPTER 7. GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE RESEARCH

7.1 Discussion of results

Although Canada is the world's largest producer and exporter of red lentil, from the perspective of milling quality, Canadian red lentil considered to be sub-standard, often resulting in discounted price and trade disputes. The recurring problem of the quality of Canadian red lentil is fundamentally related to climate. Lentil is produced in many climatic zones worldwide. The sub-tropical savannah of South Asia, and the regions with Mediterranean climates (Australia, Mediterranean basin, and west Asia) typically experience increasing daylength, rising temperatures, and reduced precipitation as harvest approaches (Vandenberg, personal communication, 2017). Lentil crops produced under these conditions have low grain moisture at harvest and as a result, seed coats are removed relatively easily during abrasive decortication, resulting in high milling efficiency. The Canadian climate during the August-September harvest period is characterized by decreasing daylength, declining temperature and often significant precipitation, which can affect seed coat quality and characteristics, rate of seed maturation, and influence the development of fungal organisms in the canopy. Especially in years with above average precipitation in August and September, the quality of Canadian red lentils is reduced, leading to increased milling losses, especially in overseas mills that are designed for milling lentils that are consistently harvested and stored dry (Vandenberg, personal communication, 2017).

This thesis represents the first comprehensive investigation of the genetic and agronomic factors that could influence future strategies for minimizing milling losses and improving milling efficiency of lentil. A series of experiments was conducted with two main areas of emphasis. The first was a determination of how milling efficiency is affected by unique Canadian lentil crop management practices, including application of harvest aid herbicides and mid-season fungicides. The second area was an exploration of how the genetics of seed characteristics, including seed coat background colour, seed shape and weight affect milling quality characteristics in lentil.

The first study determined whether newly released harvest aid herbicides applied alone, or tank mixed with glyphosate affect post-harvest seed quality and milling quality characteristics.

The results (Chapter 3) showed that, with the exception of diquat application tank-mixed with glyphosate, any application of glyphosate alone or in tank mixes with other contact herbicides significantly reduced seed germination and vigor without affecting seed dimensions. Results were inconsistent from year to year, likely due to environmental differences between site-years. For example, in 2012 both sites received a substantial amount of rain prior to harvest (Table 3.1). Higher seed moisture content (>35%) prior to harvest allows more glyphosate (>2ppm) to translocate to maturing seeds and embryo, the major sink during maturation process (Zhang et al., 2016). Additional glyphosate residue in seed might have reduced seed germination and vigor, as the results indicated that residual glyphosate was highly negatively correlated with seed germination and vigor (Table 3.6). Higher amounts of glyphosate inhibit synthesis of aromatic amino-acids, which are precursors of IAA that affects seed germination and vigor (Taiz and Zeiger, 1998). Similarly, higher amounts of glyphosate residue were negatively associated with DE and MR, an indication that more glyphosate in lentil seeds had a negative impact on the milling process by increasing seed coat adhesion to the cotyledon surface. Application of diquat alone or in combination with glyphosate consistently increased both DE and MR due to lower amounts of glyphosate translocation to seeds. Diquat is a contact herbicide and has very quick action in the dry down process. This is a novel finding as no previous research reports how desiccation influences milling quality in lentil, nor has there been an examination of the mechanism underlying the synergistic action of glyphosate with contact harvest aids on post-harvest seed quality in lentil. The results suggest that if crop desiccation is required, the application of diquat or a combination of diquat with glyphosate may be the best option for lentil growers if they wish to obtain premiums from improved milling quality. Currently, Canadian exports of red lentil are often discounted overseas because of inconsistent milling quality, especially in the years when late maturity and greater precipitation delay the harvest.

Results from the second study of the effect of fungicides (Chapter 4) on post-harvest seed quality and milling quality (DE, MR, and FR) showed that a single application of either strobilurin or chlorothalonil fungicides did not prolong plant maturity. Mid-season fungicide applications generally had no effect on seed yield or seed weight and had very little direct impact on milling efficiency in lentil. It is likely that a single application of these fungicides was insufficient to increase the “greening effect” on lentil plants in this study, although this effect has

been reported for strobilurin application on some crops in the Canadian Prairies. An important outcome of this study, however, was the observation that at one site-year where environmental conditions were conducive to stemphylium blight infection (SPG 2014), reductions of 25-34% for DE, 23-33% for MR, and > 16 % for FR were observed in comparison to the milling efficiencies observed at the uninfected sites. Stemphylium blight is widespread but is typically unrecognized as a factor in lentil production in comparison to the mid-season diseases anthracnose and the soil-borne diseases like Aphanomyces root rot. Late sowing, coupled with high humidity and temperature in the second half of the season at site-year SPG 2014, promoted more disease proliferation during the seed development and pod filling stages. Consequently, this may affect the biochemical and physical characteristics of developing seeds and seed coats, resulting in much greater milling losses. This result suggests that it may be profitable to implement appropriate crop management and genetic strategies that may control stemphylium blight and maintain post-harvest seed quality and milling quality. These strategies could include developing systems for recommending the timing, frequency, and doses of strobilurins and other potential fungicides to minimize the effect and maximize the control of stemphylium blight. Similarly, appropriate crop rotations could be designed and implemented with the goal of reducing disease infection, maintaining plant density to minimize the micro climatic conditions that sustain conidial proliferation for stemphylium blight in the lentil canopy. Efforts for breeding improved resistance to stemphylium blight could be increased as means of improving milling efficiency.

Results of the genetic study on the effect of seed coat color of red lentil on milling quality characteristics (Chapter 5) revealed that milling parameters such as DE, MR, and FR varied among the four basic background seed coat color (green, gray, tan, and brown) phenotypes. Seed coats of lentil genotypes expressing the homozygous recessive *tgc* allele (green and gray seed coat colours) had significantly increased DE and MR values compared to brown seed coat genotypes, whereas FR values were greater for brown seed coat genotypes. This indicated that increasing milling efficiency, particularly dehulling efficiency or milling recovery, was influenced by expression of the *tgc* seed coat colour allele. This may possibly be due to differential amounts or biophysical properties of polyphenolic compounds present in specific seed coat types (Mirali et al., 2017; Mirali et al., 2016a).

The results of genetic mapping of milling characters (Chapter 6) showed that genotype and genotype by site-years (environment) interactions were significant for seed weight, seed shape (diameter, thickness, and plumpness) and all milling quality characteristics (DE, MR, and FR) in inbred lentil population. Heritability estimates for seed shape were high but milling traits had moderate to low heritability. This indicates that selection for milling quality traits is not suitable in early generations. However, MAS would allow breeders to select for at least some important genomic regions in earlier generations. The correlation analysis indicated that seed diameter and seed weight were positively correlated with DE and MR, while, seed plumpness was only positively associated with FR. Seed diameter and plumpness were negatively associated with each other in lentil (Fedoruk et al., 2013), and therefore, DE and FR values were also different among genotypes based on their seed shape. Previous research also reported that seed diameter and plumpness were highly and significantly associated with milling efficiency in lentil produced in different environments (Erskine et al., 1991b; Shahin et al., 2012). We observed multiple significant QTLs for milling traits (DE, MR, and FR). The most stable QTLs for DE and FR were located only in LG7 (Table 6.7), a pattern that matched the most stable QTLs for seed diameter and plumpness in a previous study (Fedoruk et al., 2013). Significant but inconsistent QTL for MR were likely due to environmental differences between site-years that may cause overriding effects on milling characteristics, for example, the effects of stemphylium blight on milling quality noted in Chapter 4.

Multiple QTLs with continuous distribution of all milling quality traits confirmed that these traits were quantitatively inherited with polygenic control with small effects. Transgressive phenotypes for DE, MR, and FR, were observed among the RILs when compared to parental genotypes leading to RILs with higher DE, MR, and FR values than their parents. The observed transgressive segregation for milling traits could be due to the additive effects contributed by the parental genotypes to the segregated populations. These RILs line could be exploited in crosses in the breeding program. The markers identified for milling traits could be useful for future breeding efforts. Numerous potential agronomic and genetic strategies are available to improve the quality and the consistency of quality of Canadian red lentils. Red lentil cultivars represent 75% of total lentil cultivation in the world and 80% of Canadian lentil production areas. Most of the red lentils will at some point be dehulled. The seed coat represents approximately 10% of the weight of dry seeds; the seed coat fraction of the total production represents several hundred

thousand tonnes of biological materials that contain phenolic compounds, dietary fiber, and other antioxidant compounds. A deeper understanding of the relationships among phenolic compounds and their potential antioxidant activity, combined with deeper knowledge of how to maximize milling efficiency, would provide a baseline for determining the potential total economic value of red lentil. The results of this study will be of value to the entire value-added chain of the Canadian lentil industry, from breeding, to production, to milling and to satisfaction of consumer demand.

7.2 Conclusions

Diquat treatment alone or tank mixed with glyphosate consistently improved dehulling efficiency (DE) and milling recovery (MR). Glyphosate, applied alone or tank mixed with other contact herbicides, except diquat, reduced seed germination and vigor. Glyphosate residue in lentil seeds was negatively correlated with DE and MR, but positively associated with FR, suggesting that glyphosate residue in lentil seeds may simultaneously result in a stronger binding between the inner surfaces of the cotyledons, while hindering seed coat removal from the external surfaces of the cotyledons.

A single application of strobilurin or chlorothalonil fungicides did not influence seed yield, seed weight or any milling quality characteristics. Development of stemphylium blight (SB) in the canopy of the lentil crop, particularly in August, greatly reduced all milling parameters. This suggests that the timing and frequency of fungicide application is critical for effective control of SB. The adoption of appropriate crop rotations and the use of SB resistant cultivars could also mitigate the effects of SB on milling quality.

