Genetic Mapping of Milling Quality Traits in Lentil (Lens culinaris Medik.)

Maya Subedi*, Kirstin E. Bett, Hamid Khazaei, and Albert Vandenberg

ABSTRACT
Milling qualities are key traits for the red lentil (Lens culinaris Medik.) industry as price is largely determined by dhal recovery yield. Dhal milling involves removal of the seed coat and splitting of the cotyledon to produce either splits or footballs (cotyledons still attached). 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 a lentil recombinant inbred population from the cross ‘CDC Robin’ × ‘946a-46’, which have contrasting seed characteristics. The mapping population consists of 127 F7–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 polymorphism markers, seven simple sequence repeat markers, and four morphological markers were used for quantitative trait locus (QTL) mapping. The broad-sense heritability was moderate for DE and MR and relatively low for FR. 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.

Core Ideas
- Phenotypic and genotypic diversity for milling quality traits in lentil are explored.
- Milling quality traits were significantly associated with seed shape and seed weight.
- We identified single nucleotide polymorphism markers associated with milling quality traits in lentil.
- This is a novel report for the genetics of milling quality traits for lentil.

CULTIVATED LENTIL is one of the most ancient annual cool-season legume crops that originated in the Near Eastern Complex (Zohary and Hopf, 1973). Lentil seeds are rich sources of dietary protein, complex carbohydrates, and essential micronutrients including Fe, Zn, and carotene (Erskine and Sarker, 2004). It is a self-pollinated diploid crop (2n = 2x = 14) with a haploid genome size of 4063 Mbp (Arumuganathan and Earle, 1991). Two major market classes of lentil, red and green, are produced globally. Red lentils are a source of staple protein and nutritious food in many parts of the Indian subcontinent and eastern Mediterranean regions such as Turkey, Syria, and 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). Seed characteristics, including seed coat thickness, seed coat components, seed size, and seed dimensions, are important traits, influenced by both genetics and environment, which have been reported to influence the milling performance 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 incur 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 a lower percentage of loss during decortication because the proportion of hull to seed mass is lower than that of small seeds (Vandenberg, 2009). Erskine et al. (1991a) found that lentil seeds with a mean seed diameter of 4 mm lost about 8.2% of their weight during dehulling compared with losses from lentil seeds 3 mm in diameter (9.8% on average).

Seed-related morphological traits have been genetically mapped in lentil (Fratini et al., 2007; Fedoruk et al., 2013; Saha et al., 2013; Khazaai et al., 2018). Three major QTLs governing seed diameter were mapped in lentil by applying random amplified polymorphic DNA markers by Fratini et al. (2007). Verma et al. (2015) detected QTLs related to seed weight and size in lentil that were co-localized by using SSR (simple sequence repeat) markers. Multiple QTLs for lentil seed diameter, thickness and plumpness were mapped in lentil via 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 with a cultivated lentil association mapping panel (Khazaai et al., 2018).

Limited studies in genetic control for milling traits in cereal crops have revealed that milling quality traits were controlled by multiple genes with small effects (Groh et al., 2000; Kepiro et al., 2008; Swamy et al., 2012). Seed morphological characteristics have a tremendous impact on milling performance, so QTLs 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.

### Materials and Methods

#### 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, red cotyledons, and brown seed coat color (Vandenberg et al., 2002). Line 964a-46 has larger seeds and relatively flat seed shape, yellow cotyledons, and a pale green seed coat. Seeds from single F2 plants were bulked to develop advanced generations (Sharpe et al., 2013). A total of 127 F2−derived RILs of the LR-18 population were used for this study.

