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**RESEARCH ARTICLE**

# Identification of genomic regions associated with early plant vigour in lentil (*Lens culinaris*)

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**Abstract.** Lentil is one of the most important food legume species, however its genetic and genomic resources remained largely uncharacterized and unexploited. In the past few years, a number of genetic maps have been constructed and marker resources have been developed in lentil. These resources could be exploited for understanding the extent and distribution of genetic variation in genus *Lens* and also for developing saturated and consensus genetic maps suitable for quantitative trait loci (QTL) mapping and marker-assisted selection. The present study aims to enrich polymerase chain reaction-based linkage map of $F_{10}$ recombinant inbred lines (RILs) population of 94 individuals derived from cross WA8649090 × Precoz and identification of QTLs linked to early plant vigour traits. Of the 268 polymorphic markers (93 simple sequence repeats (SSR), three inter-simple sequence repeats (ISSRs) and 172 random amplified polymorphic DNA (RAPDs)), 265 (90 SSRs, three ISSRs and 172 RAPDs) were mapped on seven linkage groups, varying in length between 25.6 and 210.3 cM, coverage of 809.4 cM with an average marker spacing of 3.05 cM. The study also reported assigning of 24 new cross-genera SSRs of *Trifolium pratense* on the present linkage map. The RILs along with the parents were screened for shoot length, root length, seedling length, dry weight, number of leaves and number of branches based on two replications under polyhouse conditions. A QTL-hotspot consisting of six QTLs for shoot length (cm), root length (cm) and seedling length (cm) was observed between a map distances of 56.61 and 86.81 cM on LG1.

**Keywords.** lentil; quantitative-trait loci; molecular map; early plant vigour; *Lens culinaris*.

## Introduction

Lentil (*Lens culinaris* Medikus) is one of the important cool season food legume crops of rainfed agriculture and an essential commodity for diversifying cereal-based cropping systems worldwide (Kumar *et al.* 2013). It is a diploid ($2n = 2x = 14$), self-pollinated with genome size of ~4 Gbp (Arumuganathan and Earle 1991). Lentil has about 28% of protein content (Muehlbauer *et al.* 2006) and exhibits low glycine index and is highly recommended for patients with diabetes, obesity and cardiovascular diseases (Srivastava and Vasistha 2012).

Most of the agronomic and early vigour traits are controlled by many genes, and loci are located on different positions on genome and thus referred as quantitative trait loci (QTLs). QTL mapping provides a means to dissect complex phenotypic traits and allow to identify molecular markers linked to genomic region, so that these can be directly used in marker-assisted selection (Tanksley *et al.* 1989; Lee 1995; Schneider *et al.* 1997; Mohan *et al.* 1997). Linkage maps are useful for locating quantitative traits and in understanding the genetic make-up of a crop. Various genetic linkage maps in lentil have been constructed in the past (Zamir and Ladizinsky 1984; Tadmor *et al.* 1987; Havey and Muelbauer 1989; Tahir *et al.* 1993; Eujayl *et al.* 1997; Duran *et al.* 2004; Gupta *et al.* 2012; Fedoruk *et al.* 2013; Verma *et al.* 2015). However, during the past few years, efforts have been made towards developing DNA-based molecular markers to link directly with trait of interest for the development of improved crop varieties.

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QTL mapping technique has become a powerful tool for identifying genomic regions which affect complex plant traits by providing information on the map location, relative effect, gene action and dominance properties of each identified locus and success has been achieved through the development of DNA markers and construction of high-density linkage maps for many plant species (Lander and Botstein 1986; Tanksley 1993). Early plant vigour traits like seedling length, root length, shoot length and dry weight are economically important quantitative traits, which are believed to be controlled by multiple genes. Germination rate and early seedling growth are the major seedling-vigour-related traits. Rapid shoot and root growth were observed to be closely associated with seedling vigour. The early vigour traits are important for determining the establishment of lines in the field as early as possible. It is generally considered that carbohydrates, for early seedling growth before seedlings, gain the ability of photoautotrophy, are provided by breakdown of the starch stored in the endosperm. Thus, functionally, it should be expected that amylase activities are correlated with germination rate and early seedling growth in rice, as it was reported previously (Williams and Peterson 1973; Sasahara *et al.* 1986). Early vigour traits are considered as the indicator for drought tolerant traits as the early growth of the seedling helps to establish early and fight prolonged drought conditions during winter season. Thus, a PCR-based linkage map was developed to identify markers linked to the QTLs controlling seedling-vigour-related traits on an $F_{10}$ RIL population.