Lentil seeds with green and gray seed coat color, generally have higher DE and MR, whereas those with brown colored seed coat color have higher values for FR. To achieve higher total milling recovery yield, millers could choose green or gray seed coat, but for higher football recovery, brown seed coat could be better options.

The growing environment has a major impact on milling quality traits. Multiple QTLs with continuous distribution for DE, MR, and FR confirmed these traits are polygenic with small individual effects. SNP markers identified in this study could be used to develop strategies for genetic improvement of milling efficiency in future breeding efforts for lentil.

7.3 Future research

Each study reported in this thesis provides a basis for further research that could lead to the goal of improving the milling characteristics of the Canadian red lentil crop. Chapter 3 presents potential harvest aid herbicides applied in lentil prior to harvest as recommended. We did not determine appropriate application timing for the desiccants. Future studies could focus on this and its potential impact on post-harvest seed quality and milling efficiency. Only one lentil cultivar was included in the desiccation study. There could be genotype × treatment effects based on seed coat characteristics. In addition, both study locations (Saskatoon and Scott) were in Saskatchewan, and the results of current study showed crop desiccation effects for milling quality, seed germination and seed vigor varied among site-years due to different environmental conditions. Further studies with several additional site-years data could provide more accurate information on the variability in efficiency of desiccants on biological and milling quality characteristics. Since our results indicated that glyphosate residues, a concern of Canadian exporters, reduced both DE and MR, further research should be conducted to evaluate if lower rates of glyphosate (450 g a.i. ha^{-1}) in mixtures with other contact herbicides can simultaneously provide better milling quality, adequate crop desiccation and acceptable levels of glyphosate residues in seeds.

In Chapter 4, we applied two strobilurin systemic fungicides and chlorothalonil contact fungicide once prior to mid-flowering. These fungicides were not effective for control of a polycyclic foliar disease like stemphylium blight. Further in-depth research could be pursued on how milling efficiency is affected by fungicide effectiveness, dosage, application timing and frequency, as well as the economic basis for effective control of late season stemphylium blight. Since stemphylium blight seems to be a major potential cause of high milling losses (up to 50%), breeding programs could accelerate the development of stemphylium resistant cultivars through screening interspecies crosses and developing a complementary strategy of finding useful markers to assist with breeding for resistance. To provide reliable results, further research on biological and economic quantification of the loss in seed quality and milling efficiency attributable to stemphylium disease infestation should be continued. A limited number of genotypes of the four basic seed coat colors were compared for milling quality parameters, and significant differences in milling efficiency were found among the colours genotypes. It would be possible to further expand these comparisons to include the complete range of phenotypic and

genotypic diversity of seed coat color genotypes, including those with patterned and black seed coats. The spectrum of polyphenolic compounds in lentil seed coats can influence seed coat color, and since the color of the genotypes affected milling efficiency, future research to determine the relationship between specific polyphenolic compounds with milling efficiency may be of interest. With the availability of high throughput genome analysis for lentil, association mapping for lentil milling traits can be improved by using a wider range of diverse germplasm. The higher level of recombination contributing to lower level of linkage disequilibrium could result in finer mapping using markers.

Finally, determining the effect of storage on red lentil milling efficiency could provide valuable information on milling quality. For example, if stemphylium blight reduces milling efficiency, it is conceivable that long term storage reduces its negative effects. The methodology which we used for dehulling is laborious, expensive and time consuming, and future research on improving the efficiency of dehulling machines or laboratory methodology for dehulling procedures could improve phenotyping and selection methods.

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APPENDICES

Appendix 4.1. Summary of cultural practices conducted at Preston Ave. research field and SPG site, Saskatoon, Saskatchewan in 2013 and 2014.

Activities	Preston site		SPG site	
	2013	2014	2013	2014
Seeding	May 28	May 12	May 29	June 2
Pre-emergence herbicides Application	May 10	May 06	1st week of May	1st week of May
Post seeding herbicides	June 7	May 23	June 7	June 7
Fungicides (Treatment) application	July 17	July 15	July 17	July 27
Desiccation	August 25	August 13,	September 5	September 19
Hand Weeding	Once a week	Four weeding in whole cycle	N/A	N/A
Bird net set up	August 8	August 6	N/A	N/A
Harvest	September 4	August 19	September 13	October 9
		Chemical used		
Pre-emergence herbicides	Edge® a.i. Ethalfluralin 5% (17.3 kg $ha^{-1)}$	Edge® a.i. Ethalfluralin 5% 17.3 kg $ha^{-1)}$	Edge® a.i. Ethalfluralin 5% (17.3 kg $ha^{-1)}$	Glyphosate (17.3 kg $ha^{-1)}$
Post-emergence herbicides	Roundup® a.i. glyphosate 48.8% (2.5 L ha^{-1}) + AimEC® a.i. Carfentrazoneethyl (75 mL ha^{-1})	Roundup® a.i. glyphosate 48.8% (2.5 L ha^{-1}) + AimEC® a.i. Carfentrazoneethyl (75 mL ha^{-1})	Axial® (Pinoxaden 0.24 L $Acre^{-1}$ and Pursuit (Imazethapyr 600 g ha^{-1}	Pursuit (Imazamethapyr 600 g ha^{-1})
Desiccation	Reglone® a.i. Diquat ion 240 g L^{-1} ((2.5L ha^{-1})	Reglone® a.i. Diquat ion 240 g L^{-1} (2.5L ha^{-1})	Reglone® a.i. Diquat ion 240 g L^{-1} (2.5L ha^{-1})	Reglone® a.i. Diquat ion 240 g L^{-1} (2.5 L ha^{-1})

Appendix 4.2. Effect of fungicides on days to maturity and 1000-seed weight (g) (mean values across site-years) assessed at Preston and SPG site, SK in 2013 and 2014.

Treatment	Days to maturity				1000-seed weight (g)	
	CDC Maxim	CDC Dazil	Mean	CDC Maxim	CDC Dazil	Mean
Control	93.1	92.6	92.8	37.6	33.5	35.5
Pyraclostrobin	94.1	93.3	93.7	37.7	33.0	35.3
Azoxystrobin	95.0	93.0	94.0	37.4	33.8	35.6
Chlorothalonil	92.9	91.5	92.2	37.7	33.9	35.8
Mean	92.6	93.8		37.6[a]	33.0[b]	
LSD (0.05)				2.88		
CV (%)	6.02			7.0		

LSD denotes least significance difference. Similar letters in rows represent no significant differences between cultivars at LSD $_{0.05.}$

Appendix 5.1. Site description and cultural practices employed in the experimental trials at Sutherland and SPG, in 2013 and 2014.

Soil properties/cultural practices	Sutherland	SPG
Soil pH	6.0 - 6.6	7.4 -7.6
Organic matter content (%)	3.2 - 3.6	4.0 - 4.3
Soil type	Clay loam	Loam
Soil zone	Dark brown	Dark brown
Sowing date	May 13, 2013 and May 14, 2014	May 28, 2013 and June 3, 2014
First bloom	2nd to 3rd week of June	3nd to 4rd week of June
Harvesting time	1st week of September	2nd week of September

SPG; Saskatchewan Pulse Growers experimental field

Appendix 6.1. Analysis of variance result with F values and level of significance for seed weight, seed diameter, seed thickness, seed plumpness, dehulling efficiency (DE), milling recovery (MR) and football recovery (FR) of lentil seeds for the 127-recombinant inbred line of LR-18 lentil population and their parents evaluated at each site year in STH and SPG sites, SK in 2013 to 2014.

				Seed characteristics				Milling quality characteristics		
Site	Year	Source of variation	Df	1000-seed weight	Diameter	Thickness	Plumpness	DE	MR	FR
				F-values						
STH	2013	Genotype	128	2.12***	107.42***	10.78***	31.17***	7.47***	5.04***	22.92***
STH	2014	Genotype	128	86.40***	188.41***	20.94***	80.56***	7.66***	9.70***	9.08***
SPG	2013	Genotype	128	92.58***	175.96***	17.92***	61.65***	12.61***	15.11***	40.26***
SPG	2014	Genotype	128	38.42***	69.92***	9.10***	31.68***	3.69***	3.11***	9.55***

*** indicates significant at $P<0.001$

Appendix 6.2. Mean, standard deviation (sd), 1000-seed weight (g), diameter (mm), thickness (mm), and plumpness in the 127-lentil recombinant inbred lines of LR-18 (CDC Robin ×964a-46) population and the means for parent cultivars grown over two site-years, SK.

Year	Site	Traits	Parent means		LR-18 Population			
			CDC Robin	964a-46	mean	max	min	sd
2013	Sutherland	TSW	32.3	69.3	49.3	78.7	30.4	8.3
		SD	4.3	6.0	4.8	5.9	4.1	0.4
		ST	2.4	2.6	2.6	2.8	2.3	0.1
		SP	0.6	0.4	0.5	0.6	0.5	0.1
2013	SPG	TSW	34.7	69.2	45.1	82.0	29.0	8.4
		SD	4.0	5.9	4.8	5.7	4.1	0.3
		ST	2.1	2.6	2.5	2.7	2.3	0.1
		SP	0.5	0.4	0.5	0.6	0.4	0.0
2014	Sutherland	TSW	27.0	66.8	42.3	59.5	26.8	6.8
		SD	4.1	5.9	4.8	5.7	4.0	0.4
		ST	2.29	2.60	2.49	2.77	2.28	0.1
		SP	0.6	0.5	0.5	0.6	0.5	0.0
2014	SPG	TSW	25.1	58.5	39.7	62.6	24.5	6.91
		SD	4.0	5.5	4.6	5.5	3.9	0.4
		ST	2.3	2.6	2.5	2.8	2.3	0.1
		SP	0.6	0.5	0.5	0.6	0.5	0.0
Mean		TSW	29.8	66.0	44.1	70.7	27.7	7.6
		SD	4.1	5.8	4.8	5.7	4.0	0.4
		ST	2.3	2.6	2.5	2.8	2.3	0.1
		SP	0.6	0.5	0.5	0.6	0.5	0.1

TSW = 1000- seed weight, SD = Seed diameter, ST = Seed thickness, SP = Seed plumpness, sd = Standard deviation.

