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RESEARCH ARTICLE

Analysis of an intraspecific RIL population uncovers genomic segments harbouring multiple QTL for seed relevant traits in lentil (*Lens culinaris* L.)

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Abstract Improving seed related traits remains key objective in lentil breeding. In recent years, genomic resources have shown great promise to accelerate crop improvement. However, limited genomic resources in lentil greatly restrict the use of genomics assisted breeding. The present investigation aims to build an intraspecific genetic linkage map and identify the QTL associated with important seed relevant traits using 94 recombinant inbreds (WA 8649090 × Precoz). A total of 288 polymorphic DNA markers including simple sequence repeat (SSR), inter simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) were assayed on mapping population. The resultant genetic linkage map comprised 220 loci spanning 604.2 cM of the lentil genome, with average inter-marker distance of 2.74 cM. QTL mapping in this RIL population uncovered a total of 18 QTL encompassing nine major and nine minor QTL. All major QTL were detected for seed related traits viz., seed diameter (SD), seed thickness (ST), seed weight (SW) and seed plumpness (SP) across two locations. A considerable proportion of the phenotypic variation (PV) was accounted to these QTL. For instance, one major QTL on LG5 controlling SW (QTL 15) explained 50% PV in one location, while the same QTL accounted for 34.18% PV in other location. Importantly, the genomic region containing multiple QTL for different seed traits was mapped to a 17-cM region on LG5. The genomic region harbouring QTL for multiple traits opens up exciting opportunities for genomics assisted improvement of lentil.

Keywords Lentil · Molecular marker · Genome · Linkage map · QTL · Trait

Introduction

Lentil (*Lens culinaris* ssp. *culinaris*) is a self-pollinating grain legume grown across the Indian subcontinent, northern Africa, western Asia, southern Europe, North and South America and Australia (Akibode and Maredia 2011). It is an ancient crop that is believed to have originated in the Near East and later spread all through the Mediterranean Basin and central Asia (Cubero et al. 2009). Globally, 4.88 million tons of lentil is harvested annually, and the leading lentil-producing countries are Canada, India, Australia, Turkey and Nepal (FAOSTAT 2014). It is a diploid crop ($2n = 2x = 14$) with a large genome size of 4063 Mbp (Arumuganathan and Earle 1991). Lentil remains an excellent source of plant-based protein (up to 26%) to vegetarian people worldwide, especially in the developing world (https://www.pinterest.com/pin/556053885216362537/).

In recent years, crop genomics has witnessed remarkable developments and plenty of genomic resources have been

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generated in grain legume crops (Bohra et al. 2014). However, lentil still suffers from a dearth of genomic resources compared to the other well-researched legume crops such as soybean and common bean. Molecular markers, genetic linkage maps and QTL are the important genomic tools that remain central to any molecular breeding programme. Among the various marker systems, simple sequence repeat (SSR) marker still remains a preferred category of DNA markers given their greater abundance, co-dominant inheritance and multilocus nature (Gupta and Varshney 2000), though the trend is increasingly shifting towards high-density marker assays including single nucleotide polymorphism (SNP) (Fedoruk et al. 2013).

To understand the genetic architecture of important traits, QTL mapping provides a well-established means to allow the identification of molecular markers associated with the desirable traits. The marker trait associations (MTAs) can subsequently be harnessed in marker-assisted selection (MAS) schemes (Bohra 2013). In the context, linkage maps are useful for predicting the position of QTL/gene within the genome. Several genetic linkage maps have been constructed in lentil (see Bohra et al. 2014).

Seed traits viz. seed size and seed weight represent prime target traits in lentil breeding and manifest quantitative inheritance (Verma et al. 2015). Among legumes, researchers have performed mapping of QTL for seed weight/size in different crops including soybean (Sun et al. 2012), mungbean (Isemura et al. 2012), chickpea (Hossain et al. 2010; Upadhyaya et al. 2006) etc. In lentil, several agronomic traits such as plant height, days to flowering, winter hardiness, pod dehiscence, growth habit and yield have been genetically dissected using both inter-and intra-specific populations (Tar'an et al. 2003; Kahraman et al. 2004; Fratini et al. 2007; Tullu et al. 2008). Similarly, QTL for resistance to diseases like ascochyta blight, anthracnose and stemphylium blight have also been mapped (Ford et al. 1999; Rubeena and Taylor 2003; Tullu et al. 2006). Mapping of seed weight was also reported (Abbo et al. 1991; Verma et al. 2015).

Notwithstanding the plenty of QTL in lentil, the deployment of MTAs in lentil breeding has been limited. Seed diameter (SD), seed thickness (ST) and seed weight (SW) hold immense relevance to market class and consumers acceptance. Similarly, seed plumpness (SP) is also an important trait that influences the dehulling efficiency of the seed during milling. Precise and rapid improvement of these traits using MAS could be cost-effective and time-saving. In view of this, the present research aims to develop a genetic linkage map for cultivated lentil, and identify the DNA markers linked to the genes/QTL controlling seed-related traits using an F_8 recombinant inbred line (RIL) population.

