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Research Article

Inheritance and Linkage Map Positions of Genes Conferring Agromorphological Traits in *Lens culinaris* Medik.

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Agromorphological traits have immense importance in breeding lentils for higher yield and stability. We studied the genetics and identified map positions of some important agro-morphological traits including days to 50% flowering, plant height, seed diameter, 100 seed weight, cotyledon color, and growth habit in *Lens culinaris*. Earlier developed RILs for stemphylium blight resistance (ILL-5888 × ILL-6002), contrasted for those agro-morphological traits, were used in our study. Three QTLs for days to 50% flowering were detected with additive and epistatic effects. One QTL for days to 50% flowering, $QLG4_{83}$ (QTL at linkage group 4 at 83 cM position), accounted for an estimated 20.2% of the variation, while $QLG1_{24} \times QLG13_{52}$ and $QLG4_{84} \times QLG13_{8}$ accounted for 15.6% and 24.2% of the variation, respectively. Epistatic effects accounted for most of the variation in plant height, but the main effect of one QTL, $QLG8_{4}$, accounted for 15.3%. For seed diameter, three QTLs were detected, and one QTL, $QLG4_{82}$, accounted for 32.6% of the variation. For 100 seed weight, five QTLs were identified with significant additive effects and four with significant interaction effects. The main effect of one QTL, $QLG4_{82}$, also accounted for 17.5% of the variation in seed diameter. $QLG4_{82\text{-}83}$ which appears to affect days to 50% flowering, seed diameter, and 100 seed weight is flanked by RAPD markers, UBC 34 and UBC1. Growth habit and cotyledon color are controlled by single genes with prostrate dominant to erect and red cotyledon dominant to yellow. The QTL information presented here will assist in the selection of breeding lines for early maturity, upright growth habit, and improved seed quality.

1. Introduction

Agromorphological traits have immense importance in crop breeding. Crop adaptation, field performance, market value, and demands for specific uses are major factors that drive breeding goals. Linkage maps and QTL analysis are valuable tools for plant breeders to improve breeding efficiency by tagging genes with markers and analyzing the association between markers and traits. The inheritance of quantitative traits and tagging genes such as days to 50% flowering, plant height, seed diameter, and seed weight and qualitative trait genes such as growth habit and cotyledon color in lentil (*Lens culinaris* Medikus subsp. *culinaris*) will help breeders in the selection process and understanding interrelationships among traits.

Lentil is quantitative long-day plant flowering in progressively longer days [1]. Sometimes it is hard to determine days to maturity at or near the end of a crop season due to weather conditions. It has been reported that soybean (*Glycine max*) breeders select lines for optimum maturity based on days to flowering [2]. Sarker et al. [3] reported that flowering time is sensitive to photoperiod and temperature, and that a more complete understanding of genetic control of flowering time in lentil is needed. Roberts et al. [1] proposed four developmental phases of flowering as: preemergence, preinductive, inductive, and postinductive. When lentil plants were transferred from short days (either 8 or 10 h) to long days (16 h), or vice versa, the first two phases and the last are insensitive to photoperiod but are probably sensitive to temperature. So, it is evident that a complex phenomenon controls days to flowering in lentil.

Plant height of lentil ranges from 25 to 30 cm for the majority of genotypes but may vary from extremes of 15 to

75 cm depending on genotype and environment [4]. Most lentils grown in South Asia, Middle East, and Africa are land races. These are generally short in plant stature, prostrate in growth habit, lack uniformity in pod maturation, have a high incidence of pod shattering, and are low yielding. Tall upright lentil cultivars with high basal pod positions are always preferred by farmers for mechanical harvesting [5]. Sakar [6] reported that three genes were responsible for variation in plant height from the cross of the two lentil cultivars, Laird and Precoz. Tullu et al. [7] reported that plant height is a polygenic trait and QTL detected for plant height has environmental effect.

Growth habit has received a great deal of attention from breeders attempting to develop cultivars with more upright stature that are also lodging resistant and adaptable to mechanized harvesting. Ladizinsky [8] indicated that a single gene with incomplete dominance controls growth habit in lentil.