Appendix 6 A1 *Quantitative trait loci analysis for seed morphological characteristics*

Quantitative trait loci for seed characteristics were located at six of the seven LGs (Table 6 A1.1; Figure 6 A1.1). Multiple QTLs for 1000-seed weight were identified on LG1, LG2 and LG7. The most important and stable QTL for 1000-seed weight was located around the seed cotyledon color locus *Yc* on LG1 and explained 15.8 – 39.9% of phenotypic variation for seed weight at all site-years (Table 6 A1.1). The closest SNP markers to *Yc* were LcC13723p111 and LcC00354p7. The other major QTL for 1000-seed weight was found and linked near the SNP

markers LcC000890p1387 on LG 7. This QTL explained over 59% of phenotypic variation for 1000-seed weight in three site-years. The QTLs linked with SNP markers LcC03035p270 and LcC25386p99 on LG7 specific to individual site-year explained 12.8%, 18.2% and 16.6 % of phenotypic variation in 2013 and 2014 at SPG and in 2014 at Sutherland, respectively (Table 6 A1.1).

Five different QTLs were identified for seed diameter; two of them presented in all site-years. The most important QTL for seed diameter was shared with 1000-seed weight and located around cotyledon color locus (*Yc*) on LG1 and it explained over 33 - 46% of variation at all site-years. The other major QTLs for diameter were linked near the SNP markers LcC000890p1387 and LcC25386p99 on LG7. These three QTLs combined explained at least 60% of the phenotypic variation on seed diameter in all site-years. Additionally, minor QTLs located in LG2 near SNP marker LcC02348p98 in 2014 at STH and in 2014 at SPG explained 10.82% and 6.87% phenotypic variation for seed diameter, respectively (Table 6 A1.1).

Seed thickness QTLs were identified on all LGs, except LG2 and LG5 (Table 6 A1.1; Figure 5). There were multiple QTLs specific to each site-year. The most stable and important QTLs across three site-years was detected on LG1 and LG4. The QTL shared with seed diameter and 1000-seed weight presented around cotyledon color *Yc* locus on LG1 and explained around 8% phenotypic variation for seed thickness in three site-years. Another QTL linked with seed thickness on LG4 best associated with SNP markers LcC00977p203 and explained 11.19%, 8.45% and 12.60% phenotypic variation for seed thickness at STH 2014 and at SPG 2013 and 2014, respectively (Table 6 A1.1). Seed plumpness QTLs were present on LG1, LG2, LG4, and LG7 (Table 6 A1.1; Figure Table 6 A1.1). The consistent and important QTLs for seed plumpness were shared with same genomic region of seed diameter and seed weight on LG1 and LG7. The QTL for seed plumpness that shared with other seed shape traits was detected around cotyledon color *Yc* locus and SNP marker LcC09638p190 on LG1and linked with other SNP marker LcC03203p214 on LG7 and explained majority of phenotypic variation for seed plumpness with an average 30-60% in all site-years. The additive effects revealed that alleles contributing in increasing 1000-seed weight, seed diameter and thickness came from yellow cotyledon parent, 964a-46, whereas seed plumpness came from alleles of CDC Robin (Table 6A1.

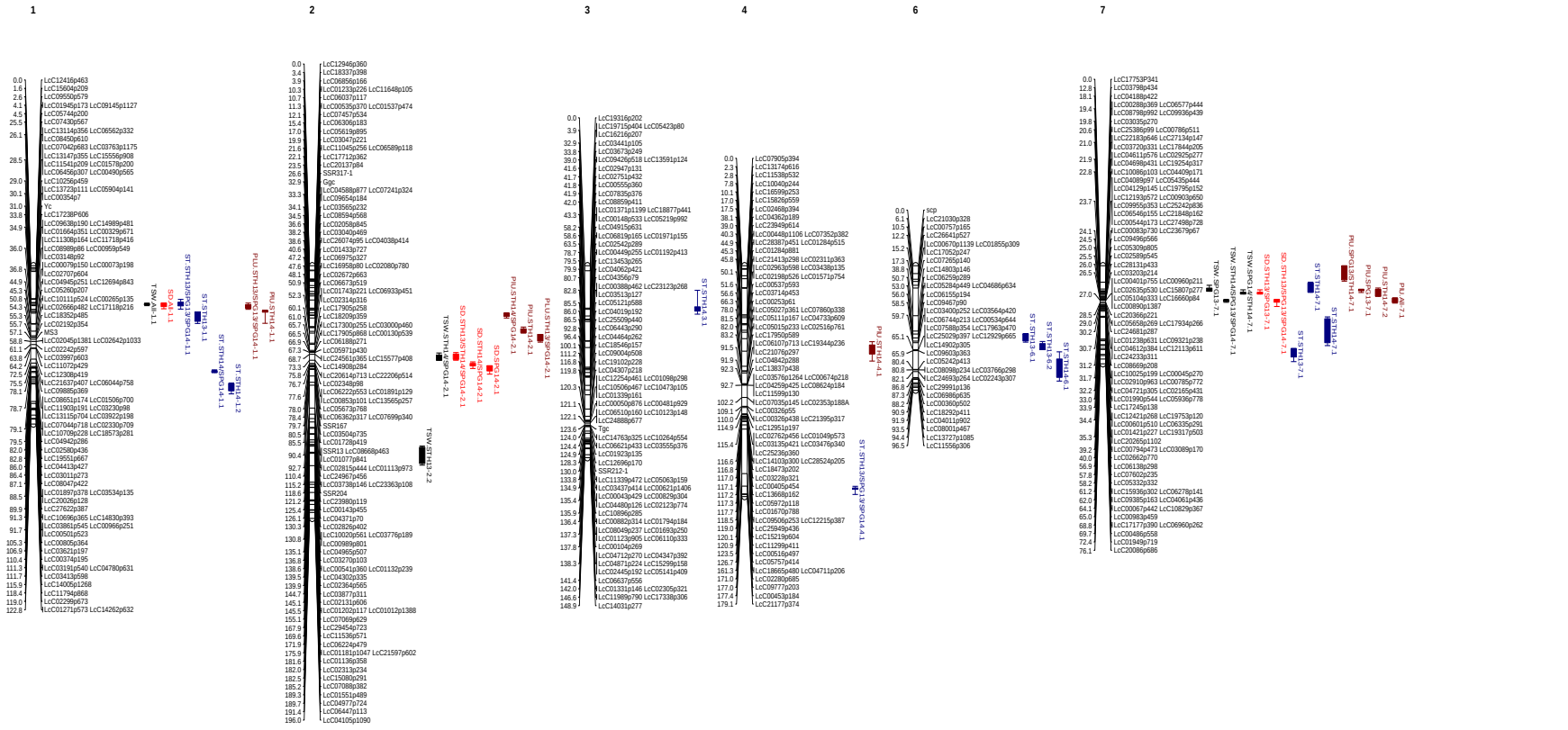
Appendix 6 A1.1. Quantitative trait loci detected for 1000-seed weight and seed dimensions in a lentil inbred recombinant population derived from a cross between CDC Robin and 964a-46 evaluated over four site-years, in SK.

Markers	Linkage group	Traits	Site-year[¥]	Position	Interval	LOD[₸]	% Exp[$]	LOD [c]	Add. effects[≠]
Yc	1	1000-seed weight	STH 2013	31.01	30.1-33.8	6.8	15.8	3.50	-2.376
			SPG 2013	31.01	29-32.9	16.0	31.2	3.40	-4.946
			STH 2014	31.01	29-31	27.8	39.9	3.63	-4.510
			SPG 2014	31.01	28.6-32	24.3	34.0	3.42	-4.143
		Seed diameter	STH 2013	31.01	29-33.4	34.45	46.00	3.46	-0.289
			SPG 2013	31.01	29-33.5	28.73	33.31	3.56	-0.204
			STH 2014	31.01	31.4-32.8	32.84	43.21	3.63	-0.250
			SPG 2014	30.01	29-33.4	29.24	37.59	3.51	-0.214
		Seed thickness	STH 2013	31.01	26.6-33.8	4.66	9.43	3.61	-0.024
			SPG 2013	31.01	26.1-33	3.98	8.76	3.53	-0.022
			SPG 2014	31.01	26.1-33.7	3.54	7.80	3.40	-0.026
		Seed plumpness	STH 2013	31.01	29.0-31.6	22.55	31.87	3.51	0.024
			SPG 2013	31.01	30.1-33.8	9.86	26.42	3.49	0.018
			SPG 2014	31.01	30.1-33.0	20.17	27.38	3.58	0.019
LcC24561p365	2	1000-seed weight	STH 2014	69.7	67.7-73.3	6.0	6.20	3.63	-1.758
			SPG 2014	69.7	67.7-73.3	6.3	6.60	3.42	-1.799
		Seed diameter	STH 2013	69.71	68.6-72.7	6.71	5.91	3.54	-0.100
			STH 2014	70.71	67.3-73.3	7.25	6.14	3.73	-0.091
			SPG 2014	71.71	67.4-73.3	4.14	4.05	3.58	-0.075
LcC22183p99	7	1000-seed weight	SPG 2014	20.61	19.9-21.9	13.0	16.61	3.55	-2.747
			STH 2014	20.61	18.7-21.9	11.2	12.82	3.63	-2.425
		Seed diameter	STH 2013	20.61	19.7-22.8	13.71	13.23	3.47	-0.144
			SPG 2013	20.61	19.1-21.9	20.73	22.89	3.49	-0.168
		Seed plumpness	STH 2014	20.61	17.3-23.7	4.46	3.17	3.54	0.010
LcC000890p1387	7	1000-seed weight	STH 2014	27.01	26.2-28.9	15.9	16.90	3.55	-2.819
			SPG 2013	27.01	23.8-28.4	12.7	22.00	3.63	-3.574
			SPG 2014	27.01	26.2-47.2	17.5	20.70	3.55	-3.112
		Seed diameter	STH 2013	27.01	25.1-28.5	19.14	16.92	3.51	-0.168

