Quantitative Trait Loci Analysis of Seed Quality Characteristics in Lentil using Single Nucleotide Polymorphism Markers

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Abstract
Seed shape, color, and pattern of lentil (Lens culinaris Medik. subsp. culinaris) are important quality traits as they determine market class and possible end uses. A recombinant inbred line population was phenotyped for seed dimensions over multiple site-years and classified according to cotyledon and seed coat color and pattern. The objectives were to determine the heritability of seed dimensions, identify genomic regions controlling these dimensions, and map seed coat and cotyledon color genes. A genetic linkage map consisting of 563 single nucleotide polymorphisms, 10 simple sequence repeats, and four seed color loci was developed for quantitative trait loci (QTL) analysis. Loci for seed coat color and pattern mapped to linkage groups 2 (Ggc), 3 (Tgc), and 6 (Scp) while the cotyledon color locus (Yc) mapped to linkage group 1. The broad sense heritability estimates were high for seed diameter (broad-sense heritability \([H^2] = 0.92\)) and seed plumpness \([H^2] = 0.94\)) while seed thickness \([H^2] = 0.60\) and days to flowering \([H^2] = 0.45\) were more moderate. There were significant seed dimension QTL on six of the seven linkage groups. The most significant QTL for diameter and plumpness was found at the cotyledon color locus \((Yc)\). The markers identified in this study can be used to help enrich breeding populations for desired seed quality characteristics, thereby increasing efficiency in the lentil breeding program.

Lentil is a crop that is consumed for its high levels of protein, vitamins, and minerals (Raghuvanshi and Singh, 2009). Lentil has become an important crop for the northern Great Plains of North America with acreage steadily increasing to meet a growing global demand for this pulse crop. Maintaining the quality of lentils for the end users is an important objective for the industry. The seed coat color and pattern are important characteristics in determining the market class and the end uses of the crop. The cotyledon color similarly decides the end use, with red cotyledon cultivars largely being milled for the dal market and yellow cotyledon cultivars being consumed whole. The diameter, thickness, and weight of the disc-shaped seeds are also considered important parameters in reaching optimum quality. The shape of the seed is usually interpreted as seed plumpness, which is a ratio of seed thickness and seed diameter. Erskine et al. (1991) showed that seed size influences dehulling efficiency and Wang (2008) found differences in milling efficiency among varieties exhibiting variation in seed shape. Their research showed that thinner lentils had higher degree of damage when dehulled while thicker and plumper varieties were less affected, resulting in a greater retention of dehulled, unsplint cotyledons (“footballs”), and dehulled split lentils. Shahin et al. (2012) found that plumper samples of the lentil cultivar CDC Blaze showed higher levels of dehulling efficiency compared to less plump samples.

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**Abbreviations:** CDC, Crop Development Centre; DTF, days to 50% flowering; \([H^2]\), broad-sense heritability; KASP, competitive allele-specific polymerase chain reaction; LG, linkage group; LOD, logarithm of the odds; MAS, marker-assisted selection; QTL, quantitative trait loci; RIL, recombinant inbred line; SNP, single nucleotide polymorphism; SPG, Saskatchewan Pulse Grower’s land; U of S, University of Saskatchewan.
Determining seed size in lentil has historically relied on measuring the 100 or 1000 seed weight (Erskine et al., 1985; Abbo et al., 1991; Tahir et al., 1995; Tullu et al., 2001). However, this method cannot distinguish different seed shape parameters such as seed thickness or seed plumpness from seed diameter. Hossain et al. (2010) noted that the traditional method of seed sizing using graded sieves can be just as effective for determining seed dimensions. This, however, still means that phenotyping seed size and especially seed plumpness can be time consuming and tedious in a breeding program. Selection of these traits using marker-assisted selection (MAS) could be a valuable, time saving venture if markers linked to the genes controlling these dimensions could be identified.