## Materials and methods

### *Plant material and DNA extraction*

A set of 94 $F_{10}$ recombinant inbred lines (RILs) derived from an intraspecific cross of *Lens culinaris* ssp. *culinaris* (WA8649090 × Precoz) were used which differed in early plant vigour traits. The material was procured from Grain Legume Genetics and Physiology Research Unit, USDA-ARS, Washington State University, Pullman, USA. Genomic DNA was extracted from young leaves of each RILs along with their parental genotypes which were grown in greenhouse during 2016–2017 following cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980). DNA quantification was done by micro-volume fluorimeter (Eppendorf) and stored in −20°C freezer for genotyping.

### *Phenotyping of RILs for early vigour traits*

The RILs were phenotyped for shoot length (SL), root length (RL), seedling length (SeL), dry weight (DW) number of leaves (NoL) and number of branches (NoB) in two replications in polyhouse at CSKHPKV, Palampur during 2016–2017. The data related to SL, RL, SeL and DW were recorded after 10 days of sowing on Petri plate using top-of-paper method. The experiment was repeated twice by sowing 20 seeds per Petri plate of each RILs. After 10 days of sowing, phenotypic data on SeL, RL, SL were recorded using a centimetre scale, whereas dry weight (g) of shoot and root was taken by separating shoot and root from cotyledon using weighing balance. The data on NoL and NoB were recorded after 45 days under polyhouse condition.

### *Genotyping analysis using SSR, ISSR and RAPD*

A set of 578 SSRs from published (Hamwieh *et al.* 2005; Sato *et al.* 2005; Saha *et al.* 2010b) and unpublished data (NIPGR, New Delhi), 250 random amplified polymorphic DNA (RAPD) decamer primers (Operon Technologies, Alameda, USA) and 30 inter-simple sequence repeats (ISSR) primers (15–23 nucleotides in length) were used to survey parental polymorphism.

The parental lines, WA8649090 and Precoz were tested for polymorphism with different sets of primer pairs in 12.5 $\mu$L reactions using Gene Amp PCR System 9700 (Applied Biosystems, Foster City, USA) and Veriti Pro-flex (Applied Biosystems). The PCR products were electrophoresed in 3% agarose gel (HiMedia) and stained with ethidium bromide (0.5 $\mu$g/mL). The amplified products were visualized and photographed using the Gel-Documentation Unit (Labnet International, USA). The polymorphic markers were used to genotype all the individual of RILs and the data generated were recorded which was further used in mapping studies.

### *Linkage and QTL analyses*

The segregation of markers was tested for goodness of fit to the expected Mendalian ratio (1:1) using chi-square ($\chi^2$) test. Highly distorted and unlinked markers were excluded from the analysis. The phenotypic data were analysed with analysis of variance (ANOVA) using CPCS1. Genotyping data were used for linkage analysis using JoinMap v4.0 (Van Ooijen 2006). Marker order was assigned using the regression mapping algorithm with maximum recombination frequency of 0.4 at minimum logarithm of odds (LOD) of 3 and jump threshold of 5. Ripple command was used after adding each marker locus to confirm marker order. The Kosambi mapping function was used to calculate the map distance (Kosambi 1994).

QTL analysis was performed using QTL Cartographer v2.5 software (Wang *et al.* 2005), (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). Composite interval mapping was performed by selecting model 6 with the default window size 10 cM, control marker number 5, and backward regression method. QTLs with a positive or negative

additive effect for a specific trait imply that increase in the phenotypic value of the trait is contributed by the alleles from Precoz or WA8649090, respectively.

## Results

### *Phenotyping of RILs for early plant vigour traits*

The nearly continuous distribution of mean phenotypic values of the traits showed that the traits were quantitatively inherited (table 1). Significant differences for all the six measured traits were observed as revealed by ANOVA (table 2).