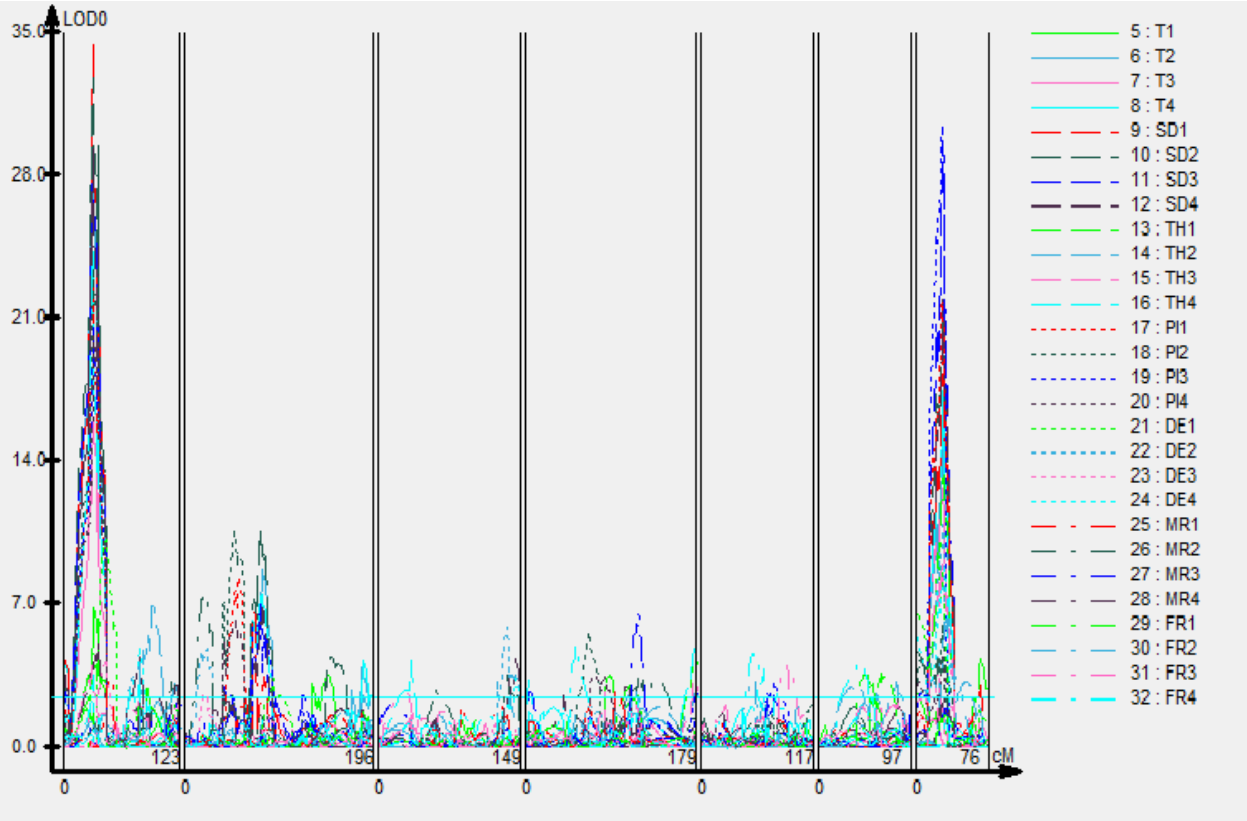
			SPG 2013	27.01	26.2-36.7	29.68	28.58	3.49	-0.185
			SPG 2014	27.01	26.3-28.7	21.92	22.27	3.63	-0.159
		Seed plumpness	STH 13	26.51	24.5-28.2	21.83	25.48	3.51	0.020
			SPG 13	26.51	24.5-28.2	30.44	38.73	3.49	0.021
			STH 14	26.51	24.5-28.2	6.09	4.24	3.54	0.012
			SPG 14	26.51	24.5-28.2	4.57	4.26	3.58	0.011
LcC10020p565	2	1000-seed weight	STH 2013	147.5	139.9-154.7	3.6	9.5	3.5	-1.758
LcC03035p270	7		SPG 2013	19.4	15.2-19.8	10.1	18.2	3.40	-3.574
LcC12421p268	7		SPG 2013	34.4	33-38	5.5	11.0	3.55	-2.789
LcC02348p98	2	Seed diameter	SPG 2013	76.71	74.6-79.7	6.86	4.17	3.49	-0.075
			STH 2014	76.71	75-79.7	10.82	7.86	3.73	-0.101
LcC06362p317	2		SPG 2014	78.41	77.6-84.1	6.89	5.38	3.49	-0.081
LcC00073p198	1	Seed thickness	STH 2013	36.81	35.9-44.8	3.59	7.35	3.23	-0.022
LcC02580p436	1		STH 2014	82.01	80.8-82.8	4.81	12.78	3.23	-0.029
LcC03861p545	1		STH 2014	92.71	91-99.4	6.97	19.64	3.23	-0.038
LcC03441p105	3		SPG 2014	32.91	19.1-37.6	4.28	9.86	3.23	-0.029
LcC00977p203	4		STH 2013	176.01	171.2-177.4	4.83	11.19	3.61	-0.027
			SPG 2013	176.01	171.4-177.4	3.70	8.45	3.40	-0.021
			SPG 2014	176.01	172.4-177.4	4.86	12.60	3.40	-0.034
LcC07588p354	6		STH 2013	64.71	60.4-65.1	3.63	7.61	3.61	-0.021
LcC03798p434	7		STH 2014	15.81	12.8-20.6	3.38	10.13	3.23	0.026
LcC04611p576	7		STH 2014	21.91	20.6-24.4	4.47	10.52	3.40	0.027
LcC17177p390	7		STH 20 13	67.01	64.1-74.4	4.49	10.14	3.40	-0.024
LcC09638p190	1	Seed plumpness	STH 2014	34.91	34.8-36	22.28	27.59	3.54	0.021
LcC026074p95	2		STH 2014	38.6	36.2-40.9	7.21	5.27	3.54	0.009
	2		SPG 2014	38.6	36.2-40.9	5.11	4.70	3.58	0.047
LcC06673p519	2		STH 2014	50.11	48.9-47.6	10.55	7.66	3.54	0.077
LcC01743p221	2		STH 2013	54.31	52.3-59.4	8.33	8.42	3.51	0.061
	2		SPG 2014	54.31	52.3-59.5	5.72	6.15	3.58	0.012
LcC00253p61	4		STH 2014	65.61	61.5-74	5.65	4.10	3.54	0.008
LcC17753p341	7		SPG 2013	3.01	0.1-7.9	22.24	36.48	3.49	0.021
	7		STH 2014	5.01	0-12	4.38	4.32	3.54	0.010
LcC03035p270	7		SPG 2013	19.81	18.8-20.6	25.12	34.64	3.49	0.020

STH[¥] University experimental plot in Sutherland, Saskatoon; SPG, Saskatchewan Pulse Growers land.

LOD[₸] logarithm of the odds; LOD [c] Significant LOD threshold from 1000 permutation test at *P<0.01* level of significance.
% Exp., percent variability explained by the locus; [$]Add. effects[€], additive effect attributed to the locus. Negative values represent alleles originating from 964a-46; positive values represent allele originating from CDC Robin.



Appendix Figure: 6 A1.1. Quantitative trait loci for 1000 seed weight, seed diameter, seed thickens and plumpness of RILs (LR-18 lentil population) derived from a cross between CDC Robin × 964a-46..QTL indicated in black symbols designated TSW refer to thousand seed weight, blue symbols designated ST refer to seed thickness, red symbols designate SD refer to seed diameter and brown symbols designated Plu refer to seed plumpness. QTL with all respective variables means QTL presented all site-years. QTLs followed with STH and SPG denote QTL present at University experimental plot in Sutherland and at Saskatchewan Pulse Growers land site and year, respectively.



LG1 **LG2** **LG3** **LG4** **LG5** **LG6** **LG 7**

Appendix Figure 6A1.2. QTL position of all tested seed quality parameters (shape, weight) and milling quality parameters in linkage group of LR-18 inbred lentil population. T = 1000-seed weight (g), SD = seed diameter (mm), TH = seed thickness (mm), PLU = seed plumpness, DE = dehulling efficiency (%), MR = milling recovery (%) and FR = football recovery (%) measured at SPG and Sutherland, in 2013 and 2014 (1,2,3,4 after each trait indicated STH 2013, STH 2014, SPG 2013, SPG 2014, respectively).

APPENDIX: A2 IMPACT OF STEMPHYLIUM BLIGHT SEVERITY ON SEED AND MILLING QUALITIES OF LENTIL

A2.1 Introduction and objectives

Lentil production is often constrained by fungal diseases that reduce both yield and seed quality (Banniza et al., 2006; Morrall et al., 2011). Stemphylium blight (SB), caused by *Stemphylium botryosum* Wallr., has been reported as one of devastating diseases in lentil crop in South Asia (Bakr and Ahmed, 1992; Chen et al., 2009). It causes loss of lentil yield up to 100% in South Asia, particularly in Bangladesh (Hosen et al., 2009). Recently high level of SB infection has been reported in lentil in central and northern districts of Saskatchewan due to warm, humid, and wet growing season (Saskatchewan Ministry of Agriculture, 2016). In 2008, about 25% of the lentil area in Saskatchewan was affected by SB (Barker, 2009). SB is more prevalent in later part of growth stages of lentil particularly under wet and humid summer in the Western Canadian Parries. It was also reported that SB infection lowers seed quality by producing deformed and stained seeds (Caudillo-Ruiz, 2016).