Uniformity of seed size, shape, and color is important for marketing of lentil. A wide range of lentil cultivars are used throughout the world. Small diameter red cotyledon types account for most of the lentil production followed by the large-seeded and small-seeded yellow cotyledon types. Lentil seeds are lens shaped and generally weigh between 20 and 80 mg. Their diameter generally ranges from 2 to 9 mm. Seed size differs according to genotype, and researchers frequently follow the classification of Barulina [9] who grouped lentils as macrosperma with large seeds that range from 6 to 9 mm in diameter and microsperma with smaller seeds that range from 2 to 6 mm in diameter. It has been reported that dry seed weight of lentil is controlled by two genes [6], whereas polygenic control of seed weight was reported by Abbo et al. [10]. Cotyledon color of lentil can be red/orange, yellow, or green. Large green lentils with yellow cotyledons are marketed to countries of southern Europe, particularly Spain, Italy, and Greece, and small red cotyledon types are exported to South Asia and the Middle East [11]. The first report of genetics of lentil cotyledon color was studied by Tschermak-Seysenegg et al. [12, 13] and by Wilson et al. [14] and confirmed that cotyledon color is controlled by a single gene and red/yellow cotyledon is dominant over yellow. Singh [15] and Slinkard [16] reported that red cotyledon color is completely dominant over green and yellow. Sharma and Emami [17] detected monogenic and digenic control of cotyledon coloration in lentil.

The objectives of the present study were to identify regions of the lentil genome associated with agromorphological traits including days to 50% flowering, plant height, growth habit, seed diameter, seed weight, and cotyledon color that could be utilized in marker-assisted breeding and to improve our understanding of the genetics of these traits.

2. Materials and Methods

2.1. Development of Inbred Lines. In order to identify and map the agromorphological trait genes, a lentil mapping population (F_6 derived F_7 recombinant inbred lines (RILs)) that was previously developed to determine the genomic locations of the genes for stemphylium blight (caused by *Stemphylium botryosum* Wallr.) resistance was used [18]. The mapping population is comprised of 206 RILs from the cross ILL-5888 ("Uthfola" the popular name and described as a *glex pilosae* microsperma type; short stature with prostrate growth habit) by ILL-6002 (developed as a pure line selection from Argentinian variety, Precoz, macrosperma type; tall and erect in growth habit). These parents were contrasting for the agromorphological traits (quantitative and qualitative traits) under study.

2.2. Phenotyping. The parents and the mapping population of 206 RILs were grown in silty loam soil at the Washington State University Spillman Agronomy Farm near Pullman, Washington (46°73′N latitude and 117°73′W longitude), USA, in the 2007 and 2008 cropping year. The experimental design was a randomized complete block with three replications. Individual plots were single rows 1 meter long and spaced 60 cm apart and within row plant spacing of approximately 3 cm. Flowering was recorded as number of days from planting to 50% of the plants in the plot with at least one open flower. Plant height was taken at the 50% flowering stage and measured from the soil surface to the tip of the central axis. Seed diameter was measured by using sliding calipers, and 100 seed weight was taken using a Mettler digital balance. Growth habit data were taken based on plant canopy spreading, and cotyledon color was determined visually. All the qualitative and quantitative trait data taken were used for statistical analysis. The combined analysis of variance (ANOVA) of the quantitative traits was done using SAS 9.1.

2.3. Genotyping, Linkage Analysis, and QTL Mapping. DNA extraction and genotyping using simple sequence repeats (SSR), randomly amplified polymorphic markers (RAPD), and sequence-related amplified polymorphic (SRAP) markers were performed following protocols described in Saha et al. [18]. Mapmaker Macintosh V2.0 was used for linkage analysis, and QTL analysis for quantitative traits were conducted following inclusive composite interval mapping (ICIM) method with the following software: QTL IciMapping v2.2 [19] and Q gene 4.2.3 [20] as described by Saha et al. [18]. The intraspecific linkage map was developed with an LOD score of 4.0 and at maximum recombination fraction 0.25. The ICIM methods were used to detect QTLs for the quantitative traits due to the greater efficiency of detection. This method uses stepwise regression models for identifying the significant flanking markers and one-way scanning to identify additive effect and two-way scanning to identify digenic epistatic effect [19].

3. Results and Discussion

The agromorphological data of two parents (ILL-5888 and ILL-6002) and 206 RILs were collected at Spillman Agronomy Farm near Pullman in 2007 and 2008 cropping years. The combined ANOVA showed highly significant differences between the parents and among RILs for the four quantitative traits analyzed (Table 1). Highly significant ($P < 0.001$) correlations exist between seed diameter and 100 seed weight, and days to 50% flowering and seed diameter and 100

Table 1: Statistical parameters of quantitative traits of RILs developed from the ILL-5888 × ILL-6002 cross grown at Pullman, WA, USA, in 2007 and 2008 cropping years (combined).