### *Linkage map construction*

Among 853 SSRs, RAPD and ISSRs markers (433 (*Lens* genomic SSRs) + 145 (SSRs from other legumes, namely red clover, *Medicago* and pea) + 25 ISSR + 250 RAPD) screened for parental polymorphism, a total of 268 (31.41%) yielded scorable amplicons. Of all the scored SSR markers, 265 (98.88%) were mapped on seven linkage groups (LGs), whereas three of the markers were found unlinked. All the markers were analysed to detect segregation distortion by calculating $\chi^2$ values. Highly distorted and unlinked markers were excluded from the analysis. Following linkage analysis, 265 markers were assigned positions on seven LGs at LOD 4.0 covering a total genetic distance of 809.4 cM with an average marker density of 3.05 cM. The number of markers mapped per LG varied from eight on LG7 to 76 on LG3. However, the maximum number of markers (76) were mapped on LG3, the average density was highest for LG1 (2.05 cM), which harboured 56 markers (table 3). The markers were unevenly distributed on all the LGs, except for LG1, which showed cluster of five markers with 1 cM distance; similarly LG3 and LG6, which showed cluster of two markers with 1 cM distance. The largest gap of 39.9 cM was observed on LG2 followed by 31.1 on LG5 (figure 1).

**Table 1.** Mean performance of parents and RILs for different traits in RIL (WA8649090 × Precoz) population.

| Trait | WA8649090 | Precoz | Range (RIL) | Mean | SD |
|---|---|---|---|---|---|
| SL (cm) | 3.2 | 5.4 | 1.5–9.8 | 5.65 | 1.64 |
| RL (cm) | 1 | 2.9 | 0.3–8.9 | 4.6 | 1.4 |
| SeL (cm) | 4.3 | 8.7 | 1.55–17.6 | 9.575 | 2.59 |
| DW (mg) | 0.13 | 0.2 | 0–0.21 | 0.105 | 0.032 |
| NoL (PL) | 151 | 124 | 97–155 | 126 | 10.91 |
| NoB (PB) | 15 | 7 | 2.5–17.5 | 102.53 | 2.53 |

### *QTLs for early plant vigour traits*

The constructed linkage map was used to detect the QTLs by using phenotypic data obtained on various plant vigour traits under controlled conditions. A total of 14 QTLs were detected (LOD ≥ 3.0) with additive gene effect ranging from −5.14 to 4.52 and explained phenotypic variation (PVE) from 9.2 to 21.4% (table 4). Of the total 14 QTLs found in the study, LG1 contained six QTLs (three for SeL (*qSeL01*, *qSeL02* and *qSeL03*) and one each for SL (*qSL01*), RL (*qRL01*) and NoB (*qNOB02*)) with a LOD score range of 3.1–4.8, explaining 12.3–21.2% of the phenotypic variation (table 3). LG2 contained only one QTL for DW *qDW01*, while LG3 harboured two QTLs one each for number of leaves *qNOL01* and NoB *qNOB02*; LG4 had a total of two QTLs (both for number of leaves *qNOL02*, *qNOL03*), LG6 contained two QTLs (Each for DW trait *qDW02* and *qDW03*) and LG7 contained only one QTL *qSeL01* responsible for SeL (figure 1).

**Table 3.** Distribution of 265 genomic markers on seven LGs of an intraspecific linkage map of (WA8649090 × Precoz) *L. culinaris*.

| LGs | Markers mapped | Map length (cM) | Average marker density (cM) | Skewed markers (%) |
|---|---|---|---|---|
| LG1 | 56 | 115.3 | 2.05 | 15 (26.78) |
| LG2 | 21 | 108.7 | 5.17 | 17 (80.95) |
| LG3 | 76 | 210.3 | 2.76 | 9 (11.84) |
| LG4 | 22 | 38.1 | 1.73 | 02 (9.09) |
| LG5 | 57 | 141.9 | 2.48 | 17 (29.82) |
| LG6 | 25 | 169.5 | 6.78 | 08 (32) |
| LG7 | 8 | 25.6 | 3.2 | 03 (37.5) |
| Total | 265 | 809.4 | 3.05 | 71 (26.79) |