Quantitative traits have been mapped in lentil for the purpose of associating molecular markers with phenotypic traits. However, very few molecular markers are used in lentil breeding because many of the molecular markers are not reproducible in multiple populations or are difficult and too expensive to screen (Vail, 2010; Ford et al., 2009). The recent development of high-throughput highly parallel multiplex assays allows for the genotyping of many individuals with thousands of single nucleotide polymorphism (SNP) markers all at once. These assays can greatly increase the chances of developing useful functional markers that would be available for MAS. These types of platforms have already been developed for crops such as barley (Hordeum vulgare L.), corn (Zea mays L.), apple (Malus domestica Borkh.), and others (Close et al., 2009; Yan et al., 2009; Chagné et al., 2012). Recently, we developed a lentil 1536-SNP Illumina GoldenGate array that is reproducible and locus specific in the L. culinaris subsp. culinaris genetic background (Sharpe et al., 2013). This array was constructed using SNPs discovered in expressed sequence tag sequences from nine L. culinaris subsp. culinaris and two Lens ervoides (Brig.) Grande accessions. A linkage map was developed using this assay in the lentil recombinant inbred line (RIL) population LR-18. The LR-18 population was originally developed to study the inheritance of disease resistance (Tar’an et al., 2003). With these SNP resources, it is now possible to map quantitative traits, such as seed weight and plumpness, which are segregating in this population.

Past studies of seed-related quantitative trait loci (QTL) in several crops have uncovered loci controlling more than one related trait. In lentil, a flowering time locus was shown to be linked with the seed coat pattern locus (Sarker et al., 1999). Flowering or, more specifically, preanthesis and postanthesis periods influence seed size in different crops (Gupta et al., 2006). For example, preanthesis changes in vegetative organs can affect the amount of assimilates that are partitioned to the seed while they are developing. Similarly, postanthesis processes can affect the time for maturation or grain filling, which could change the seed size. Loci controlling flowering time or other flower morphology traits have also been associated with seed weight or seed dimension loci in model legume crops (Ohito et al., 2005; He et al., 2010; Wang et al., 2012). In chickpea (Cicer arietinum L.), Hovav et al. (2003) studied how a major flowering time gene, PPD, affected seed weight. They found that earlier flowering resulted in reduced seed weight, which could lead to lower yield and quality. This would affect how cultivars are selected, because it could be difficult to select for early flowering without affecting seed size. Therefore, it is important to note that when selecting markers linked to QTL for seed size, some other traits can influence or even mask the QTL of interest.

The purpose of this study was to develop a gene-based SNP linkage map based on a population segregating for cotyledon and seed coat color and pattern as well as seed shape. The map was used to detect the genomic regions associated with seed size and shape and days to 50% flowering (DTF) that were evaluated in two different locations in Saskatchewan, Canada. The objective was to develop functional SNP markers associated with QTL of these traits for routine MAS in the breeding program.

Materials and Methods

Plant Material

The RIL population, LR-18, was developed from a cross between ‘CDC Robin’ (Vandenbergh et al., 2002) and the breeding line 964a-46 from the lentil breeding program at the Crop Development Centre (CDC), University of Saskatchewan (U of S) (Tar’an et al., 2003). CDC Robin produces seeds that are small in diameter but relatively plump with red cotyledons. Line 964a-46 produces seeds that are large in diameter but not as plump (Fig. 1), and the cotyledons are yellow.

A total of 139 F$_{2}$-derived RILs were assessed at two different locations: one in Saskatoon (Preston) and one 15 km southeast of Saskatoon on the Saskatchewan Pulse Grower’s land (SPG) in both 2009 and 2011. The RILs were grown in 1 m$^2$ microplots in a randomized complete block design with three replicates. In 2009, the trials were sown at the SPG and Preston sites on 12 May and 20 May, respectively, while in 2011 they were sown on 14 May and 19 May at Preston and SPG, respectively.

Genetic Map

The LR-18 population had already been mapped with a set of 484 markers from a 1536-SNP Illumina GoldenGate assay, 56 single SNPs genotyped using fluorescence-based competitive allele-specific polymerase chain reaction (KASP) assays (KBioscience, Hoddeston, UK), and 9 simple sequence repeats (Sharpe et al., 2013). The genes controlling cotyledon color (Yc), seed coat pattern (Scp), and two seed coat ground color genes (Ggc and Tgc) were segregating so could be scored as individual loci in LR-18 (see Phenotyping below) and were included in the map as additional markers. A further 13 polymorphic SNPs were genotyped using KASP assays designed around SNPs in additional lentil genes. These markers were combined and analyzed using maximum likelihood analysis and a minimum logarithm of the odds (LOD) of 6 in JoinMap 4.0 (Van Ooijen, 2006) to identify linkage...
groups (LGs). These groups were compared to those of the original map (Sharpe et al., 2013) to confirm LGs and ordered using regression mapping using the Kosambi mapping function to develop the map used for QTL analysis. All genotyping information, including the contig sequences of the SNP markers, can be found in the U of S Pulse Crop Breeding Group’s KnowPulse database accessible through a web portal (http://knowpulse2.usask.ca/portal/; accessed 20 July 2012). Sequence data for the additional markers is available in Supplemental File S1.