#### Field Experiments

Field experiments were conducted at the Saskatchewan Pulse Growers (SPG) farm (52.05°N, 106.41°W) and at the University of Saskatchewan experimental farm in Sutherland (52.14°N, 106.61°W) during 2013 and 2014. The site details are described in Supplemental Table S1. The plants were grown in 1-m2 microplots with 130 seeds m−2 in a randomized complete block design with three replicates. Individual microplots were hand-harvested when 95% of the lower canopy pods had turned yellow to brown in color. The mean temperature and total monthly precipitation for each month of the growing season at Saskatoon, SK, Canada, in both years were obtained from Environment Canada (2014) (Supplemental Table S2; sourced from http://climate.weather.gc.ca/climate_data/daily_data_e.html?StationID=7707, accessed 21 Mar. 2018).

#### Seed Weight and Seed Dimension Characteristics

Hand-harvested plants were forced-air-dried at 39 to 42°C for 4 d and threshed. The threshed seeds were cleaned and seed weight was determined by weighing 250 randomly selected seeds. Seed diameter, thickness, and plumpness were estimated by passing 100 g of the seed sample through round-hole and slotted-hole sieves (Fedoruk et al., 2013) and then calculating seed diameter, thickness, and plumpness via the equations described in Fedoruk et al. (2013). Seed diameter was measured by passing the seed sample through a set of 10 round-hole sieves ranging from 6.75 mm (17/64˝) down to 3.6 mm (8/64˝) in 0.25 mm (1/64˝) increments. Seed thickness was measured by passing the same sample through a set of nine slotted-hole sieves from 3.1 mm (8/64˝) down to 1.80 mm (4/64˝) in 0.2 mm (0.5/64˝) increments. Samples were shaken for 1 min on a flatbed shaker (Lab Line Instruments, Melrose Park, IL). The seed fractions remaining in each round and slotted-hole sieve were weighed. The seed diameter (D) and seed thickness (ST) for each sample were calculated via the following formulae in Khazaai et al. (2018):

\[
\text{% on sieve} = \frac{\text{Mass on sieve}}{\text{Mass of total sample}} \times 100; \quad [1]
\]

\[
D = \sum \frac{\% \text{ on round sieve}}{100} \times \text{sieve hole size}; \quad [2]
\]

\[
ST = \sum \frac{\% \text{ on slot sieve}}{100} \times \text{sieve slot size}; \quad [3]
\]
Seed plumpness = \frac{ST}{D}, \quad [4]

**Measurement of Milling Quality Traits**

Milling quality traits were determined by following a procedure described in 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 with 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 them into football and split fractions. All fractions were weighed and expressed as a proportion of the total original milled sample via the following formula proposed by Wang (2005) and Bruce (2008):

\[
DE = \left(1 - \frac{W_{U\text{whole}} + W_{U\text{split}}}{W_{\text{sample}}}\right) \times 100; \quad [5]
\]

\[
MR = \frac{W_{\text{milled}}}{W_{\text{sample}}} \times 100; \quad [6]
\]

\[
FR = \frac{W_{\text{football}}}{W_{\text{sample}}} \times 100, \quad [7]
\]

where \(W_{U\text{whole}}\) is the weight of undehulled whole seeds (in g), \(W_{U\text{split}}\) is weight of undehulled split seeds (in g), \(W_{\text{sample}} \) is the weight of the seed sample, \(W_{\text{milled}}\) is the total weight of milled seeds (the weight of dehulled whole seeds plus the weight of dehulled split seeds in g), and \(W_{\text{football}}\) is the weight of footballs (dehulled whole seeds, in g). Dehulling efficiency, MR, and FR are expressed as percentages.

**Data Analysis**

The location and year of the field trials were treated as environments (site-years). ANOVA was performed by following the PROC MIXED procedure of SAS version 9.4 (SAS Institute, 2015). Homogeneity and normality were checked before subjecting the data to ANOVA. Genotypes were considered to be fixed, whereas site–year and replications nested within site–years were considered to be random effects. Parental lines data were removed from the dataset prior to estimating the variance components of the RILs to omit confounding effects. The VAR COMP procedure was used to determine the variance components where genotypes, locations, years, replicates, and their interactions were treated as random effects to determine the genetic variability.