Statistical parameters	Days to 50% flowering (days)	Plant height (cm)	Seed diameter (mm)	100 seed weight (g)
MSE	32.6^{***}	38.6^{***}	0.83^{***}	1.78^{***}
Mean ± sd	51.3 ± 3.5	25.1 ± 3.8	4.9 ± 0.5	3.2 ± 0.6
Range	47–58	15.7–34.3	4.0–6.3	2.0–4.9
ILL-5888	53	18.2	3.7	2.16
ILL-6002	60	27.8	6.5	5.59
Shapiro-Wilk value and probability	0.875, $P = 0$	0.992, $P = 0.466$	0.959, $P = 0.001$	0.965, $P = 0.003$

MSE: Mean sum of squares, and $^{***}P < 0.001$.

seed weight (Table 2). Plant height was highly significantly correlated with 100 seed weight ($P < 0.001$) and significantly correlated with days to 50% flowering and seed diameter ($P < 0.05$) (Table 2). The photoperiod sensitivity between two parental lines was also observed due to high genetic differences (data not shown).

To map the genes, an intra-specific lentil map of 14 linkage groups comprised of 23 SSRs (simple sequence repeats), 30 RAPDs and 108 SRAPs (sequence-related amplified polymorphisms) and two morphophysiological markers (cotyledon color and growth habit) was used [18].

3.1. Qualitative Trait Loci

3.1.1. Cotyledon Color. ILL-5888 and ILL-6002 have red and yellow cotyledon colors, respectively. The RILs segregated into a 1 : 1 ratio of red to yellow (Table 3). The clear 1 : 1 segregation ratio of the RILs was consistent with reports of the inheritance of cotyledon color by Tschermak-Seysenegg et al. [12, 13] and Wilson et al. [14]; that is, cotyledon color is controlled by a single gene. All F_1 seeds were red and the F_2 segregated in a 3 : 1 ratio of red to yellow (99 : 32), indicating that red cotyledon is completely dominant over yellow cotyledon. Singh [15] and Slinkard [16] also reported that red cotyledon color is completely dominant over green and yellow. The cotyledon color gene (*Yc*) was positioned on LG8 and flanked by RAPD marker UBC40b and SSR marker GLLC511a at 16.4 cM and 13 cM from *Yc*, respectively (Figure 1).

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

Ladizinsky [8] made crosses within and between lines of *L. culinaris* and *L. orientalis* that differed for growth habit: erect tall with few branches, erect bushy with many branches, and prostrate. He reported that prostrate growth habit was incompletely dominant over erect growth habit. Emami and Sharma [21] also showed that prostrate is dominant over erect growth habit. On the contrary, Kumar [22] and Mishra [23] reported erect growth habit as completely dominant over prostrate type. These suggest that multiple alleles may confer growth habit, so different inheritance patterns may be observed in different crosses.

3.2. Quantitative Traits and QTLs. The QTLs and epistatic interactions were identified in different linkage groups, but one of the QTLs, $QLG4_{82\text{-}83}$ (QTL at linkage group 4 at 82-83 cM), accounted for 20.2%, 32.6%, and 17.5% phenotypic variation for days to 50% flowering, seed diameter, and 100 seed weight, respectively.

3.2.1. Days to 50% Flowering. ILL-5888 flowered in 53 days compared to 60 days for ILL-6002. The range for days to 50% flowering among RILs was 47 to 58 days with a mean of 51.3 ± 3.5 days (Table 1). The frequency distribution of the RILs and the normality test (Shapiro-Wilk, $P = 0$) of days to 50% flowering showed two discrete classes and the absence of a normal distribution pattern (Table 1, Figure 2(a)).

The ICIM analysis for days to 50% flowering (Table 4) showed the presence of additive and epistatic interaction effects that accounted for a significant amount of phenotypic variation. LOD_A determines the significance of total variation at the interaction data point, whereas the LOD_{AA} value indicates epistatic effects between QTLs.

Three significant QTLs were detected with two on linkage group 4 and one on linkage group 13. The QTL detected at the 83 cM position of linkage group 4 ($QLG4_{83}$) showed significant additive effects (1.506) that accounted for 20.2% of the phenotypic variation (Table 4). The flanking markers, UBC34 and UBC1, were 1.4 and 1.6 cM from $QLG4_{83}$ (Figure 1). The other two QTLs, $QLG4_7$ and $QLG13_{50}$, accounted for 14.4% and 10.4% of the variation, respectively (Table 4). QTL, $QLG4_7$, was 7 cM from the GLLC 556 marker on linkage group 4, and $QLG13_{50}$ was flanked by two SRAP markers, ME5XR7b and F8XEM58b, on linkage group 13 separated by 2.9 cM and 7.2 cM, respectively (Figure 1).