Phenotyping

The number of DTF was recorded for all plots at all locations. The harvested seed samples of each plot were measured for seed diameter and seed thickness using round-hole and slotted sieves, respectively, as described by Hossain et al. (2010). Seed diameter was measured by passing at least 50 g of a sample through a set of seven round-holed sieves from 5.8 mm (15/64 inch) down to 3.6 mm (9/64 inch) in 0.25 mm (1/64 inch) increments. Thickness was measured by passing the same samples through a set of six slotted sieves from 2.8 mm (7.5/64 inch) down to 2.0 mm (5/64 inch) in 0.2 mm (0.5/64 inch) increments. All samples were shaken through the sieves for 1 min on a flat-bed shaker before weighing the seed retained on each sieve. Seed diameter and thickness for each sieved fraction of the sample was calculated as a weighted mean using the formulas

\[
% \text{ on sieve} = \frac{\text{mass on sieve}}{\text{mass total sample}} \times 100,
\]

\[
\text{mean seed diameter} = \frac{\sum(\% \text{ on round sieve/100} \times \text{ sieve hole size})}{\text{num samples}}, \quad \text{and}
\]

\[
\text{mean seed thickness} = \frac{\sum(\% \text{ on slotted sieve/100} \times \text{ sieve slot size})}{\text{num samples}},
\]

in which mass in measured in grams and hole size and slot size are measured in millimeters. Seed plumpness was calculated by dividing the seed thickness (Eq. [3]) by the seed diameter (Eq. [2]) for seed from each plot. The values were then checked for correlation with the values generated by the seed screening method.

Yellow or red cotyledon color (Yc) (Slinkard, 1978) was determined for each of the RILs. Seed coat ground color phenotypes (brown, grey, tan, and green) were recorded to genotype the four allelic combinations of the Ggc and Tgc loci and the two seed coat pattern phenotypes (absent or patterned) were recorded to genotype the Scc locus (Vandenberg and Slinkard, 1990).

Statistical Analysis

The years and locations of the field trials were treated as site–years. All statistical analyses were performed using the software R v2.11.1 (R Development Core Team, 2011). A linear mixed model was fit using replications and site–years as random factors while the genotypes were considered fixed. The R package nlme was used to fit the linear mixed model using the lme function (Pinheiro and Bates, 2000). The R package lme4 was used to calculate the variance components under a mixed model using the function lmer (Bates et al., 2011).

Quantitative Trait Loci Analysis

Quantitative trait loci analysis was conducted using MapQTL 5.0 (Van Ooijen, 2004) and the LR-18 genetic linkage map. One thousand permutation tests were run to determine the LOD threshold value. A value of 3.0 was determined and used to declare significant QTL. Interval mapping was used for each location and year. Markers that showed high LOD values were selected as co-factors and through composite interval mapping were reanalyzed for QTL.

Results

Genetic Map

An additional set of 17 markers, consisting of 13 SNPs and the four seed color genes, were added to the Sharpe et al. (2013) genetic map of the LR-18 population. At total of 561 markers were mapped resulting in a map with a total length of 697 cM in seven LGs (Fig. 2). The map had an average distance of 1.2 cM between markers. The length of each LG varied: LG 2 was the longest with a total length of 150 cM whereas LG 7 was the shortest with a length of 57 cM. The cotyledon color gene, Yc, mapped to LG 1 while the two seed coat ground color
Genes, Ggc and Tgc, mapped to LG 2 and LG 3, respectively. The seed coat pattern gene (Scp) mapped to LG 6.