Materials and methods

Plant materials

A RIL population comprising 94 individuals derived from an intraspecific cross of *Lens culinaris* ssp. *culinaris* (WA 8649090 × Precoz) was used for the construction of genetic linkage map. The parents differed from each other significantly with respect to various agro-morphological and seed related traits. The RILs were grown in greenhouse during 2014–2015 for collecting leaf samples for DNA extraction.

This mapping population was phenotyped for nine important quantitatively inherited traits in which four were seed related characters viz. SD (mm), ST (mm), SP and SW (g). Besides seed traits, phenotypic observations were also recorded on days to flowering (DF), number of primary branches (PB), days to maturity (DM), plant height (PHT, cm) and seed per plant (SPP). The material was raised with two replications at two different locations i.e. Palampur (L1) during 2014–2015 and Akrot (L2) during 2011–2012 in Himachal Pradesh, India. The data on PHT, DM and PB were recorded only in one location (L1). The recommended cultivation practices were followed. The location-wise data of all these traits are provided in Supplementary Table 1.

Traits SD (mm) and ST (mm) were measured by averaging ten seeds selected randomly using scale and vernier calliper, respectively. SP was calculated as the ratio of ST and SD. Phenotypic data were also recorded for DF as number of days from planting to appearance of first flower. Similarly, DM were calculated as days from sowing to 75% maturity of the plants, while PHT was measured in cm by centimeter scale. For SW (g), 100 seeds were weighed with the help of electronic balance.

SSR analysis

SSRs were assayed on mapping parents to search for DNA polymorphism. The polymorphic SSRs were then used for genotyping the mapping population. For amplification of genomic DNA, a reaction mixture of 12.5 µl volume was prepared using 7.15 µl of sterilized distilled water, 1.0 µl template DNA (25 ng/µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 1.0 µl $MgCl_2$ (25 mM), 1.25 µl 10 × PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.0 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5U/µl). The amplifications were carried out in Gene Amp PCR System 9700® (Applied Biosystems, CA, USA) and 2720 Thermal Cycler (Applied Biosystems, CA, USA).

The amplified products were electrophoresed in 3% agarose gel (HiMedia, Mumbai, India) and stained with ethidium bromide (0.5 µg/ml). The gels were visualized and photographed using the Gel-Documentation Unit (Bio-Rad, Hercules, CA, USA).

RAPD and ISSR analyses

Due to the lack of adequate SSR polymorphism, RAPD and ISSR primers were also screened to gain the sufficient number of polymorphic DNA markers. A total of 250 RAPD decamer primers (Operon Technologies, Alameda, CA, USA) and 30 ISSR primers (15–23 nucleotides in length) were screened on mapping parents. The primers that produced easy-to-score and polymorphic fragments were then used to genotype the mapping population. Information on polymorphic markers generated from additional 42 RAPD primers (kindly supplied by Fred Muehlbauer, USDA, ARS, USA) was also used in the study. For amplification of genomic DNA, a reaction mixture of 12.5 µl volume was prepared using 7.15 µl of sterilized distilled water, 1.0 µl template DNA (25 ng/µl), 1.0 µl primer (RAPD/ISSR), 1.0 µl $MgCl_2$ (25 mM), 1.25 µl 10X PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 1.0 µl dNTP mix (0.2 mM each of dATP, dGTP, dCTP and dTTP) and 0.1 µl *Taq* polymerase (5U/µl). The amplification was carried out in a 2720 Thermal Cycler (Applied Biosystems, CA, USA).

PCR products were mixed with 2 µl of gel loading dye (0.25% bromophenol blue and 40% sucrose), resolved on 1.5% agarose gel for RAPDs and 1.8% gel for ISSRs and electrophoresed at 100 V for 90 min in 1× Tris acetate-EDTA (TAE) buffer (40 mM Tris, 40 mM Acetic acid Glacial, 1 mM EDTA, pH 8.0). The gels were stained with ethidium bromide (0.5 µg/ml) and the PCR products were visualized and photographed using the Gel-Documentation Unit (Bio- Rad, Hercules, CA, USA).

Linkage analysis

Genotyping data were used to perform linkage analysis using JoinMap v. 4.0 (Van Ooijen 2006). Marker order was determined using the regression mapping algorithm with maximum recombination frequency of 0.4 at minimum logarithm of odd (LOD) score of 3 and jump threshold of 5. The Kosambi mapping function was used to calculate the map distance (Kosambi 1944). To check segregation distortion, Chi square ($\chi 2$) values were calculated using "Locus genotype frequency" function of JoinMap v. 4.0. Final linkage maps were drawn using MapChart v. 2.5 (Voorrips 2002).

QTL analysis

Genotypic and phenotypic data scored on the RIL population were subjected to QTL analysis by using QTL Cartographer v. 2.5 (Wang et al. 2012) (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm). Composite interval mapping (CIM) was performed by selecting Model 6 with the default parameters such as 10 cM window size, control marker number 5, and backward regression method.