Phenotypic variance (\(\sigma^2p\)) was estimated as reported by Falconer and Mackay (1996) [Eq. 8]:

\[
\sigma^2p = \sigma^2g + \frac{\sigma^2gy}{y} + \frac{\sigma^2gl}{l} + \frac{\sigma^2gyl}{ly} + \frac{\sigma^2e}{lyr}, \quad [8]
\]

where \(\sigma^2g\) is the estimated genotypic variance, \(\sigma^2gy\) is genotype \(\times\) year interaction variance, \(\sigma^2gl\) is the genotype \(\times\) location interaction variance, and \(\sigma^2gyl\) is genotype \(\times\) year \(\times\) location interaction variance, \(\sigma^2e\) is the error variance, \(y\) is the number of years tested, \(l\) is the number of locations, and \(r\) is the number of replicates per location. The broad-sense heritability (\(H^2\)) of the traits was estimated as: \(\sigma^2g (\sigma^2p)^{-1}\).

Correlations between milling quality parameters and seed morphological traits were determined from the means of the three replications in each site–year (PROC CORR procedure in SAS). Frequency distribution figures were drawn with the Hist. Command in R version 1.0.136 (R Core Team, 2014).

**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 Illumina Golden Gate array (Illumina, San Diego, CA) as described by Sharpe et al. (2013). The final map comprised 534 SNP markers, 7 SSR markers, and 4 morphological markers (see Supplemental Table S3): cotyledon color (Yc), seed coat ground color (Ggc and Tgc are independent genes that determine background color in lentil (Vandenberg and Slinkard, 1990)), and seed coat pattern (Scp). The map spanned 697 cM of the lentil genome at a 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/chado/genotype/Lens (accessed 4 Apr. 2018).

Quantitative trait locus analysis was performed by applying the QTL Cartographer version 2.5 software package (Wang et al., 2012). Interval mapping was used to select markers with high logarithm of odds values that were then used as cofactors for composite interval mapping in the CIM procedure. The CIM analysis used 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 logarithm of odds at 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 to be different if the 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. Increases in the phenotypic value of the specified traits contributed by the alleles from CDC Robin and 964a-46 are presented with positive and negative additive effect values, respectively.

**Results**

**Milling Quality Traits: DE, MR, and FR**

The genotype, site–year, and genotype \(\times\) site-year interactions effects were significant (\(P \leq 0.001\)) for the
percentages of DE, MR, and FR (Table 1). The mean DE, MR, and FR values were significantly greater in the 2013 growing season than in 2014 (Table 2; Fig. 1). The DE, MR, and FR values of the RIL population over the 2 yr ranged from 80.0 to 99.1, 66.5 to 87.5, and 7.8 to 59.3%, respectively (Table 2). Frequency distributions showed continuous variation for milling traits (Fig. 1 and Fig. 2). Dehulling efficiency and MR percentages were slightly skewed toward the large-seeded parent 964a-46, but the FR percentage was skewed toward the small-seeded parent CDC Robin. The mean values of MR and the FR of some individual RILs were higher than both parental values indicating that some RILs exhibited transgressive segregation for milling quality traits (Fig. 1). The heritability was moderate for DE and MR and relatively low for FR (Table 3). Phenotypic data across the years and locations are presented in Supplemental Table S4.