Identification of causal agent and factor affecting conidia development of stemphylium blight have been explored under controlled as well as field conditions (Morrall, 2003; Banniza et al., 2006; Morrall et al., 2011). Our study on efficacy of fungicides on milling quality traits revealed that natural SB infected plots significantly lowered milling efficiency parameters (Chapter 4). However, no previous reports exist on implication of SB infection on lentil seed and milling quality parameters. This study was conducted with an objective of assessing the impact of stemphylium blight infection at vegetative and reproductive stages of lentil on seed yield, morphological and milling quality characteristics by artificially inoculating field grown two lentil cultivars with *Stemphylium botryosum* conidia at seedling, flowering, and pod-set stages.

A.2.2 Materials and Methods

A.2.2.1 Plant Materials

Two lentil cultivars, CDC Maxim, and CDC Robin, obtained from the Crop Development Center of the University of Saskatchewan (U of S), were used in this study. CDC Maxim is a small-seeded imidazolidinone tolerant (Clearfield type) red lentil. It is early-to medium-maturing and has a good resistance to both *Ascochyta* and *Anthracnose* diseases. CDC Robin is a

conventional type lentil cultivar with partial resistance to *Ascochyta* and *Colletotrichum spp* (Chongo et al., 1999; Buchwaldt et al., 1999). CDC robin is small red market class cultivar and has a moderate resistance to stemphylium blight (Vandenberg et al., 2002).

A.2.2 Site description experimental design

A field experiment was conducted at the Preston Ave. experimental site (52º 07' 35.5"N 106º 37' 19.6" W), Saskatoon, SK, in 2016 to assess the impact of stemphylium blight severity at different growth stages on milling quality characteristics of lentil. This study comprised of three main treatment factors. The first factor was inoculated/un-inoculated treatment. Plants were inoculated with *Stemphylium botryosum* conidia at three growth stages (seedling, early flowering, and podding) and un-inoculated treatment involved the plot sprayed by fungicide and the plot sprayed with water only. The second factor comprised of the plots covered with green polyethylene (low tunnels) and uncovered plot, and the third factor had lentil cultivars: CDC Robin and CDC Maxim. We assumed that the un-inoculated plots without fungicide application would provide estimation for natural level of infection and the polythene cover treatment would create a favorable micro-climate for rapid multiplication of conidia, and consequently those plants will have a high level of disease infection.

A.2.3 Experimental procedures and environment conditions

The crop was seeded on May 26, 2016 into 4m long and 1m wide plot with three rows spaced 30 cm apart, using a seeding density of 130 seeds m^{-2}. The plot inoculation was carried out by spraying 1.5 L suspension containing 1×10^{4} conidia mL^{-1} of isolate SB19 per plot using a 3.8 L Glimore $^{@}$ Chapin pro series-26011XP model hand sprayer (Victor, New York, USA). All nozzles used to apply suspension were calibrated to deliver 100 L ha^{-1} of spray water volume. A surfactant (Tween-20 $^{@}$) of 2 ml L^{-1} was mixed with conidian suspension before application. After each inoculation, Priaxor (167 g L^{-1} fluxapyroxad + 333 g L^{-1} of pyraclostrobin) fungicide (180 mL $acre^{-1}$) was applied only on sprayed control plots to minimize natural infection. Fungicide was applied with a CO_2-pressurized backpack sprayer (110-015 AirMix nozzle, 241 kpa, 45 cm spacing). Inoculum conidian suspension was obtained from the Pulse Pathology Laboratory of the University of Saskatchewan. Low tunnels were established immediately after inoculation, which was 30 days after seeding in 2016. Covered plots were opened only at the time of inoculation and disease rating. After each inoculation, plots were

irrigated using misting for 1 h to maintain high humid condition within each covered tunnel. Date of inoculation and other cultural practices are listed in Table A2.1.

The average monthly mean temperature ranged between 15.7 ºC and 19.8 ºC; and 18.6 ºC to 20 ºC for covered and non-covered treatments, respectively. Relative humidity ranged between 78 - 87.5% for non- covered and 90-98% for covered plots. Covered plots were remained mostly humid and hotter throughout season. The total amount of precipitation received for the season was 244.2 mm. July and August received higher than normal amount of precipitation (58.6 mm and 70.2 mm, respectively). The high precipitation combined with warmer growing conditions provided an ideal environment for proliferation of stemphylium blight disease (Table A2.2).

Table A2.1. Dates of treatment applied at the three growth stages in experimental plots at the Preston Ave. research field, Saskatoon, SK in 2016.

Activities	Stages	Dates
Sowing		26 May, 2016
Inoculation	Seedling	27 June 2016 (30 das)
	Early Flowering	14 July 2016 (50 das)
	Podding	04 August 2016 (77 das)
Fungicides sprayed	Seedling	28 June 2016 (32 das)
	Early Flowering	14 July 2016 (51 das)
	Podding	05, August 2016 (78 das)
Desiccation with diquat	90% of lower pods turning brown in color	26 August 2016
Harvesting		02 September 2016

Note: das = days after sowing

Figure A2.1. Test plots with covered and uncovered treatments at Preston research field, 2016.

Table A2.2. Temperature and relative humidity collected from non-covered and covered with green-polyethylene at Preston research field, SK in 2016.

Month		Un-Covered			Covered		
		Max	Min	Average	Max	Min	Average
May	Temp (°C)	32.6	12.6	15.7	34	13.4	17.1
	RH (%)	100	33.5	85.8	100	67.7	94.7
June	Temp (°C)	30.2	12.8	16.1	25.8	14	17.3
	RH (%)	100	40.1	86.1	100	80.5	98.5
July	Temp (°C)	29.9	10.5	18.7	31.3	10.9	20.5
	RH (%)	100	40.6	87.5	100.0	60.1	90.1
August	Temp (°C)	29.0	2.37	19.8	30.7	2.69	18.6
	RH (%)	100	24.9	78.1	100	26.8	96.2
				Precipitation (mm)			
May				41.6			
June				49.7			
July				58.6			
August				70.2			
Sept				24.1			
Total				244.2			

A.2.3 Data collection

Prior to inoculation, plant emergence data were collected by counting the number of seedlings in the middle rows. Disease severity data were collected prior to each inoculation on a weekly basis using a semi quantitative scale developed by Banniza et al. (2006). A quantitative scale ranging from 0 to 10 with 10% increments of plant damages was used for disease rating. The scales are: 0 = healthy plants, 1 = 1-10% (plant infected with tiny lesions), 2 = 10-20% (plant damaged by a few chlorotic lesions), 3 = 21-30% (expanding lesions on leave and onset of leaf drop), 4 = 31-40% (1/5th of nodes affected by legions and leaf drop), 5 = 41-50% (2/5th nodes affected by lesions); 6 = 51-60% (3/5th nodes affected); 7 = 61-70% (4/5th of nodes affected), 8 = 71-80% (all leaves dried up), 9 = 81-90% (all leaves dried up but stem green) and 10 = 91-100% (plant completely dead). Five randomly selected plants were used to assess disease severity per treatment. Altogether 8 disease ratings were taken. Quantitative data of disease severity were transformed to percentage using the class mid points. The average rating per experimental unit was used to determine the area under the disease progress curve (AUDPC) as a measure of repeated quantitative disease ratings explained by Shaner and Finney (1977):

$$AUDPC=\sum n\ ^{i}=1[(Yi+1+Yi)\ /2]*\ [Xi+1-Xi]$$

Where Y_i is disease severity in percentage at the i^{th} observation, X_i is number of days after inoculation at the i^{th} observation, and n is the total number of observations. Harvest aid Reglone$^{@}$ was applied to desiccate the crop to facilitate uniform harvesting. Harvesting was done with a combine harvester. Harvested seeds were subjected to a forced air drier at 39-42^{o}C for 3 days to bring seed moisture down. After drying, seed yield was estimated in a hectare basis. Randomly selected seeds from the original sample from each plot were assessed for 1000-seed weight (TSW) by weighing 250 seeds and multiplied by 4. The percentage of stained or wrinkled seeds was visually assessed from 100 randomly selected seeds (Canadian Grain Commission, 2014). Seed dimension (seed diameter, seed thickness and plumpness) and all milling quality parameters were determined using same procedure described in Chapter 3.

A.2.4 Data analyses

Data were tested for normality and homogeneity of variance. Heterogeneity of variance was subsequently modeled using repeated/group command with the mixed model procedure of SAS whenever necessary. Data were analyzed using the PROC MIXED procedure of SAS software version 9.4 (SAS Institute Inc., 2016), using blocks as random and the inoculation timings, covered/uncovered and cultivar as fixed factors. Significance of variance was declared at 5%. Means were separated by Least significance differences (LSD) at $P<0.05$. Correlation among measured traits was performed using mean of four replicates by PROC CORR to assess the relationship between disease severity and milling quality parameters. Single degree of freedom contrasts was performed to make specific comparisons of interest. Graphs were plotted using Sigma Plot V2. (Sigma Plot, 2010)

A.3 Results and discussion

A.3.1 Disease severity and disease development (AUDPC)

The analysis of variance revealed a highly significant effect of inoculation timing, cover treatments, interaction of inoculation timing and cover treatments on SB disease severity and area under disease progress curve (AUDPC) ($P \leq 0.001$) (Table A2.3). Cultivars did not differ in disease severity. Plants under cover treatments were significantly ($P \leq 0.001$) affected by disease severity (65.2%) compared to the non-covered treatment (52.1%). Fungicides sprayed, and non-sprayed control plots had significantly ($P \leq 0.001$) lower disease than inoculated plots at all

stages (Figure A2.2). Contrast analysis showed inoculated plots had significantly ($P \leq 0.001$) higher disease level (42% vs 22%) than control plots. Plants at water-sprayed uninoculated control were significantly ($P \leq 0.001$) infected compared to the fungicide-sprayed plots (Figure A2.2). AUDPC in both fungicide sprayed, or non-sprayed control plots were significantly ($P \leq 0.001$) lower than all inoculated treatments. Mean comparisons revealed that the levels of AUDPC in plants inoculated at the seedling and early flowering stage were significantly ($P \leq 0.001$) higher than uninoculated plants or plants inoculated at pod-set stage (Figure A2.2). The SB development at seedling stage inoculated plots was significantly ($P \leq 0.001$) increased (62%) than fungicide applied plots at later stages (Figure A2.2).