The $QLG4_{84} \times QLG13_8$ and $QLG1_{24} \times QLG13_{52}$ interactions accounted for 24.2% and 15.6% of the phenotypic variation, respectively, and were due to combined additive and epistatic effects. Five other QTL interaction pairs had significant epistatic effects that accounted for 4.6 to 8.0% phenotypic variation each, but the additive effects of the individual QTLs were nonsignificant (Table 4). It should be noted that the $QLG4_{84} \times QLG13_8$ interaction probably

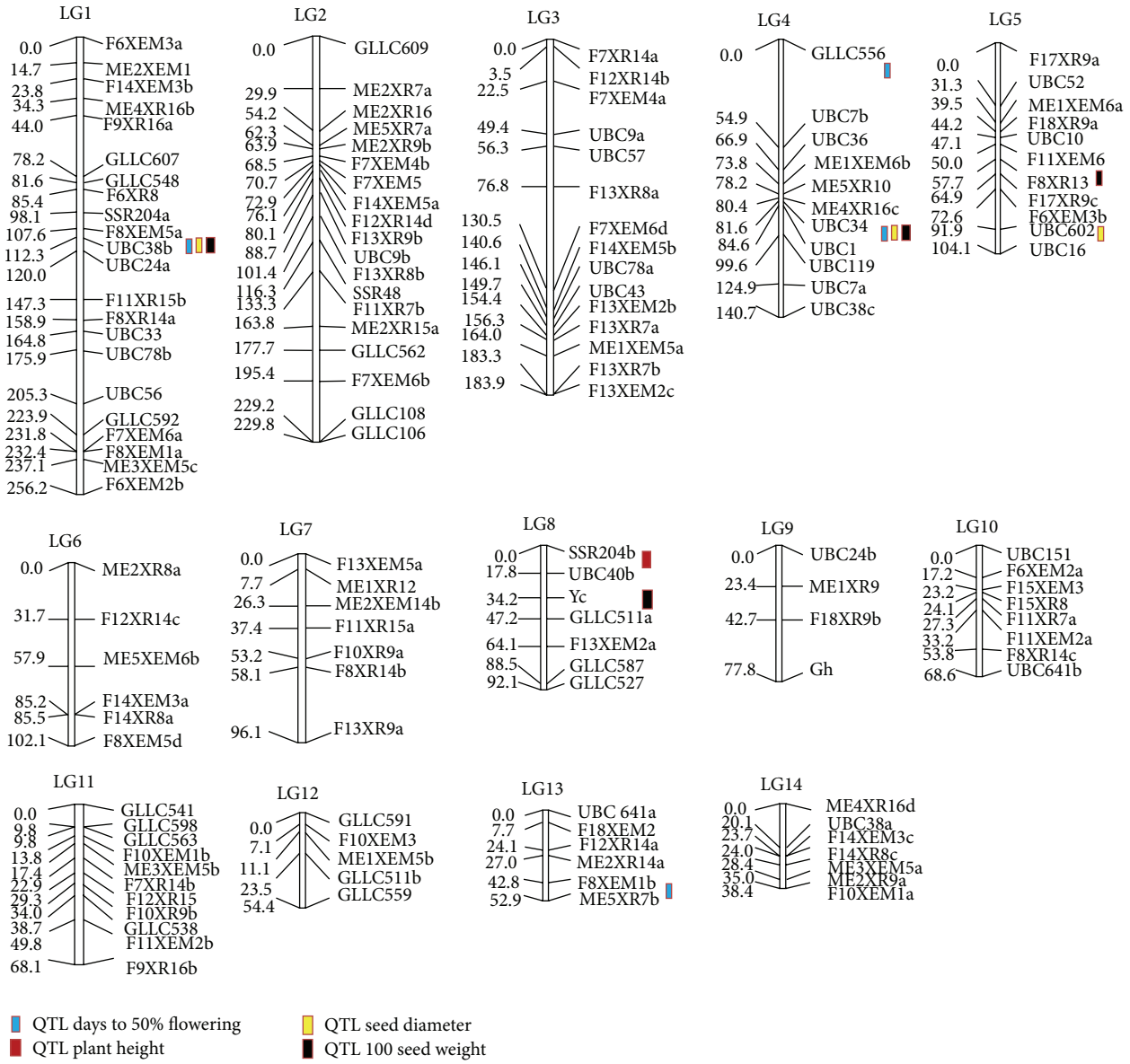

Figure 1: Intraspecific linkage map of lentil at an LOD score of 4.0 and at maximum recombination fraction 0.25. The linkage groups are named (LG1–LG14). Loci names are indicated on the right side, and genetic distances are on the left side of the vertical bar. SSR markers are named as SSR or GLLC-SSR, RAPDs are UBC, and SRAP are F or ME as forward and R or EM as reverse primer.

involves the main effect of QTL $QLG4_{83}$, and the $QLG1_{24} \times QLG13_{52}$ interaction probably involves the QTL $QLG13_{50}$.