**Table 2.** Variation analysis for different traits in RIL (WA8649090 × Precoz) population.

| | SL | RL | SeL | DW | NoL | NoB |
|---|---|---|---|---|---|---|
| Mean | 4.112553 | 2.966596 | 6.693511 | 0.035106 | 128.3968 | 7.434681 |
| SD | 1.644982 | 1.399033 | 2.596383 | 0.032087 | 10.9187 | 2.539174 |
| Coefficent variation | 39.99904 | 47.15955 | 38.78955 | 91.39857 | 8.50387 | 34.1531 |
| Minimum | 1.5 | 0.3 | 1.55 | 0 | 97 | 2.5 |
| Maximum | 9.08 | 8.9 | 17.6 | 0.21 | 155 | 17.5 |
| Range | 7.58 | 8.6 | 16.05 | 0.21 | 58 | 15 |

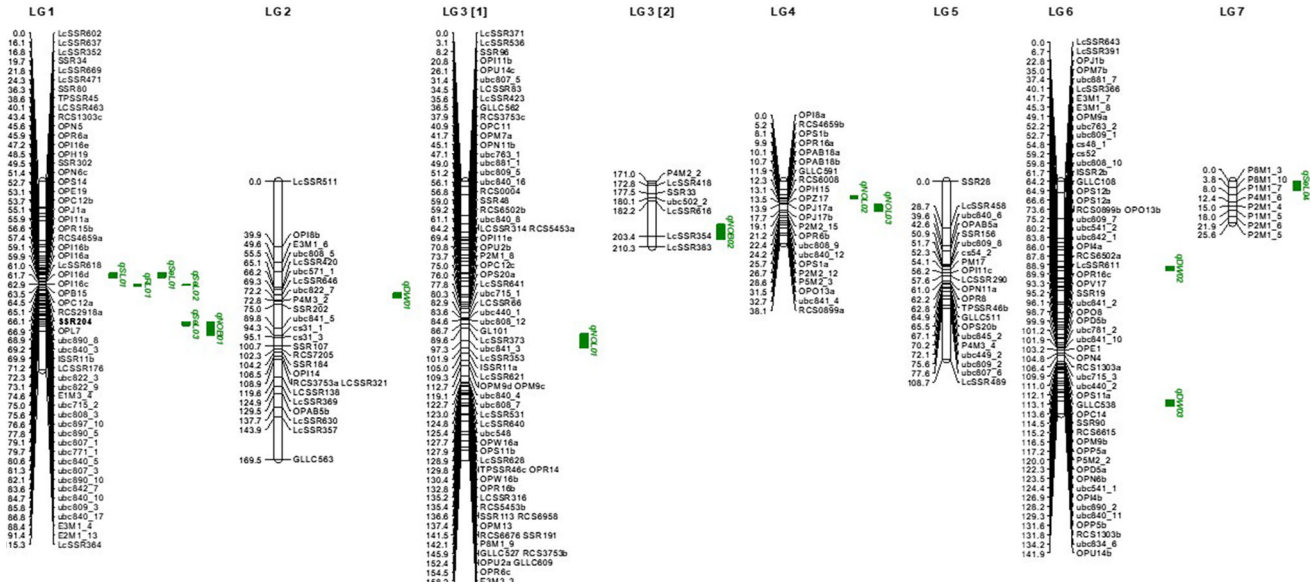


**Figure 1.** Intraspecific linkage map of lentil based on SSR markers showing location of QTLs for early vigour traits identified in WA8649090 × Precoz mapping population.