**Table 1. ANOVA results with F-values for dehulling efficiency, milling recovery, and football recovery of lentil seeds for 127 lines of the LR-18 recombinant inbred lines and their parents grown at two locations (Sutherland and Saskatchewan Pulse Growers test sites) in Saskatchewan in 2013 and 2014.**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Dehulling efficiency</th>
<th>Milling recovery</th>
<th>Football recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>128</td>
<td>4.73***</td>
<td>3.77***</td>
<td>3.84***</td>
</tr>
<tr>
<td>Site–year</td>
<td>3</td>
<td>752.97***</td>
<td>919.18***</td>
<td>378.98***</td>
</tr>
<tr>
<td>Genotype × site–year</td>
<td>384</td>
<td>2.60***</td>
<td>2.95***</td>
<td>11.07***</td>
</tr>
<tr>
<td>CV (%)</td>
<td>6.80</td>
<td>8.74</td>
<td>5.66</td>
<td></td>
</tr>
</tbody>
</table>

*** Significant at the 0.001 probability level.

**Table 2. Mean, minimum, maximum, and SD for dehulling efficiency (DE, in %), milling recovery (MR, in %), and football recovery (FR, in %) for the LR-18 lentil recombinant inbred line (RIL) population and the means of the parent cultivars grown over two site–years in Saskatchewan in 2013 and 2014.**

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

† SPG, Saskatchewan Pulse Growers test site; SD, standard deviation.

**Fig. 1. Box and whisker plots for the distribution of milling traits [dehulling efficiency (A), milling recovery (B), and football recovery (C)] in the 127 F7 derived LR-18 inbred lentil population grown at two locations [Sutherland (STH) and Saskatchewan Pulse Growers (SPG) sites] in 2013 and 2014. The letters X and Y represent the values of 964a-46 and CDC Robin, respectively.**

**Correlations between Seed Characteristics and Milling Traits**

Seed weight had a positive significant correlation with DE and a negative correlation with FR (Table 4). Seed diameter was significantly positively correlated with DE in all site–years. Two of the four site–years had significant correlations between seed diameter and FR. Seed thickness was positively significantly correlated with DE but had a negative association with FR in two site–years. Seed plumpness was significantly and 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 4).

**Quantitative Trait Locus Analysis for Milling Quality Traits**

Quantitative trait loci for DE were located on four of seven LGs (LG1, LG2, LG3, and LG7) (Table 5; Fig. 3). Ten significant QTLs specific to each site–year were identified for DE. The most stable QTL linked 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 others were detected at both sites in 2014 and explained 26.8% (combined for the two site–years), 19.5 and 20.4% phenotypic variation for DE, respectively (Table 5). Additional QTLs, explaining 10% phenotypic variation for DE on average, were also located on LG1, LG2, and LG3, and these QTLs were specific to each site–year (Table 5). The QTL linked with SNP marker 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 QTLs for higher DE came from 964a-46, the parent with green seed coats, larger diameter, and higher DE (Table 5).

There were multiple and significant QTLs for MR located on all LGs except LG5 and LG6 (Table 5; Fig. 3). These QTLs were not stable and were specific to individual site–years (Table 5). The QTL for MR associated with SNP marker 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 QTL linked with SNP marker LcC03047p221 on LG2 and another associated with SNP marker LcC13668p162 on LG4 were present only for SPG 2013 and SPG 2014 and explained over 16% phenotypic variation in MR. Additional MR QTLs linked with multiple SNP markers were coincident with QTLs for DE on LG1, LG3, and LG7, and explained 10% of the phenotypic variation for MR on average (Table 5). One QTL that was only found in SPG 2014 was associated with same region of the Yc locus and explained over 11% phenotypic variation for MR. The alleles contributing to increased MR percentage came from the 964a-46 parent (Table 5).

Multiple and significant QTLs for FR were present on LG4, LG5, LG6, and LG7 (Table 5; Fig. 3). 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 QTLs for DE on LG7 across three site–years. These QTLs explained 20 to 35% of the phenotypic variation for FR across three site–years. Five additional FR QTLs associated with the SNP markers LcC00537p593 (LG4), LcC00149p593 (LG5), LcC06259p289 (LG6) and SSR marker SSR156 (LG5) were identified across two site–years and explained over 10% of the variation. The alleles contributing to increasing the percentage of FR came from the plump, brown-seeded parent, CDC Robin (Table 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. We phenotyped milling quality traits (DE, MR, and FR) in an inbred lentil mapping population and then searched for genomic regions controlling these traits. To our 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.