Study of the inheritance of flowering in Precoz under both Indian and Syrian environments determined that a dominant gene *Sn* played a major role in early flowering [3]. Tahir and Muehlbauer [24] identified four QTLs on different linkage groups, and Sarker et al. [3] reported single and polygenic systems control of days to 50% flowering based on F_2 segregation in different lentil crosses. Tullu et al. [7] identified two QTLs (LG4 and LG12) from one location data and five QTLs (LG1, LG4, LG5, LG9, and LG12) from another location data for days to 50% flowering.

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

3.2.2. Plant Height. The RILs varied in height from 15.7 cm to 34.3 cm with a mean of 25 ± 3.8 (Table 1). Frequency

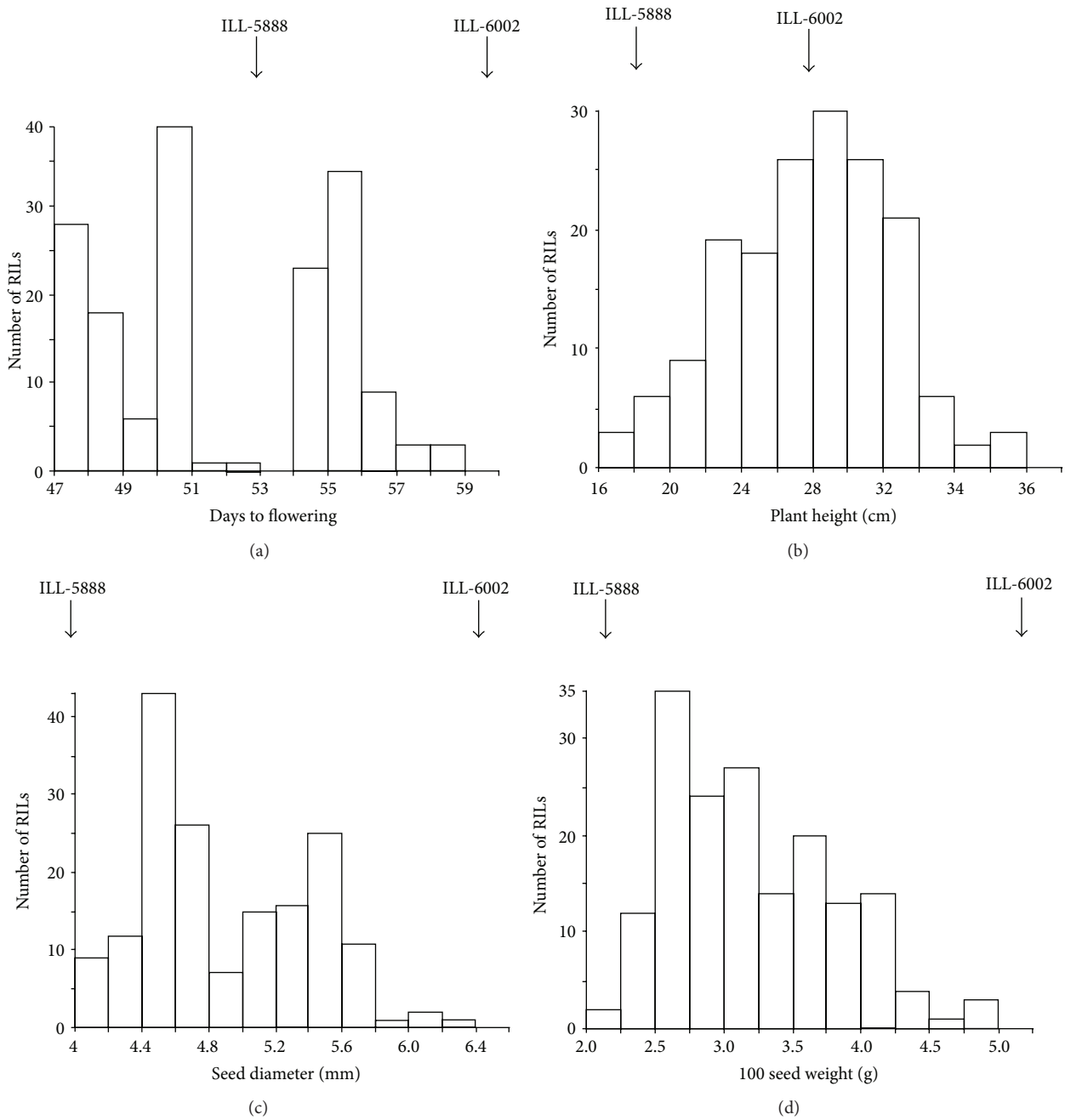

Figure 2: Frequency distribution of 2007 and 2008 combined cropping year data of the RILs for (a) days to 50% flowering, (b) plant height, (c) seed diameter, and (d) 100 seed weight of lentil.

Table 2: Correlation between quantitative agromorphological traits (days to 50% flowering, plant height, seed diameter, and 100 seed weight).

Trait	Days to 50% flowering	Plant height	Seed diameter	100 seed weight
Days to 50% flowering	1.0	0.217*	0.393***	0.411***
Plant height		1.0	0.207*	0.261***
Seed diameter			1.0	0.885***
100 seed weight				1.0

***$P < 0.001$, *$P < 0.05$.

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

RILs/parents	Traits and gene symbol	Number of RILs	Expected segregation ratio	χ^2 value
F_6 derived F_7 RILs	Red cotyledon (*Yc*)	94	1 : 1	2.14*
	Yellow cotyledon (*yc*)	75		
Parents: ILL-5888	Red cotyledon (*Yc*)			
ILL-6002	Yellow cotyledon (*yc*)			
F_6 derived F_7 RILs	Prostrate growth habit (*Gh*)	92	1 : 1	1.73*
	Erect growth habit (*gh*)	75		
Parents: ILL-5888	Prostrate growth habit (*Gh*)			
ILL-6002	Erect growth habit (*gh*)			

*$P < 0.250 > 0.100$.

Table 4: Summary of QTL analysis for days to 50% flowering variation in RILs from the ILL-5888 × ILL-6022 cross.