**Table 4.** QTLs for various early plant vigour traits identified using QTL Cartographer.

| Trait | LG | QTL name | Position | LOD score | Marker interval | Additive | PVE |
|---|---|---|---|---|---|---|---|
| SL | 1 | qSL01 | 56.61 | 3.9 | OPI11a-OPI16b | 0.66 | 14.2 |
| RL | 1 | qRL01 | 63.51 | 3.1 | OPI16c-OPC12a | 0.57 | 13.2 |
| SeL | 1 | qSeL01 | 56.61 | 5.6 | OPI11a-OPI16b | 1.29 | 21.2 |
| | 1 | qSeL02 | 63.51 | 4.8 | OPI16c-OPC12a | 1.34 | 19.4 |
| | 1 | qSeL03 | 86.81 | 4.2 | ubc809_3-E3M1_4 | −1.17 | 14.2 |
| | 7 | qSeL04 | 2.01 | 3.1 | P8M1_3-P1M1_7 | 0.94 | 12.2 |
| DW | 6 | qDW02 | 52.21 | 2.7 | OPM9a-cs48_1 | 0.01 | 9.5 |
| | 6 | qDW03 | 134.21 | 3.1 | RCS1303b-OPU14 | 0.01 | 10.9 |
| | 2 | qDW01 | 69.31 | 2.7 | ubc51_1-ubc822_7 | 0.01 | 9.2 |
| NoL | 3 | qNOL01 | 95.61 | 3.9 | LcSSR373-LcSSR353 | −5.14 | 21.4 |
| | 4 | qNOL02 | 10.11 | 3.2 | OPS1b-OPAB18b | 3.80 | 11.4 |
| | 4 | qNOL03 | 15.91 | 3.4 | OPJ17a-P2M2_15 | 4.52 | 15.4 |
| NoB | 3 | qNOB02 | 203.41 | 3.1 | LcSSR616-LcSSR383 | 0.84 | 10.3 |
| | 1 | qNOB01 | 86.81 | 3.7 | ubc809_3-LcSSR364 | −0.98 | 12.3 |

## Discussion

Development of linkage maps is extremely useful for locating quantitative traits and also helps to understand the genetic architecture of the trait. Genetic linkage maps in lentil have been constructed previously by many workers (Zamir and Ladizinsky 1984; Tadmor *et al.* 1987; Havey and Muelbauer 1989; Tahir and Muehlbauer 1994; Eujayl *et al.* 1997; Duran *et al.* 2004; Gupta *et al.* 2012; Fedoruk *et al.* 2013; Verma *et al.* 2015). Early plant vigour traits, like SeL, RL, SL and DW are economically important quantitative traits, which are believed to be controlled by multiple genes. Rapid shoot and root growth were observed to be closely associated with seedling vigour (Williams and Peterson 1973; Sasahara *et al.* 1986).

### *Linkage map construction*

The polymorphism observed (31.41%) was in accordance with majority of the earlier studies in different legumes (Radhika *et al.* (2007) in chickpea (22.1%), Chaitieng *et al.* (2006) in adzuki bean (26.8%), Hwang *et al.* (2009) in soybean (27.02%) and Yang *et al.* (2012) in lotus (37.0%)). However, polymorphism varied from as low as 6.5% in tomato (Shirasawa *et al.* 2010), 23.2% in cucumber (Zhang *et al.* 2012) to as high as 32.8% in *Catharanthus* (Shokeen *et al.* 2011) and 50% in *Vitis* (Riaz *et al.* 2004). The possible reason that affects the level of polymorphism exhibited by the parents of the mapping population is the type of markers used, reproduction behaviour (self-pollinated or cross-pollinated, interspecific or intraspecific cross), type of population

($F_2$/BC/RIL) etc. Intersubspecific populations used for map construction in genus *Lens* have exhibited higher polymorphism (Vaillancourt and Slinkard 1993; Tahir and Muehlbauer 1994; Eujayl *et al.* 1998). Segregation distortion observed in the present study was significantly higher 26.79% (71/265), which was comparable to different levels of segregation distortion in lentil mapping populations observed by different workers (9.5% and 17.8%, Hamwieh *et al.* 2005; 14.0%, Rubeena Taylor *et al.* 2006; 12.0%, Phan *et al.* 2007; 20.0%, Tullu *et al.* 2008; 48.0%, Tanyolac *et al.* 2010; 22.0%, Saha *et al.* 2010a, b; 23.81%, Gupta *et al.* 2011; 33.85%, Verma *et al.* 2015). A clustering of distorted loci has often been reported within the LGs constructed in several other species (Graner *et al.* 1991; Kammholz *et al.* 2001).