**Phenotypic Data**

The mean temperature and the total precipitation for Saskatchewan were collected for each month of the growing season (May–August) for both years from the Environment Canada website (http://climate.weather.gc.ca/index_e.html; accessed 20 July 2012) and are listed in Table 1. The 2009 growing season had greater precipitation (215 mm) but a lower mean temperature (13.8°C) than 2011, which had total precipitation of 197 mm and a mean temperature of 15.5°C.

The seed diameter, thickness, and plumpness values that were determined using the sieve screening method were correlated with the values measured with a caliper. Seed diameter ($r = 0.90$), seed thickness ($r = 0.88$), and seed plumpness ($r = 0.91$) all had significant ($P < 0.05$) correlations between the two methods of measurement.

Analysis of variance revealed that the genotype effect was highly significant ($P \leq 0.001$) for all seed dimension traits and flowering time (Table 2). Site–year was significant for seed thickness, seed plumpness, and DTF and the genotype × site–year interaction was significant for all traits.

The mean seed thickness and seed plumpness in the 2009 growing season were greater than in 2011 while there was no difference in seed diameter among site–years (Fig. 2). The 2009 SPG site had the greatest seed thickness and also the greatest seed plumpness compared to the other site–years. Days to flowering had the most site–year variability amongst all the traits measured. The SPG site had longer DTF for both 2009 and 2011 compared to the Preston site (Fig. 2).

Pearson’s correlation coefficients were calculated for all the traits measured within each site–year (Table 3). Seed diameter and seed plumpness showed the highest significant correlation with all site–years averaging a negative correlation of $r = -0.90$. All site–years for seed diameter and seed thickness were significant but remained below $r = 0.40$. Only two of the four site–years had a significant correlation between seed thickness and seed plumpness. Days to 50% flower was significantly correlated with all seed dimension traits for all site–years and had the highest correlation with seed plumpness, averaging $r = 0.36$.

Variance components were used to calculate the heritability of each trait (Table 4). Seed diameter and plumpness were highly heritable (0.92 and 0.94, respectively) while seed thickness had a more moderate heritability (0.60) and the heritability of DTF was even lower (0.45).

**Quantitative Trait Loci Analysis**

Quantitative trait loci were located on six of the seven LGs (Table 5; Fig. 3). Three different QTL were identified for seed diameter, all of them present in all site–years.
Table 1. Mean temperature (°C) and precipitation (mm) for the 2009 and 2011 growing seasons at Saskatoon, SK.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean temperature, °C</td>
<td>8.7</td>
<td>10.9</td>
<td>14.8</td>
<td>15.5</td>
<td>15.8</td>
<td>18.4</td>
<td>15.9</td>
<td>17.2</td>
</tr>
<tr>
<td>Total precipitation, mm</td>
<td>6.9</td>
<td>17.5</td>
<td>75.5</td>
<td>94.4</td>
<td>50.3</td>
<td>68.6</td>
<td>82.4</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Table 2. Analysis of variance results, including F-values, for lentil seed dimensions and days to 50% flowering (DTF) for genotype and environmental effects.

<table>
<thead>
<tr>
<th>Effect</th>
<th>df</th>
<th>Seed diameter</th>
<th>Seed thickness</th>
<th>Seed plumpness</th>
<th>DTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>146</td>
<td>114.0***</td>
<td>15.5***</td>
<td>150.8***</td>
<td>8.26***</td>
</tr>
<tr>
<td>Site–year</td>
<td>3</td>
<td>2.9 ns†</td>
<td>16.5***</td>
<td>445.5***</td>
<td>468.04***</td>
</tr>
<tr>
<td>Genotype × site–year</td>
<td>438</td>
<td>2.1***</td>
<td>1.9***</td>
<td>2.6***</td>
<td>1.96***</td>
</tr>
<tr>
<td>CV²</td>
<td>8.11</td>
<td>5.05</td>
<td>8.55</td>
<td>8.54</td>
<td></td>
</tr>
</tbody>
</table>

***Significant at the 0.001 probability level.
†ns, not significant.

The most important QTL was located around the cotyledon color locus (Yc) on LG 1 and explained over 23% of the variation at all site–years. The closest SNP marker to Yc was LcC13114p356, which was located just over 1 cM away. The other major QTL for seed diameter were linked near the SNP markers LcC00853p101 and LcC00890p1387 on LG 2 and LG 7, respectively. These three QTL combined explained at least 60% of the variation for seed diameter for all site–years. The additive effect results indicated that the seed diameter allele for increased diameter at all three markers came from the large seed diameter parent, 964a-46 (Table 5).