Trait	QTL	Additive effect	LOD	PVE+ (%)	
Days to 50% flowering	$QLG4_7$	−1.262	4.84	14.38	
	$QLG4_{83}$	1.506	10.24	20.18	
	$QLG13_{50}$	1.078	4.99	10.40	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE+ (%)
	$QLG1_{24} \times QLG13_{52}$	0.637	13.25	3.48	15.57
	$QLG2_0 \times QLG12_{24}$	−0.914	6.74	6.59	7.26
	$QLG2_{30} \times QLG8_{92}$	0.858	6.09	5.89	6.39
	$QLG2_{82} \times QLG3_{150}$	−0.937	7.01	6.56	7.98
	$QLG2_{132} \times QLG8_{18}$	0.731	4.73	4.43	5.07
	$QLG3_{50} \times QLG7_{94}$	0.744	3.81	3.74	4.56
	$QLG4_{84} \times QLG13_8$	0.912	19.22	6.57	24.16

+Phenotypic variation explained.

distribution and the normality test for plant height showed the presence of a normal distribution (Shapiro-Wilk, $P = 0.466$) and indicated polygenic control (Table 1, Figure 2(b)).

A significant QTL (LOD = 4.9) was detected on LG 8 at the 4 cM position ($QLG8_4$), and the closest marker, SSR204b, was 4 cM away at the proximal end. QTL $QLG8_4$ accounted for an estimated 15.3% of the phenotypic variation for plant height (Table 5, Figure 1).

The epistatic effects of $QLG3_{76} \times QLG8_{36}$ and $QLG3_{82} \times QLG4_6$ accounted for 17.6 and 17.3% phenotypic variation, respectively, but additive effects of the interacting QTLs were insignificant (Table 5). The interaction of $QLG1_{44} \times QLG8_{14}$ accounted for an estimated 14.6% of phenotypic variation by combined additive and epistatic gene action (Table 5). Six other pairs of QTLs displayed epistatic interactions each affecting 6.8 to 10.7% of the phenotypic variation (Table 5).

Epistatic effects of QTLs accounted for a major portion of the phenotypic variation for plant height in our population. Genes close to F13XR8 and *Yc* markers played a major role for the $QLG3_{76} \times QLG8_{36}$ interactions, whereas, for the $QLG3_{82} \times QLG4_6$ interactions, genes close to the same SRAP marker F13XR8 and GLLC 556 accounted for a significant portion of the variation (Table 5, Figure 1).