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 × 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 (Supplemental Table S2). Variability in seasonal soil moisture levels would have caused differences in DE, MR, and FR, as high moisture prior to harvest significantly affects the dehulling process (Subedi et al., 2017). Complex traits are strongly influenced by 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 lentil. Erskine et al. (1991b) noted that genotype had greater impact on DE than location when they evaluated 23 diverse microesperma red lentil cultivars in three locations of Lebanon and Syria. Their estimation methodology for DE was quite different from ours, as they determined DE value by summing 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 daylength. 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 a 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 or early July and continues until soil moisture becomes depleted in the root zone. Flowering and seed development occur simultaneously and acropetally from multiple growing points on primary, secondary, and tertiary branches in lentil during the second half of the growing season, which would have been extended in 2014 because more moisture was available. In addition, because of high late-season humidity, the lentil canopy at 2014 test sites was also severely affected by stemphylium blight, which is caused by Stemphylium botryosum. In lentil, late-season canopy

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### Table 4. Pearson's correlation coefficients among 1000-seed weight, seed dimensions, and milling quality parameters of the LR-18 lentil recombinant inbred line population grown at the Sutherland (STH) and Saskatchewan Pulse Growers sites (SPG) in Saskatchewan in 2013 and 2014 (n = 129).

<table>
<thead>
<tr>
<th>Site-year</th>
<th>Traits</th>
<th>TSW</th>
<th>SD</th>
<th>TH</th>
<th>PLU</th>
<th>DE</th>
<th>MR</th>
<th>FR</th>
<th>Site-year</th>
</tr>
</thead>
<tbody>
<tr>
<td>STH 2013</td>
<td>TSW</td>
<td>–</td>
<td>0.96***</td>
<td>0.48***</td>
<td>–0.78***</td>
<td>0.52***</td>
<td>0.42***</td>
<td>–0.20*</td>
<td>STH 2014</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>0.93***</td>
<td>0.61***</td>
<td>–0.74***</td>
<td>0.32***</td>
<td>0.02ns</td>
<td>–0.15 ns</td>
<td>–0.36***</td>
<td>SPG 2014</td>
</tr>
<tr>
<td>SPG 2013</td>
<td>SD</td>
<td>0.60***</td>
<td>–</td>
<td>0.34***</td>
<td>–0.90***</td>
<td>0.49***</td>
<td>–0.39***</td>
<td>–0.17 ns</td>
<td>STH 2014</td>
</tr>
<tr>
<td></td>
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<td>0.51***</td>
<td>0.51***</td>
<td>0.34***</td>
<td>–0.88***</td>
<td>0.35***</td>
<td>0.06 ns</td>
<td>–0.03 ns</td>
<td>SPG 2014</td>
</tr>
<tr>
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<td>TH</td>
<td>0.37***</td>
<td>0.34***</td>
<td>–</td>
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<td>0.19*</td>
<td>–0.23**</td>
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</tr>
<tr>
<td>SPG 2013</td>
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<td>–0.93***</td>
<td>0.01 ns</td>
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<td>–0.37***</td>
<td>0.07 ns</td>
<td>STH 2014</td>
</tr>
<tr>
<td>STH 2013</td>
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<td>–0.90***</td>
<td>–0.01 ns</td>
<td>–</td>
<td>–0.33***</td>
<td>–0.07 ns</td>
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<tr>
<td></td>
<td>MR</td>
<td>0.41***</td>
<td>0.62***</td>
<td>0.20*</td>
<td>–0.57***</td>
<td>–</td>
<td>0.88***</td>
<td>–0.18*</td>
<td>STH 2014</td>
</tr>
<tr>
<td>STH 2013</td>
<td>FR</td>
<td>0.36***</td>
<td>0.45***</td>
<td>0.28***</td>
<td>–0.39***</td>
<td>–</td>
<td>0.92***</td>
<td>–0.03 ns</td>
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<td></td>
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<td>0.09 ns</td>
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<td>0.09 ns</td>
<td>–0.39***</td>
<td>SPG 2014</td>
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<tr>
<td>SPG 2013</td>
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<td>–0.15 ns</td>
<td>0.51***</td>
<td>–0.37***</td>
<td>0.35***</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>STH 2013</td>
<td>–0.43**</td>
<td>–0.46***</td>
<td>0.05 ns</td>
<td>0.52***</td>
<td>–0.04 ns</td>
<td>0.60***</td>
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* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