The linkage map generated in the present study spanning 809.4 cM with a mean marker distance of 3.05 cM, which was denser than reported previously (6.0 cM, Eujayl *et al.* 1998; 6.9 cM, Rubeena Ford and Taylor 2003; 15.87 cM, Duran *et al.* 2004; 13.5 cM, Phan *et al.* 2007; 8.9 cM, Tullu *et al.* 2008; 8.4 cM, Tanyolac *et al.* 2010; 11.6 cM, Saha *et al.* 2010b; 7.1 cM, Gupta *et al.* 2011 and 19.3 cM, Gupta *et al.* 2012; 5.48 cM, Verma *et al.* 2015) and sparse as compared to map published by Hamwieh *et al.* (2005). Similarly, genomic region mapped by us is comparable to the earlier published maps (784.1 cM; Rubeena Ford and Taylor 2003), (1192 cM; Kahraman *et al.* 2004), (1868 cM; Tullu *et al.* 2008), (1565.2 cM; Saha *et al.* 2010b), (1183.7 cM; Verma *et al.* 2015). Among the seven LGs, markers distribution was random such as densely packed LGs were LG3 and LG1, however LG7 and LG2 contained only eight and 21 markers, respectively (figure 1), which can be explained by the fact that SSRs are ubiquitously and randomly distributed in the plant genomes (Ramsay *et al.* 1999; Elsik and Williams 2001) and was also in accordance with Areshechenkova and Ganal (1999). The markers were unevenly distributed on all the LGs, except for LG1, which showed cluster of five markers with 1 cM distance; similarly LG3 and LG6, which showed cluster of two markers with 1 cM distance. The largest gap of 39.9 cM was observed on LG2 followed by 31.1 on LG5 (figure 1). This may be due to the occurrence of fewer marker polymorphisms in these gaps or regions thus resulting in lower marker density. The low density of markers in these distal regions may be attributed to homozygous regions with lower recombination frequency (Castiglioni *et al.* 1999; Souza *et al.* 2013) and centromeres, centromeric heterochromatin and in some instances telomeres experience up to 10-fold less recombination (Tanksley *et al.* 1989). A nonrandom distribution of markers due to centrally located clusters has been reported in barley (Langridge *et al.* 1995), sugar beet (Hallden *et al.* 1996) and chickpea (Winter *et al.* 2000). Whereas in cereals, where cytogenetic markers are available, the crossing over frequency in the distal regions of the chromosomes has been shown to be higher than in the regions proximal to the centromere (Lukaszewski 1992; Alonso-Blanco *et al.* 1993).

The present linkage map can increase the marker density, allowing comparison of locations of genes of interest across maps, as was evidenced in common bean (Freyre *et al.* 1998) and barley (Marcel *et al.* 2007). A high level of correspondence was observed for location of mapped SSRs in the present study and the earlier studies. For example, Hamwieh *et al.* (2005) and Verma *et al.* (2015) have also mapped SSR80 (LG1) on the same linkage group. SSR107 and SSR184 were assigned position on LG2 in the present linkage map which is in complete agreement with Hamwieh *et al.* (2005), Phan *et al.* (2007), Tullu *et al.* (2008), Gupta *et al.* (2011, 2012), Kaur *et al.* (2013) and Verma *et al.* (2015). Similarly, GLLC markers (GLLC556 and GLLC607) were present on the same LGs as reported by Saha *et al.* (2010a). Further, they reported the location of GLLC511 on LGVIII, however, in present study it was present on LG5.