Tahir and Muehlbauer [24] reported that a gene linked to the *Aat-p* locus was responsible for increased plant height. Tullu et al. [7] reported that PI 320937 is taller than "Eston" but both contributed to reduced height of lentil plants, and they identified different QTLs of plant height at two locations, though there is a nonsignificant genotype × environment interaction. They concluded that the chance of simultaneous detection of QTLs at both environments was small due to lack of powerful statistical methods to detect minor QTLs.

3.2.3. Seed Diameter. The parents, ILL-5888 and ILL-6002, had seed diameters of 3.7 mm and 6.5 mm, respectively, and all the RILs were intermediate to the parents. The highest and the lowest seed diameters of the inbred lines were 4.0 and 6.3 mm, respectively, with a mean of 4.9 ± 0.5 mm (Table 1). Frequency distribution of seed diameter of the RILs is bimodal but continuous (Figure 2(c)) indicating that a single major gene or QTL along with minor QTLs was involved in determining seed diameter. Three different QTLs, $QLG1_{112}$, $QLG4_{82}$, and $QLG5_{98}$, were detected for seed diameter on LG1, LG4, and LG5, respectively. $QLG4_{82}$, at the 82 cM position of linkage group 4, accounted for 32.6% of the phenotypic variation through large additive effect of 0.293 with an LOD score of 22.2 (Table 6). The $QLG4_{82}$ QTL has the most significant effect by far and may be responsible for the bimodal frequency distribution of the mapping population observed for this trait (Table 6, Figure 2(c)). Two flanking markers, UBC34 and UBC1, were 0.4 cM and 2.6 cM from the QTL, respectively (Figure 1). The other two QTLs, $QLG1_{112}$, and $QLG5_{98}$ accounted for 4.5 and 3.6% of the phenotypic variation for seed diameter, respectively (Table 6). Significant

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

Trait	QTL	Additive effect	LOD	PVE $^{+}$ (%)	
Plant height	$QLG8_4$	−1.478	4.93	15.33	
	Interacting QTLs	Epistatic effect	LOD_A	LOD_{AA}	PVE^{+} (%)
	$QLG1_{44} \times QLG8_{14}$	−0.958	8.42	4.12	14.57
	$QLG1_{44} \times QLG8_{22}$	−0.976	4.39	4.36	6.84
	$QLG1_{86} \times QLG7_{76}$	1.114	3.26	3.21	8.65
	$QLG1_{224} \times QLG5_{30}$	1.21	7.19	7.06	9.92
	$QLG3_{76} \times QLG8_{36}$	−1.590	11.52	11.34	17.61
	$QLG3_{82} \times QLG4_6$	−1.557	6.64	6.58	17.29
	$QLG3_{164} \times QLG10_{52}$	−1.103	6.17	5.93	8.84
	$QLG4_{66} \times QLG14_{16}$	−1.232	6.48	6.42	10.56
	$QLG5_{72} \times QLG11_{68}$	1.267	7.98	6.66	10.69

$^{+}$Phenotypic variation explained.

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

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

$^{+}$Phenotypic variation explained.

epistatic interactions of $QLG2_{62} \times QLG2_{132}$, $QLG2_{62} \times QLG2_{136}$, and $QLG13_0 \times QLG14_0$ with relatively minor effects were observed (Table 6).

Cubero [25] reported that a polygenic system governs seed shape and size in faba bean (*Vicia faba* L.). In chickpea (*Cicer arietinum* L.), small seed size was found to be dominant in desi × kabuli crosses [26], but Niknejad et al. [27] found polygenic control of seed size and partial dominance for large seededness. Davies et al. [28] reported that three genes control the seed size in peas (*Pisum sativum* L.). In our study, one major QTL with two minor QTLs and three minor epistatic interactions indicate that genetic control of seed diameter in lentil is controlled by a combination of genetic effects.

3.2.4. 100 Seed Weight. Seed weight is a major yield component. The 100 seed weight of ILL-5888 was 2.16 g and ILL-6002 was 5.59 g. The range of 100 seed weight of the RILs was 2.0 to 4.9 g with a mean of 3.2 ± 0.1 g (Table 1). As with seed diameter, the 100 seed weight of the RILs was intermediate to the two parents. The frequency distribution showed a skewed distribution toward light seed (Figure 2(d)). Five QTLs for 100 seed weight were identified on four linkage groups (LG1, LG4, LG5, and LG8) that additively accounted for 5.6 to 17.5% of the phenotypic variation. Two QTLs, $QLG4_{82}$, and $QLG1_{113}$ accounted for 17.5% and 12.8% of the phenotypic variation with an LOD score of 15.3 and 11.5, respectively (Table 7). $QLG4_{82}$ was flanked by UBC34 and UBC1 at 0.4 and 2.6 cM, respectively, and $QLG1_{113}$ was flanked by UBC38b and UBC 24a at 1 cM and 7 cM, respectively (Figure 1). The epistatic interactions of $QLG2_{158} \times QLG2_{210}$, $QLG4_{22} \times QLG4_{56}$, $QLG5_{74} \times QLG5_{78}$, and $QLG5_{76} \times QLG5_{84}$ accounted for a significant portion of the phenotypic variation, each accounting for about 9% of the variation (Table 7).