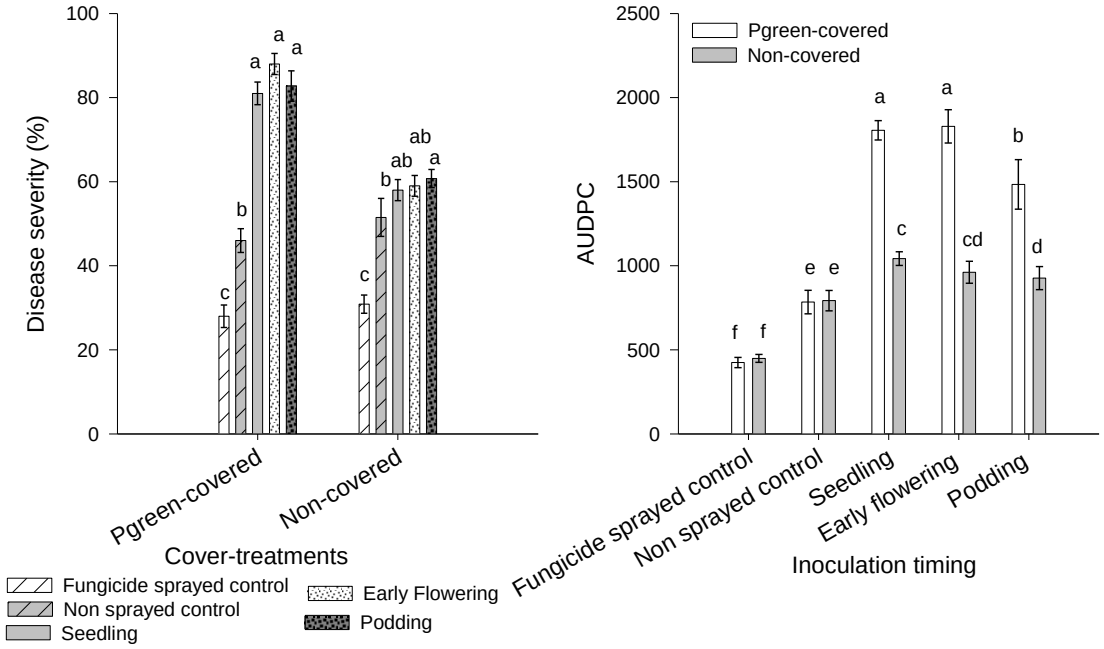


Figure A2.2. Interaction of cover treatments and inoculum timing on final disease severity levels and AUDPC of lentil assessed at Preston site in 2016. Error bars represent the standard errors mean. Similar letter above error bars in each group indicates means were not significantly different at LSD $_{(0.05)}$ for disease severity =11.3. and for AUDPC= 198.7

Table A2.3. P-values derived from analysis of variance from mixed model for disease severity, AUDPC, seed yield, 1000-seed weight, stained seed, seed diameter, seed thickness, seed plumpness, assessed in responses of stemphylium blight inoculation of lentil cultivars at Preston site, 2016.

Source of variation	Df	Disease severity	AUDPC	Seed yield	1000-seed weight	Stained seed	Seed diameter	Seed thickness	Seed plumpness
Cultivar (C)	1	0.1430	0.8906	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
Cover (CR)	1	<.0001***	<.0001***	<.0001***	0.3518	0.1289	0.7829	0.0008***	0.0023**
Stage (ST)	4	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	0.0004***	<.0001***	0.0080***
C × CR	1	0.1926	0.3723	<.0001***	0.0002***	0.0015**	0.7202	0.0008***	<.0001***
C × ST	4	0.2861	0.1748	0.0795	0.0299*	0.5421	0.7213	0.0228*	0.1958
CR × ST	4	<.0001***	<.0001***	0.9583	0.4500	0.9691	0.0797	0.2520	0.3224
C × CR × ST	4	0.9387	0.4903	0.5528	0.5922	0.2824	0.6143	0.4726	0.3455
Contrasts									
Fungicide sprayed control vs. Inoculated		<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	0.0069**	<.0001***	0.0072**
Non-sprayed control vs. Inoculated		<.0001***	<.0001***	0.0627	0.0324*	0.0107*	0.7828	0.8477	0.8432
Fungicide sprayed control vs Non - sprayed control		<.0001***	<.0001***	0.0035**	0.0066**	0.0410*	0.0440	<.0001**	0.0180*

*, ** and *** denotes significant at *P<0.05, 0.01* and *0.001*, respectively.

A.3.2 Seed yield, 1000 seed weight and percentage of deformed and stained seeds

Covered treatments, and inoculation timing at different growth stages had significant effect on seed yield, 1000-seed weight, and percentage of stained and deformed seeds *(P≤0.001)*. The cultivar by cover interactions effect was only significant on seed yield (Table A2.3). Treatments under covered plots had significantly reduced seed yield, compared to the non-covered treatments *(P=0.001)* (Table A2.4; Table A2.5). As expected, cultivar differed in their seed yield. CDC Maxim at non-covered plots had significantly higher yield than CDC Robin (*P<0.05)* (Figure A2.3). Fungicide sprayed control plot had significantly higher seed yield than the non-sprayed control or inoculated treated plots (Table A2.4). Linear contrast revealed both control plots had significantly higher yield by 492.9 kg ha^{-1} and 166.28 kg ha^{-1} respectively than inoculated plots (Table A2.4). Fungicide sprayed control plots produced significantly higher yield (326.6 kg ha^{-1}) compared with un-sprayed control plots (Table A2.3).

Table A2.4. Mean seed yield, 1000-seed weight, seed dimensions of two lentil cultivars assessed under stemphylium disease inoculation treatment field at Preston research site, 2016.

Inoculation treatment	Seed yield (kg ha^{-1})	1000 seed weight (g)	Seed diameter (mm)	Seed thickness (mm)	Seed plumpness
Fungicide-sprayed control	2114.00^{a}	27.06^{a}	4.38^{a}	2.32^{a}	0.53^{a}
Non-sprayed control	1787.40^{b}	25.42^{ab}	4.32^{b}	2.24^{b}	0.52^{ab}
Seedling	1645.90^{b}	24.78^{b}	4.35^{ab}	2.24^{b}	0.52^{ab}
Early flowering	1591.82^{b}	24.07^{b}	4.25^{c}	2.20^{c}	0.52^{ab}
Podding	1625.81^{b}	24.23^{b}	4.35^{ab}	2.24^{b}	0.51^{b}
LSD (0.05)	214.82	1.17	0.06	0.03	0.008
Cultivar					
CDC Maxim	2022.60^{a}	28.60^{a}	4.60^{a}	2.29^{a}	0.50^{b}
CDC Robin	1483.40^{b}	21.60^{b}	4.06^{b}	2.20^{b}	0.54^{a}
LSD (0.05)	135.86	0.74	0.04	0.02	0.004
Covering treatment					
Covered	2206.85^{a}	25.28^{a}	4.33^{a}	2.27^{a}	0.53
Non-covered	1299.12^{b}	25.94^{a}	4.33^{a}	2.23^{b}	0.51
LSD (0.05)	135.86	-	-	0.02	0.05

Means followed by same letters within columns are not significantly different at *P<0.05*, LSD denotes Fisher's least significant difference (LSD).

Among inoculation treatments, fungicide sprayed control plot had significantly ($P \leq 0.001$) heavier seed weight than other treatments (Table A2.4). CDC Robin had significantly higher percentage of stained and deformed seeds than CDC Maxim. This result was higher for CDC Maxim under covered CDC and for CDC Robin under on non-covered treatments (Table A2.5; Figure A2.3). Result indicated that irrespective of cultivar or cover treatments, all inoculated plots had lower 1000-seed weight with higher percentages of stained and deformed seeds compared to the controls. Linear contrast also supports this result as both fungicide sprayed or non-sprayed control plots significantly ($P \leq 0.001$) reduced stained or deformed seeds by 12.3% and 6.3% respectively compared to the inoculated treatment (Table A2.3; Figure A2.3).

Table A2.5. Cover treatment by cultivar interaction effect for seed yield, 1000-seed weight, stained seed, seed thickness and plumpness of lentil assessed at Preston research site, 2016. Value represent mean value of each variable.

Cultivar	Cover-treatment	Seed yield	1000-Seed weight	Seed thickness	Seed plumpness
CDC Maxim	Covered	1345.2^{c}	28.1^{b}	2.29^{a}	0.50^{c}
	Non-covered	2699.9^{a}	29.2^{a}	2.29^{a}	0.50^{c}
CDC Robin	Covered	1713.7^{b}	22.5^{c}	2.16^{c}	0.55^{a}
	Non-covered	1253.0^{c}	20.7^{d}	2.24^{b}	0.53^{b}
LSD (0.05)		192.4	1.04	0.03	0.007

Means followed by same letters within columns are not significantly different at $P<0.05$, LSD denotes Fisher's least significant difference (LSD).