Seed thickness QTL were detected on all LGs except LG 3 (Fig. 3). There were multiple QTL that were specific to each site–year. The QTL that was most stable throughout the different site–years was located on LG 7, explaining an average of 8.4% of the variation in three site–years. The additive effects showed that the allele contributing to the QTL for increased seed thickness came from CDC Robin (Table 5).

Seed plumpness QTL were present on LG 1, LG 2, and LG 4 (Fig. 3). Seed plumpness shared the same QTL on LG 1 with seed diameter at the cotyledon color (Yc) locus (Table 5). The plumpness QTL present on LG 7 also shared the same marker locus (LcC00890p1387) with a seed diameter QTL. The third plumpness QTL linked to the SNP marker LcC00853p101, once again mapped to the same location as a seed diameter QTL on LG 2. The QTL on LG 1 and LG 7 together explained the majority of the variation with their combined values explaining over 50% of the variation for each site–year. The QTL located on LG 2 explained less than 10% of the variation.

Quantitative trait loci identified for DTF were located on LG 1, LG 2, and LG 7. The QTL on LG 1 was the only one that was significant in multiple site–years and was located in the same genomic region as the Yc marker and, therefore, the seed diameter and seed plumpness QTL.

Discussion

In this study, seed diameter, seed thickness, seed plumpness, and DTF were phenotyped in the lentil mapping population LR-18 and then analyzed for QTL. This is the first study to map seed dimension QTL in an intraspecific lentil population. This is also the first reported study in lentil using SNP markers for QTL mapping.

The genetic map developed in this study is the most condensed linkage map of lentil to date. The physical size of the lentil genome is estimated to be 4086 Mb (Arumuganathan and Earle, 1991). Based on the size of the map developed in this study, an average 5.9 Mb cM⁻¹ was covered. The morphological markers that mapped in this study have also previously been mapped onto separate
Table 5. Quantitative trait loci identified for lentil seed dimensions and days to 50% flowering (DTF) over four site–years.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Linkage group</th>
<th>Trait†</th>
<th>Site‡</th>
<th>Position</th>
<th>LOD§</th>
<th>% Exp.¶</th>
<th>Add. effects§§</th>
</tr>
</thead>
<tbody>
<tr>
<td>LcC20026p128</td>
<td>1</td>
<td>Thickness</td>
<td>Pres 2011</td>
<td>71.4 cM</td>
<td>12.35</td>
<td>25.5</td>
<td>0.03</td>
</tr>
<tr>
<td>LcC09777p203</td>
<td>4</td>
<td>Thickness</td>
<td>SPG 2009</td>
<td>61.25 cM</td>
<td>4.01</td>
<td>11.1</td>
<td>-0.01</td>
</tr>
<tr>
<td>LcC06044p758</td>
<td>1</td>
<td>Diameter</td>
<td>Pres 2009</td>
<td>63.4 cM</td>
<td>8.51</td>
<td>23.5</td>
<td>-0.02</td>
</tr>
<tr>
<td>LcC05579p160</td>
<td>5</td>
<td>Diameter</td>
<td>SPG 2011</td>
<td>7.5 cM</td>
<td>28.64</td>
<td>35.6</td>
<td>0.02</td>
</tr>
<tr>
<td>LcC04409p171</td>
<td>7</td>
<td>Diameter</td>
<td>SPG 2011</td>
<td>13.3 cM</td>
<td>3.08</td>
<td>7.2</td>
<td>-0.01</td>
</tr>
<tr>
<td>LcC02348p98</td>
<td>7</td>
<td>Diameter</td>
<td>Pres 2009</td>
<td>13.3 cM</td>
<td>3.08</td>
<td>7.2</td>
<td>-0.01</td>
</tr>
<tr>
<td>LcC00890p1387</td>
<td>2</td>
<td>Diameter</td>
<td>Pres 2009</td>
<td>7.5 cM</td>
<td>26.79</td>
<td>32.4</td>
<td>0.01</td>
</tr>
<tr>
<td>LcC00835p101</td>
<td>2</td>
<td>Diameter</td>
<td>SPG 2011</td>
<td>7.5 cM</td>
<td>29.71</td>
<td>35.6</td>
<td>0.02</td>
</tr>
<tr>
<td>LcC05284p449</td>
<td>6</td>
<td>Thickness</td>
<td>Pres 2009</td>
<td>45.3 cM</td>
<td>4.01</td>
<td>11.1</td>
<td>-0.03</td>
</tr>
<tr>
<td>LcC05323p332</td>
<td>7</td>
<td>Thickness</td>
<td>SPG 2011</td>
<td>7.5 cM</td>
<td>3.7</td>
<td>12.35</td>
<td>25.5</td>
</tr>
<tr>
<td>LcC05579p160</td>
<td>5</td>
<td>Thickness</td>
<td>SPG 2009</td>
<td>7.5 cM</td>
<td>3.7</td>
<td>12.35</td>
<td>25.5</td>
</tr>
<tr>
<td>LcC06044p758</td>
<td>1</td>
<td>DTF</td>
<td>Pres 2011</td>
<td>7.5 cM</td>
<td>3.7</td>
<td>12.35</td>
<td>25.5</td>
</tr>
<tr>
<td>LcC020026p128</td>
<td>1</td>
<td>Thickness</td>
<td>Pres 2011</td>
<td>71.4 cM</td>
<td>3.86</td>
<td>10.1</td>
<td>-0.02</td>
</tr>
<tr>
<td>LcC23363p108</td>
<td>2</td>
<td>DTF</td>
<td>SPG 2011</td>
<td>76.9 cM</td>
<td>3.48</td>
<td>6.2</td>
<td>-0.24</td>
</tr>
</tbody>
</table>