### *QTLs for early plant vigour traits*

All the 14 QTLs reported in this study were found to be clustered across all the LGs, except LG5. Clustering of QTLs has been reported for various agronomic traits in many agriculturally important crops like sorghum (Lin *et al.* 1995), common bean (Blair *et al.* 2006), wheat (Quarrie 1996), cotton (Qin *et al.* 2008), soybean (Xu *et al.* 2005), rice (Wang *et al.* 2012). Clustering of QTLs can arise due to pleiotropic effect of a single regulatory gene (Aastveit and Aastveit 1993). Of the total 14 QTLs, six were clustered together and called 'QTL-hotspot' (QTL cluster 1 and QTL cluster 2), as this region contained several consistent QTLs with very high phenotypic variation (12.3–14.2% PVE) and its introgression will definitely improve early plant vigour traits (figure 1). On LG1, four QTLs were identified in the regions of about ≤8 cM that governs the different early plant vigour traits. QTL cluster 1 contained QTLs for SL trait (*qsL04*.2% PVE) at LOD score 3.9 with additive effect of 0.66 contributed by allele from WA8649090 parent. SeL trait (*qSeL01* with 21.2% PVE; *qSeL02* with 19.4% PVE); RL traits at LOD value of 3.1 (*qRL01* 13.2% PVE) with additive effect of 0.57 mainly contributed by the allele from Precoz parent (table 4). Overall, the region harboured four QTLs for three different traits explaining 13.2–21.2% phenotypic variation. Therefore, introgression of this region will not only improve the SL trait but also RL and SeL traits in lentil. QTL cluster 2 present on LG1 contained genetic loci for SeL traits (qSeL03 with 14.2% PVE) and number of branches trait (qNOB01 with 12.3% PVE). This is in similarity with the findings of Tahir and Muehlbauer (1994), who identified QTLs influencing early flowering and plant height using isozyme analysis and Gupta *et al.* (2011) detected QTLs affecting earliness, plant height, winter hardiness and resistance to ascochyta blight on LG1 and LG2 in lentil. Since no consensus genetic map of lentil exists, it is not always easy to compare the results obtained in earlier studies in relation to the chromosomal

location of the QTL analysed. Using the single marker analysis and isozymes studies, a QTL related to days to flowering was previously located in linkage group 1, while QTL related to days flowering and plant height was reported by other workers on linkage group 5 (Tahir *et al.* 1993; Tahir and Muehlbauer 1994; Fratini *et al.* 2007; Tullu *et al.* 2008).

Of the six phenotypic traits, SeL was conferred by highest number of alleles contributed by Precoz at three loci, namely *qSeL01*, *qSeL02* and *qSeL03*, on LG1, also another traits namely; number of branches was conferred by the allele contributed by WA8649090 at single loci *qNOB01* on LG7 LOD score range of 3.7–5.6, explaining 12.2–21.2 per cent of the phenotypic variation (table 4). This showed that SeL and number of branches are influenced by QTLs additive effect. These positive additive effects (*qSeL01* and *qSeL02* has 1.29 and 1.34 respectively) govern an increasing SeL and the negative additive effect (*qSeL03* and *qNOB01* has −1.17 and −0.98) governs a decreasing SeL and number of branches. In addition, three significant QTLs (two on LG6 and one on LG2) were detected for DW with a LOD score range of 2.7–3.1. These three QTLs had a same additive effect of 0.01 g and contributed by the allele from parental line Precoz at three loci namely *qDW02*, *qDW03* and *qDW01*, which governs the early plant vigour character for DW, explaining 9.2–10.9% of the phenotypic variation.

Of the six traits analysed, major QTLs were identified for two traits, three QTLs for number of leaves and one for number of branches (table 4). QTLs for number of leaves (*qNOL01* with negative additive effect of −5.14, *qNOL02*, *qNOL03* with positive additive effect, namely 3.80 and 4.52) were present on LG3 and LG4 explaining 11.4–21.4% of the phenotypic variation at LOD score range of 3.2–3.9, while for number of branches QTL (qNOB02 with additive effect of 0.84) was present on LG3 with 10.3% of phenotypic variation explained at the 3.1 LOD value. Of these traits, one significant QTL for number of branches with 12.2% PVE was present on LG7 at LOD value of 3.1 with an additive effect of 0.94.

In conclusion, with the long-term goal of understanding the genetic basis of early plant vigour traits, our study focussed on identification of major QTLs for six traits in lentil. Hence summarizing the results, it is envisaged that the present linkage map, fortified with 265 SSR markers and 14 QTLs identified for early plant vigour traits would provide a means to breeders for further genetic enhancement of the crop species. The map constructed covered a significant portion of the genome; a further saturation of this map with additional markers (SSRs or SNPs) would be more useful for its efficient utilization. A denser genetic linkage maps with large number of markers by the inclusion of SNPs would facilitate the identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The knowledge of marker-trait association may also lead to the identification of genes influencing agronomic traits.

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