Table A2.6. Inoculation timing by cultivar interaction effect for 1000 seed-weight and seed thickness of lentil assessed at Preston research site, 2016. Value represent mean value of each variable.

Treatments	1000-seed weight (g)		Seed thickness (mm)	
	Covered	Non -covered	Covered	Non-covered
Sprayed control	27.86^{a}	26.26^{ab}	2.36^{a}	2.28^{b}
Non-sprayed control	25.38^{ab}	25.46^{ab}	2.25^{bc}	2.23^{bcd}
Seedling	24.53^{b}	25.04^{b}	2.25^{bc}	2.24^{bcd}
Early flowering	24.16^{b}	23.98^{b}	2.21^{cd}	2.19^{d}
Podding	24.50^{b}	23.95^{b}	2.26^{bc}	2.21^{cd}
LSD (0.05)	1.65		0.05	

Means followed by same letters within columns are not significantly different at $P<0.05$, LSD denotes Fisher's least significant difference (LSD).

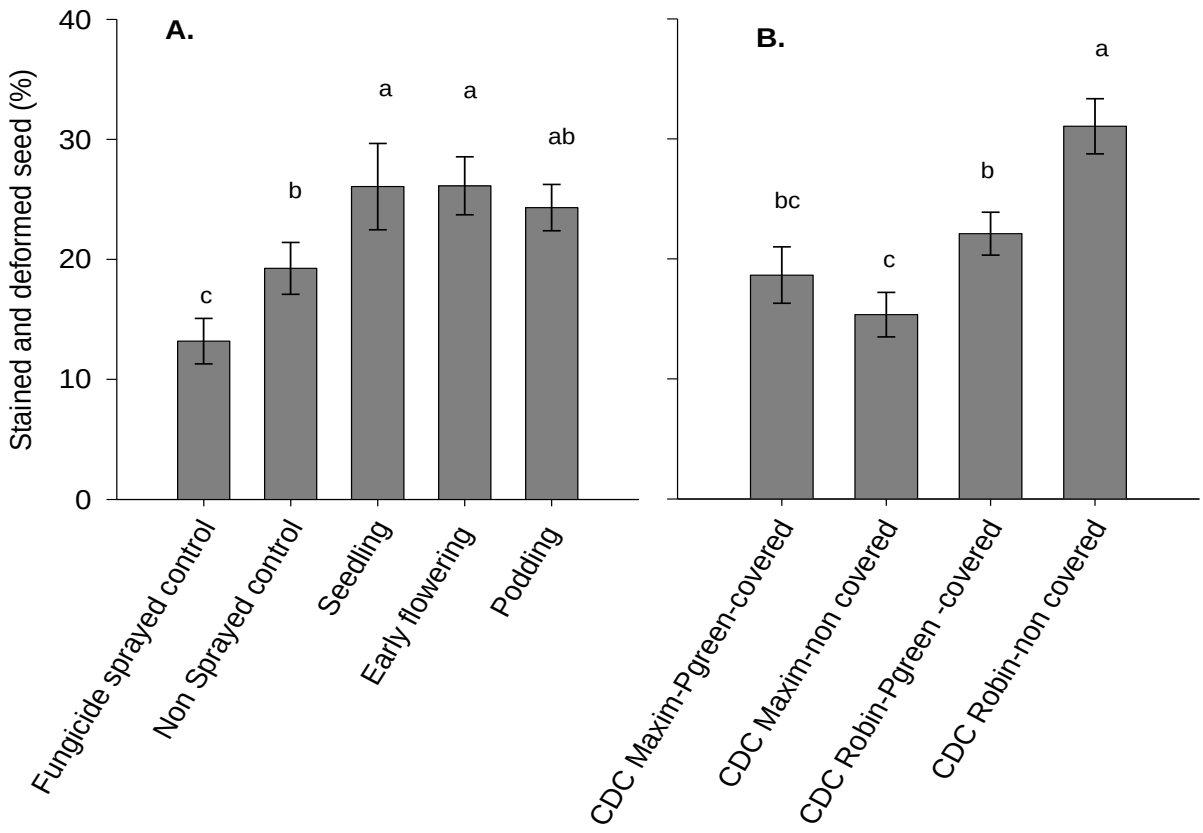


Figure A2.3. Effect of inoculation timing (Figure A) and cultivar by cover treatment (Figure B) on percentage of stained and/or deformed seeds of lentil cultivars, CDC Maxim and CDC Robin, assessed at Preston site, SK in 2016. Error bars are standard errors of the treatment means. Similar letter above error bars indicates mean were not significantly different at LSD (0.05) for inoculation timing = 5.1 and cultivar and cover treatments =5.2.

A **B** **C**

Figure A2.4. Amount of stained and deformed seeds at fungicide sprayed control (A), inoculated at seedling (B), and early flowering (C) plots.

A.3.3 Seed morphological characteristics: seed diameter, thickness, and plumpness

The results showed that cultivar, and inoculation timing treatments had significant impact on seed diameter *(P=0.001)* (Table A2.3). As expected, CDC Maxim produced bigger seed compared to CDC Robin. Irrespective of cultivar and cover treatments, inoculation at early flowering and podding stages had significantly ($P \leq 0.001$) reduced seed diameter than the control treatments (Table A2.4). This result is also supported by linear contrast analysis, which showed fungicide sprayed un-inoculated treatment significantly ($P \leq 0.001$) increased seed diameter compared to the inoculated treatments (Table A2.4).

Seed thickness and plumpness also significantly differed between cultivars, cover treatments and among inoculation treatment timing ($P \leq 0.001$). Interaction effect of cultivar by cover treatments was significant ($P \leq 0.001$) for both seed thickness and plumpness (Table A2.3). As expected, CDC Maxim produced significantly thick but less plump seeds than CDC Robin. Both seed thickness and plumpness were slightly higher under covered (low tunnels) compared to the non-covered (Table A2.4; Table A2.5). Irrespective of cover treatment or cultivars, fungicide sprayed control plots produced significantly ($P \leq 0.001$) thick and plump seeds compared to other treatments (Table. A2.4).

A.3.4 Milling characteristics: dehulling efficiency, milling and football recovery

Highly significant differences were observed among inoculation treatments and cultivars on percent dehulling efficiency ($P<0.001$). However, cover treatment had no effect on DE (Table A2.7). Irrespective of cultivar and covers, both sprayed and non-sprayed un-inoculated control had significantly higher DE compared to inoculated treatments (Figure A2.5). In general, CDC Maxim had significantly ($P<0.001$) higher DE compared to CDC Robin. Linear contrast results showed sprayed uninoculated treatment significantly increased DE (7.5%) than inoculated treatments.

Results revealed that cultivar and inoculation timing had significant ($P<0.001$) effect on MR. Although cover treatment had no impact on milling recovery, interaction of cultivar by cover treatment had a significant effect. CDC Maxim had significantly higher MR than CDC Robin. As found in interaction effect, CDC Maxim at non-covered treatment produced significantly ($P<0.001$) higher MR while CDC Robin produced higher MR under covered treatments (Figure A2.6).

Table A2.7. P-values derived from analysis of variance from mixed model for dehulling efficiency, milling and football recovery assessed in responses of stemphylium blight inoculation of lentil cultivars at Preston site, 2016.

Source of variation	Df	Dehulling efficiency	Milling recovery	Football recovery
Cultivar (C)	1	<.0001***	<.0001***	<.0001***
Cover(CR)	1	0.1725	0.2273	0.0042**
Stage (ST)	4	0.0007***	0.0003***	0.2481
C ×CR	1	0.062	0.0097**	<.0001***
C ×ST	4	0.5362	0.6235	0.3188
CR ×ST	4	0.8277	0.8946	0.3982
C ×CR×ST	4	0.4565	0.4438	0.6617
Contrasts				
Fungicide sprayed control vs. Inoculated		<.0001***	<.0001***	0.0255*
Non-sprayed control vs. Inoculated		0.0114*	0.0069**	0.3187
Fungicide sprayed control vs Non - sprayed control		0.1665	0.1372	0.2981

*, ** and *** denotes significant at *P<0.05, 0.01* and *0.001*, respectively.

Cover treatments and cultivar had significant *(P<0.001)* impact on FR. However, inoculation timing had no effect (Table A2.7). On average, non-covered plot produced higher percentage of FR, but differences were mediated by cover by cultivar interaction. CDC maxim on non-covered treatment yielded higher FR compared to covered treatments. In contrast, CDC Robin yielded statistically comparable FR under both conditions (Figure A2.6). Although inoculation timing had no effect in general, contrast analysis showed sprayed uninoculated treatment produced significantly higher FR (2%) compared to inoculated treatments (TableA2.7).