†Diameter, seed diameter; Plumpness, seed plumpness; Thickness, seed thickness.
‡Pres, Preston plots in Saskatoon; SPG, Saskatchewan Pulse Grower’s land.
§LOD, logarithm of the odds.
¶% Exp., percent of variability explained by this locus.
§§Add. effects, additive effect attributed to this locus. Negative values represent alleles originating in 964a-46; positive values represent alleles originating from CDC Robin.

Studies in other crops have revealed that seed size related traits, such as seed width, seed length, and seed weight, are complex and highly influenced by the environment (Cobos et al., 2007; Breseghello and Sorrells, 2007). Previous studies of lentil involving seed weight QTL (Fratini et al., 2007; Abbo et al., 1991) have not addressed environmental interactions. In the current study, significant genotype × environment interactions were detected. One of the routine observations that is made for lentil seed development is that seed diameter appears to reach its maximum size quickly and so is somewhat buffered against variability in the environment. The QTL analysis supports this as all three seed diameter QTL were observed in all environments. Seed thickness, however, does appear to be more subject to environmental variability, with ideal growing conditions resulting in thicker seed. The 2009 and 2011 growing seasons differed in Saskatchewan. The mean temperature for the months of May to August in 2009 was 13.8°C while for 2011 it was 15.5°C (Environment Canada, 2012). The total precipitation from May to August for 2009 and 2011 were 215 mm and 197 mm, respectively. The 2009 growing year had nearly 50 mm greater precipitation during the seed filling months of July and August compared to 2011. The greater moisture availability after flowering in the 2009 growing season would have contributed to increased seed thickness and plumpness. There were no significant differences in variability of seed diameter, however, across the different environmental conditions. In season precipitation distribution may also affect seed dimensions differentially on the same plant since flowering is acropetally indeterminate.

There was considerable variation observed in DTF among the site–years. Lentil plants have a long day quantitative response that is also influenced by temperature. At the latitude of Saskatoon, adapted lentil plants normally begin flowering around 1 July, regardless of planting date, and the plants continue to flower well into August. This would result in differences in the number of days it will take to reach flowering from site to site but will also
Figure 3. Genetic linkage map of lentil indicating seed color genes (Yc, Tgc, Ggc, and Scp) and quantitative trait loci (QTL) for seed diameter, seed thickness, seed plumpness, and days to flowering from the LR-18 population (CDC Robin × 964a-46). Quantitative trait loci are marked next to the loci they are associated with and the respective site-years they were significant in. The thin lines represent the regions that were significant using interval mapping while the boxes represent the regions significant under composite interval mapping. Quantitative trait loci boxes marked with an asterisk (*) represent QTL that had a significant \( P > 0.05 \) logarithm of the odds value for all site-years. There were no QTL on linkage group (LG) 3. SPG, Saskatchewan Pulse Grower’s land.
compress variation in DTF within a site. The plots at SPG in 2009 had an extra week to accumulate biomass before flowering, relative to those at the Preston site, which could have resulted in more assimilates being transmitted to the developing seed and therefore the thicker and plumper seeds that were seen at that location (Fig. 2).