† Data above and below the diagonal are correlation coefficients at Sutherland and SPG in 2013 and 2014, respectively.
‡ ns, nonsignificant; TSW, 1000-seed weight; SD, seed diameter; TH, seed thickness; PLU, seed plumpness; DE, dehulling efficiency; MR, milling recovery; FR, football recovery.
infection with 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. 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 et al., 1991b; Wang, 2008; Shahin 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 modifications 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 milling traits had moderate to low heritability for DE, MR, and FR. However, the contribution of genetic factors to total variability for DE and MR were higher than location, year, and their interaction (Table 3). 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 et al. (1991b) reported medium to high heritability for DE in lentil in a Mediterranean climate where seed development occurs during a dry period with increasing temperatures that is not typically affected by wet weather as experienced in temperate regions.

We 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 QTLs for seed shape, particularly seed diameter, seed weight, and seed plumpness, were detected by Fedoruk et al. (2013) (Fig. 3 and Supplemental Fig. S1). This suggests that seed shape and milling traits, particularly DE and FR, are inherited together through linkage, 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 Sutherland 2014 on LG2 (Fig. 3) but on the same genomic region on LG2, Fedoruk et al. (2013) found 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 (Supplemental Fig. S1). This might have been caused by significant but moderate correlations between seed diameter or seed thickness and DE or MR (Table 5).

Seed coat chemistry may also influence DE or MR. In some site–years, we observed QTLs 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 the polyphenolic compounds present in seed coats of lentil. These compounds are also influenced by environment (Mirali et al., 2017). There is also evidence that the polyphenol profile changes over time; for example, storage of seeds with green seed coats results in biochemical changes that make the seed coats more brittle (Mirali et al., 2016).

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–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 in lentil breeding programs. Phenotyping for these traits is a time-consuming, laborious, and expensive endeavor and the heritability is moderate to low. As a result, breeding material cannot be selected for milling quality traits in early generations. In contrast, marker-assisted selection 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 one biparental 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.

Conclusions
We identified multiple QTLs related to milling quality in a biparental 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 develop plump seeds with greater diameter, and higher seed weight in cases where this does not interfere with market preference.

Supplemental Information
Supplemental Table S1: Site description and cultural practices.
Supplemental Table S2: Mean daily temperature and total monthly precipitation.
Supplemental Table S3: Genotyping calls.
Supplemental Table S4: Seed and milling characteristics.
Supplemental Fig. S1: QTLs for seed characteristics.

Conflict of Interest Disclosure
The authors declare that there is no conflict of interest.

Acknowledgments
The authors are grateful to Michael J. Fedoruk and Rob Stonehouse for providing genomic data and the linkage map for this study. Special thanks go to the field crew (Brent Barlow, Helen Atuku, Anoja Weerasinghe, Densmaw Chulumbaatar, Jannatul Ferdose, Scott Ife, and Lijiljana Pelemis) of the Pulse Crop Research Field Laboratory, University of Saskatchewan for providing technical assistance. This research was supported by the Agriculture Development Fund of the Saskatchewan Ministry of Agriculture, the Department of Plant Sciences of the University of Saskatchewan, the Saskatchewan Pulse Growers, and the Government of Canada through the Natural Sciences and Engineering Research Council of Canada Industrial Research Chair in Lentil Genetic Improvement.

References