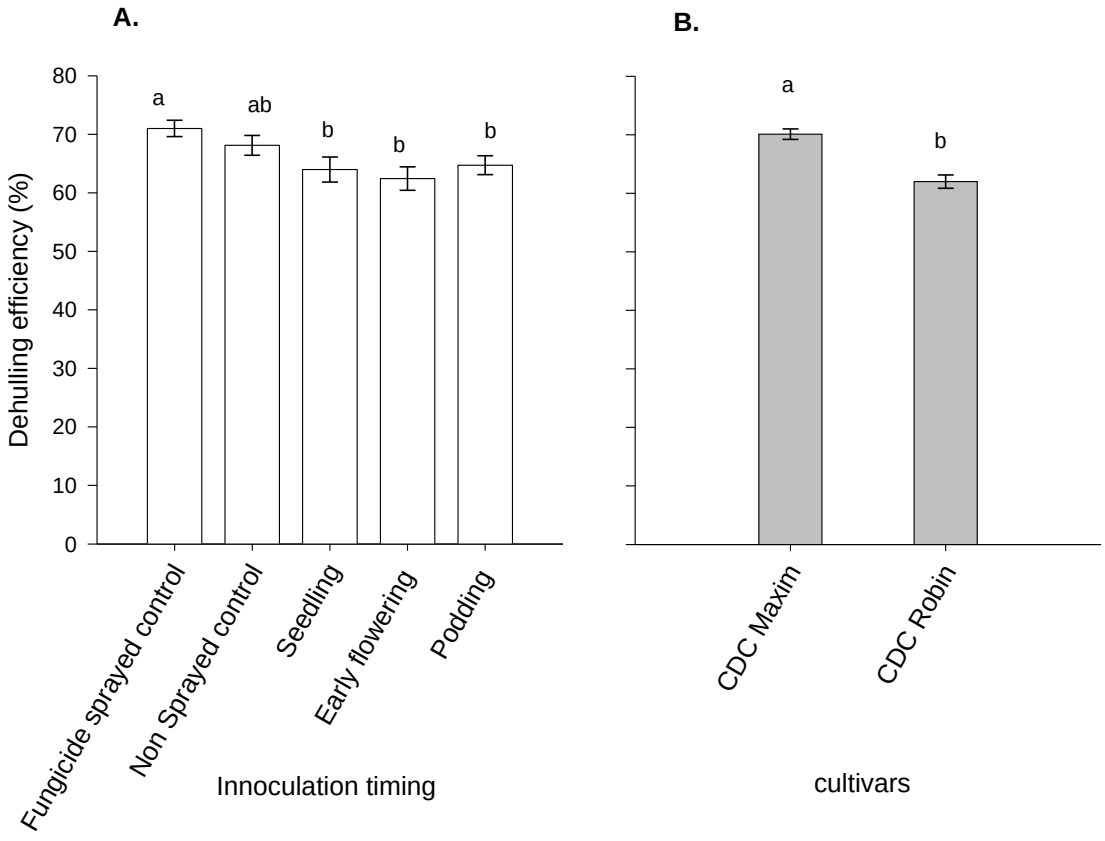


Figure A2.5. The effect of inoculation timing and cultivar on dehulling efficiency assessed at Preston site, SK in 2016. Error bars are standard errors of the treatment means. Similar letter above error bars indicates means were not significantly different at LSD $_{(0.05)}$ for inoculation timing = 4.12, and cultivars= 2.61

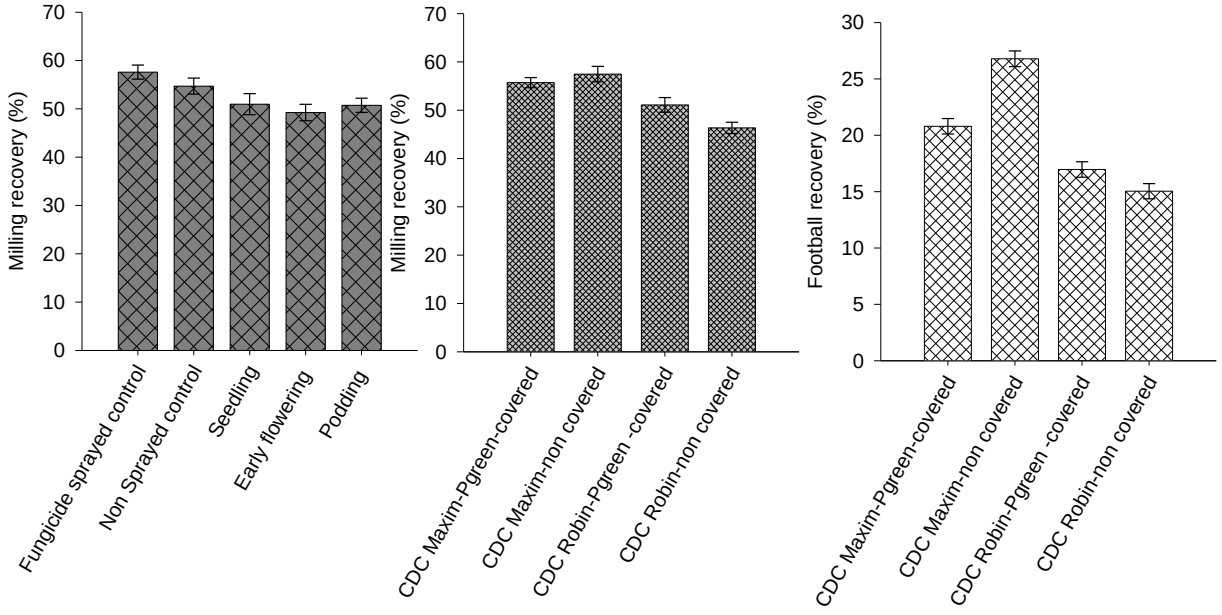


Figure A2.6. Effect of inoculation timing (Figure A) and interaction between cover by cultivar effect (Figure B) on milling recovery (%), and interaction of cover by cultivar effect on football recovery (%) (Figure C) of CDC Maxim and CDC Robin lentil cultivar assessed at Preston site, SK in 2016. Error bars are standard errors of the treatment mean. Similar letter above error bars indicates means were not significantly different at LSD (0.05) for inoculation timing (Figure A) =3.8.; for cover by cultivar interaction (Figure B) = 3.4 and cover by cultivar interaction for football recovery (Figure C) =1.9.

A.3.5 Correlation among disease components, seed dimension and milling traits

A significant and positive correlation was observed between disease severity, AUDPC and percent stained and deformed seeds. Disease severity was negatively correlated with seed yield, seed weight, seed thickness and all milling parameters, DE, MR, and FR (Table A2.8). The trend was similar for AUDPC, which was negatively associated with seed yield, TSW, seed thickness, and all milling quality traits. Percentage of stained and deformed seeds was significantly but negatively correlated with all seed characteristics but strongly correlated with milling quality characteristics (Table A2.8). Seed yield was positively and significantly correlated with seed characteristics but negatively correlated with milling traits. Seed weight was strongly but positively associated DE, MR, and FR. Conversely, seed weight was negatively correlated with seed plumpness (Table A2.8). Seed diameter was negatively but strongly correlated with seed plumpness but positively correlated with all milling quality traits. Seed thickness was positively correlated with all milling parameters. Seed plumpness was negatively

correlated with DE and MR*)*, but positively associated with FR. DE was strongly and positively correlated with MR, and FR. Similarly, MR was also positively correlated with FR (Table A2.8).

Table A2.8. Correlation matrix for disease severity, AUDPC, days to maturity, stained seeds, seed yield, 1000-seed weight, seed diameter, thickness, plumpness, dehulling efficiency, milling and football recovery affected by *Stemphylium botryosum* field isolate SB19 at 1 x 10^4 conidia mL^{-1} at Preston site, Saskatoon, SK 2016.

	$DS^{\bar{T}}$	$AUDPC^{\bar{T}}$	$STN^{\bar{T}}$	$Yield^{\bar{T}}$	$TSW^{\bar{T}}$	$SD^{\bar{T}}$	$TH^{\bar{T}}$	$Plu^{\bar{T}}$	$DE^{\bar{T}}$	$MR^{\bar{T}}$
$AUDPC^{\bar{T}}$	0.96***									
$STN^{\bar{T}}$	0.50***	0.40**								
$Yield^{\bar{T}}$	-0.56***	-0.58***	-0.47***							
$TSW^{\bar{T}}$	-0.32**	-0.22*	-0.80***	0.54***						
$SD^{\bar{T}}$	-0.15^{ns}	-0.08^{ns}	-0.64***	0.47***	0.94***					
$TH^{\bar{T}}$	-0.35**	-0.25*	-0.84***	0.40***	0.90***	0.75***				
$Plu^{\bar{T}}$	-0.03^{ns}	-0.07^{ns}	0.33^{ns}	-0.39**	-0.72***	-0.90***	-0.40*			
$DE^{\bar{T}}$	-0.43**	-0.22**	-0.94***	0.45***	0.85***	0.76***	0.80***	-0.55**		
$MR^{\bar{T}}$	-0.48***	-0.29***	-0.95***	0.49 ***	0.86***	0.75***	0.82***	-0.52**	0.98***	
$FR^{\bar{T}}$	-0.28**	-0.20***	-0.73***	0.75 ***	0.87***	0.83***	0.72***	0.68**	0.78***	0.80***

*, ** and *** denote significant at *P<0.05, 0.01* and *0.001*, respectively, $DS^{\bar{T}}$= disease severity (%); $DTM^{\bar{T}}$=Days to maturity, $STN^{\bar{T}}$=Percentage of staining and deformed seeds; $Yield^{\bar{T}}$=Seed yield $TSW^{\bar{T}}$= 1000-seed weight (g), $SD^{\bar{T}}$=seed diameter (mm), $TH^{\bar{T}}$=Seed thickness, $Plu^{\bar{T}}$=seed plumpness, $DE^{\bar{T}}$=dehulling efficiency (%), $MR^{\bar{T}}$=milling recovery (%) and $FR^{\bar{T}}$=football recovery (%).

A2.3.5 Conclusions

In conclusion, stemphylium blight infection was severe at early flowering stage. Diseases severity level was higher under covered treatments. Seed yield and 1000-seed weight were affected by SB infection. Severe SB infection increased percentage of stained or deformed seeds, and eventually infection significantly reduced milling quality parameters. The results suggest that SB is the main leading cause to reduce milling quality of red lentil in typical climatic conditions of the Northern climatic regions. Therefore, disease management strategies for SB infection throughout the season should be warranted. These strategies could be exploring potential fungicides, their application timing and frequency of application to effectively control diseases. Breeding strategies such as SB resistance cultivar or cultivar suitable for high humid and late planting season could be developed.

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