No studies in lentil seed development have described the underlying mechanisms that control why certain traits in lentil seed development are more susceptible to environmental differences. However, there are a number of studies in other legume crop species where seed development has been more closely examined (e.g., Le et al., 2007). Domoney et al. (2006) also highlighted that there are largely two distinct phases in legume seed development that influence seed diameter and seed thickness and, therefore, seed plumpness. The first developmental phase, cell division, depends on the embryo genotype, which controls the cotyledon cell number and is largely insensitive to environmental variability. Due to these low environmental interactions and high heritability, seed diameter and, therefore, seed plumpness can be considered to be highly influenced by loci that are regulated in this developmental phase. The second phase, cell expansion, is highly influenced by the environment and is regulated by loci involved in photosynthate partitioning. Seed thickness would be largely determined in this developmental phase. Loci that could influence the rate of photosynthate partitioning to the seed could be contributing factors to this genetic variability.

The level of environmental variation for each trait was reflected in the heritability estimates: both seed diameter and plumpness had very high heritability, but the heritability of seed thickness was more moderate. Results presented in this study showed much higher heritability for seed size and shape relative to other legume crops. In soybean \([Glycine\ max\ (L.)\ Merr.],\ \) Cober et al. (1997) found the heritability for seed size (cross-sectional area) ranged from 0.26 to 0.50, and for seed shape (ratio of minimum to maximum seed diameter) heritability ranged from 0.59 to 0.75 among four populations. In common bean \((Phaseolus\ vulgaris\ L.),\ \) the heritability for seed shape has been estimated at 0.61 (Genchev, 2006). However, both soybean and common bean have different morphological seed characteristics compared to lentil. Like many legume species, their seed shape is determined by a three axial dimension (length \(\times\) width \(\times\) thickness) instead of the two axial (diameter \(\times\) thickness) ratio of lentil. Lentil DTF had the lowest heritability (0.45), which is probably a reflection of the noticeable difference between the average days to flowering among the site–years. These results for flowering time agree with the findings of Tullu et al. (2008) who found that DTF in their lentil population had even lower heritability of 0.31.

One of the justifications for using MAS is that the trait that is being selected has a low heritability. Traits with high heritability such as those observed for seed diameter and seed plumpness in this study are not typical candidates for MAS. However, the lentil breeding program at the CDC often makes three-way or multiparent crosses. Some male parents of the final cross would be heterozygous, which would result in heterogametic progeny (Singh, 1994). Marker-assisted gamete selection of the resulting F\(_1\) s could help enrich the populations for beneficial alleles thereby increasing the efficiency of subsequent field level selection. Not to mention, sieving seed samples is resource intensive and requires at least a 50-g sample so is not practical for early generation selection.

Quantitative trait loci analysis was used to dissect the quantitative nature of these traits and identify regions of the genome that contributed to the genetic variability in this population. It was observed that all seeds with large diameter had yellow cotyledons and the RILs with smaller diameter seeds had red cotyledons suggesting a high level of linkage between seed diameter and cotyledon color. It was not surprising, therefore, to discover that the seed diameter QTL that explains the highest level of variation was linked to the \(Yc\) locus. These findings agree with Abbo et al. (1991) who mapped a seed weight QTL to the \(Yc\) locus in both intraspecific \([L.\ culinaris\ subsp.\ culinaris\ \times\ L.\ culinaris\ subsp.\ orientalis\ (Boiss.)\ Ponert]\) and interspecific \((L.\ culinaris\ subsp.\ culinaris\ \times\ L.\ ervoides)\) populations. In chickpea, seed weight and \(\beta\)-carotene, a carotenoid that controls cotyledon color, also share the same QTL (Abbo et al., 2005). However, when Fratini et al. (2007) mapped the cotyledon color marker in an F\(_2\) population derived from an intraspecies cross between \(L.\ culinaris\ subsp.\ culinaris\ \times\ L.\ culinaris\ subsp.\ orientalis,\ \) they found no association between this locus and seed diameter. Tullu et al. (2001) also found that there was large variation in seed weight compared to cotyledon color when analyzing a lentil core collection. This suggests that the linkage between seed diameter and cotyledon color may be specific to certain populations, including LR-18. Furthermore, within the lentil breeding program at the CDC, a small number of lines derived from crosses between large, yellow cotyledon and small, red cotyledon parents have been identified that appear to have had this linkage broken, with the result being large reds and small yellows.