Genetics of seed weight of common bean (*Phaseolus vulgaris*) has been under investigation since the early studies of Johannsen [29]. According to Motto et al. [30], based on a classical quantitative genetics study, seed weight of common bean is quantitatively inherited and affected by at least ten genes with additive effects. In mung bean (*Vigna radiata)*, seed weight is controlled by genes with additive and nonadditive actions and low seed weight is dominant [31]. It has been reported that dry seed weight of lentil is controlled by two genes [6]. Abbo et al. [10] found that seed weight of lentil is under polygenic control with additive and dominant gene action and partial dominance of low seed weight alleles. In our study, two QTLs accounted for relatively high levels of variation supporting the results of Sakar [6], and the frequency distribution was skewed toward low seed weight and indicated the polygenic nature of the control of seed weight and partial dominance for low seed weight, the results supporting Abbo et al.'s [10] findings.

4. Conclusion

Agromorphological traits have immense importance in breeding lentils for high yields, yield stability, and market

Table 7: Summary of QTLs, additive and epistatic effects for 100 seed weight in RILs from the ILL-5888 × ILL-6002 cross.

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

$^+$Phenotypic variation explained.

acceptability. The ICIM (inclusive composite interval mapping) method opens the door for understanding quantitative inheritance with epistatic interactions. Now, it is possible to identify digenic interactions while developing polygenic interaction models to improve the efficiency and accuracy of QTL detection. Taking these interactions into account, it will be possible to formulate breeding and selection procedures for important agronomic and market value traits.

We report here the detection of three significant QTLs ($QLG4_7$, $QLG4_{83}$, and $QLG13_{50}$) for days to 50% flowering, one significant QTL ($QLG8_4$) for plant height, three significant QTLs ($QLG1_{112}$, $QLG4_{82}$, and $QLG5_{98}$) for seed diameter, and five significant QTLs ($QLG1_{107}$, $QLG1_{113}$, $QLG4_{82}$, $QLG5_{55}$, and $QLG8_{34}$) for 100 seed weight.

$QLG4_{82\text{-}83}$ accounted for 20.2%, 32.6%, and 17.5% of the phenotypic variation for days to 50% flowering, seed diameter, and 100 seed weight, respectively. $QLG4_{82\text{-}83}$ was flanked by two RAPD markers UBC 34 and UBC 1 at 0.4–1.4 and 1.6–2.6 cM, respectively. The three traits involved are all positively correlated with each other indicating the possibility for linkage or pleiotropic effect in this LG 4 QTL region.

Some interacting QTLs accounted for significant phenotypic variation for quantitative traits through additive or epistatic interactions or both. $QLG1_{24} \times QLG13_{52}$ and $QLG4_{84} \times QLG13_8$ accounted for 15.6% and 24.2% through additive and epistatic effects for days to 50% flowering. For plant height, $QLG3_{76} \times QLG8_{36}$, $QLG3_{82} \times QLG4_6$, $QLG4_{66} \times QLG14_{16}$, and $QLG5_{72} \times QLG11_{68}$ interactions accounted for 17.6%, 17.3%, 10.6%, and 10.7% of the phenotypic variation, respectively. $QLG1_{44} \times QLG8_{14}$ both additively as well as epistatically accounted for 14.6% of the phenotypic variation for plant height. Growth habit and cotyledon color were each controlled by single genes with prostrate growth dominant over erect plant type and red cotyledon dominant over yellow cotyledon.

Understanding genetics of the quantitative traits will help to develop the breeding strategy for selection. The significant correlations among days to 50% flowering, seed diameter, and 100 seed weight and the association between the gene-rich QTL region ($QLG4_{82\text{-}83}$) will help the breeders in selecting plants for early maturity and improved seed quality. The putative QTLs will be useful to locate the genes in the genome that are important for the traits and provide the guidance for marker-assisted selection and cloning of the genes.

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