All QTL for seed diameter, seed plumpness, and DTF were significant in all site–years. Seed thickness QTL were detected on six of the seven LGs; however, only three QTL were significant in multiple site–years. These results highlighted the importance of genotype \(\times\) environment interactions for seed thickness. Although seed thickness was significantly correlated with seed diameter and DTF at all site–years, no QTL for seed thickness were shared with the other seed dimension or DTF QTL.

For seed plumpness, all three QTL reported were also located in the same region as seed diameter QTL. Sharing the same QTL for seed diameter and seed plumpness was expected because the correlations between the two traits were high, ranging from –0.88 to –0.92, and were significant for all site–years. The seed plumpness calculation is also a function of seed diameter. Salas et al. (2006) evaluated seed shape traits in soybean and found that there were certain QTL regions that controlled multiple seed traits such as...
seed length, height, weight, and volume. This suggests that certain seed quality and morphological traits in lentil and in other legume crops are inherited together, either through linkage or pleiotropy, making it difficult to select for each trait independent of the other. In retrospect, the LR-18 population may not have been ideal for studying seed plumpness in lentil. The high correlation between diameter and seed plumpness suggests that in this population seed diameter highly influences the level of plumpness and that key genes for thickness are not segregating in this population. A possible solution would be to use a mapping population where the two parents have nearly the same seed diameter but differ in their seed thickness.

Since seed diameter and seed plumpness were correlated, it seems that dividing the values of seed diameter and seed thickness to get seed plumpness may have caused artificial inflation in the correlation between those traits. There are an increasing number of phenotyping software options such as Tomato Analyzer (Rodriguez et al., 2010) and SmartGrain (Tanabata et al., 2012) that could be used to phenotype seed plumpness in lentil, independent of seed diameter. In addition, methods have been developed at the Canadian Grain Commission to estimate seed plumpness in lentil using a series of digital images (Shanin et al., 2006). These methods could help contribute to a more accurate phenotyping of seed shape characteristics in lentil.

Days to 50% flowering was significantly correlated with all the seed size and shape traits albeit all correlation values were below 0.50 (Table 2). The only QTL significant in multiple site-years was located on LG 1 at the Yc locus, the same as seed diameter and seed plumpness. This suggests that if this marker were used to select for increased seed diameter and plumpness, indirect selection for increased DTF would result. In soybean and common bean, DTF and seed size QTL have also been mapped to coincident regions (Watanabe et al., 2004; Pérez-Vega et al., 2010). Another example is the AP2 gene in Arabidopsis, which is known to control both flower development and seed size (Jofuku et al., 1994).

Many genes are associated with DTF, any of which could contribute to genetic differences in DTF in a given population. Fratini et al. (2007) mapped a single QTL for DTF near Scp on LG 6 but found no seed weight or seed diameter QTL nearby. No QTL for DTF were observed on LG 6 in the LR-18 population suggesting the gene controlling DTF in their population was different. The Saskatchewan growing season for lentil is relatively short at 90 to 100 d so there is little variation for DTF within populations derived from crosses between adapted parents. It is, therefore, not surprising that only one DTF QTL was consistently significant in the LR-18 population.

With the availability of high throughput genome scans for lentil, association mapping can now be applied to diverse material. The higher levels of recombination contributing to the lower levels of linkage disequilibrium found in more diverse germplasm could result in finer mapping of markers. This could also result in less co-inheritance of traits. These types of studies could lead to developed of improved strategies for MAS breeding for specific seed dimensions of lentil.

### Supplemental Information Available

Supplemental material is available at http://www.crops.org/publications/tpg.

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