Results

Parental polymorphism and population genotyping

Three different types of marker systems viz. SSR, RAPD and ISSR were employed in the present investigation. The SSR markers were *Lens* specific and also from *Trifolium pratense*. Of the total 725 SSR screened on the parental combination, 47 polymorphic SSRs yielded scorable amplicons of expected size. The amplicons generated using SSR primer in parents and RIL are shown in Fig. 1. In parallel, ISSR markers were also screened on parental genotypes to detect DNA polymorphism. Three of the polymorphic ISSRs yielded 5 clear, scorable and reproducible fragments (1.67 markers/primer). The size of the RAPD markers varied from approximately 300–2400 bp.

Construction of an intraspecific genetic linkage map

Of the 288 markers (223 RAPD, 5 ISSR, 60 SSR) assayed on parents and RIL, 220 were placed on to the linkage map (Fig. 2), whereas 68 (51 RAPD, 2 ISSR and 15 SSR) remained unlinked (Table 1).

The present genetic linkage map was constructed at LOD score of 2.5 with a maximum recombination value of 0.40. The map comprised a total of 220 markers including 172 RAPDs, 3 ISSRs and 45 SSRs. Of the total 172 mapped RAPDs, 90 markers were mapped previously in the same population by Kahraman et al. (2004). The linkage analysis established eight linkage groups (LG1-8) spanning a total map length of 604.2 cM with individual LGs varying between 23.1 cM and 156.5 cM. The number of mapped loci per LG ranged from 8 to 57. The average inter marker distance was 2.74 cM (Table 2).

RAPD markers were distributed across all LGs in the genome. Similarly, SSRs were mapped on all LGs except LG1. ISSR markers were mapped on three different LGs (LG8, LG5 and LG6). The distribution of DNA markers across different LGs was unequal and the size of the LG did not necessarily reflect the number of mapped loci. For instance, LG6 with 51 markers covered 85.10 cM with an

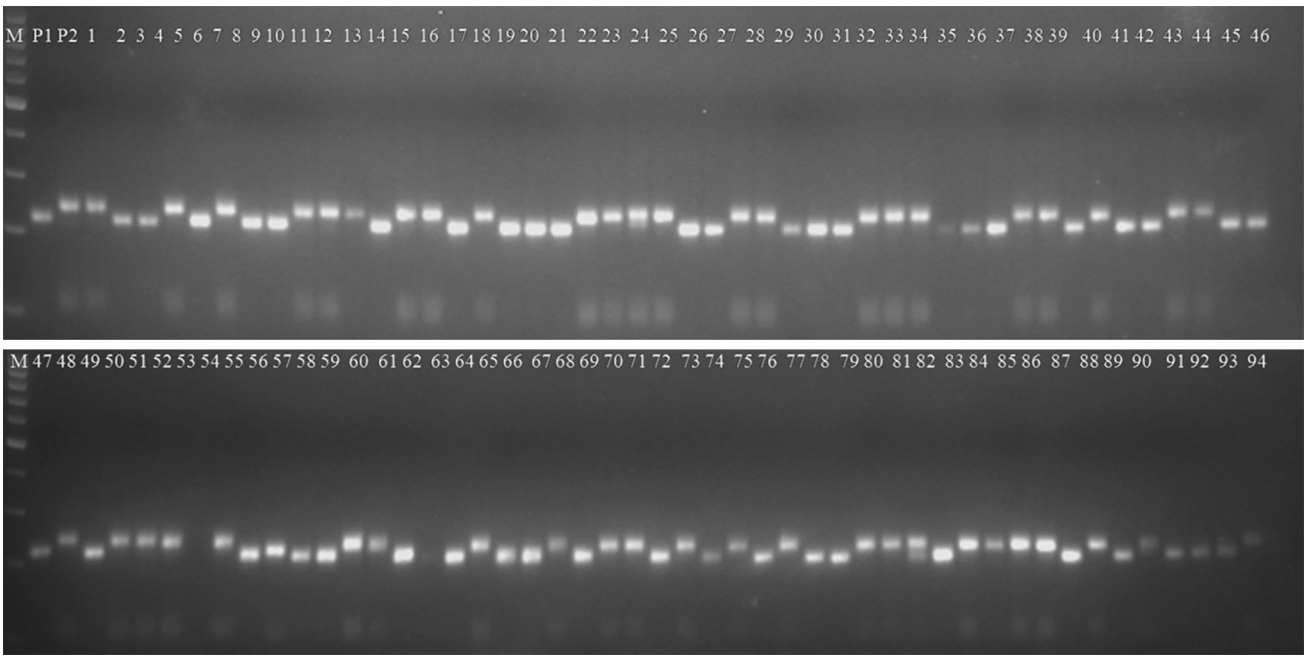

Fig. 1 Genotyping profile of SSR marker (SSR-113). The gel image illustrates the segregation pattern of SSR 113 in mapping parents and recombinant inbred individuals. Lane M, 100 bp standard DNA ladder; lane P1, WA 8649090; lane P2, Precoz; lanes 1–94, mapping individuals

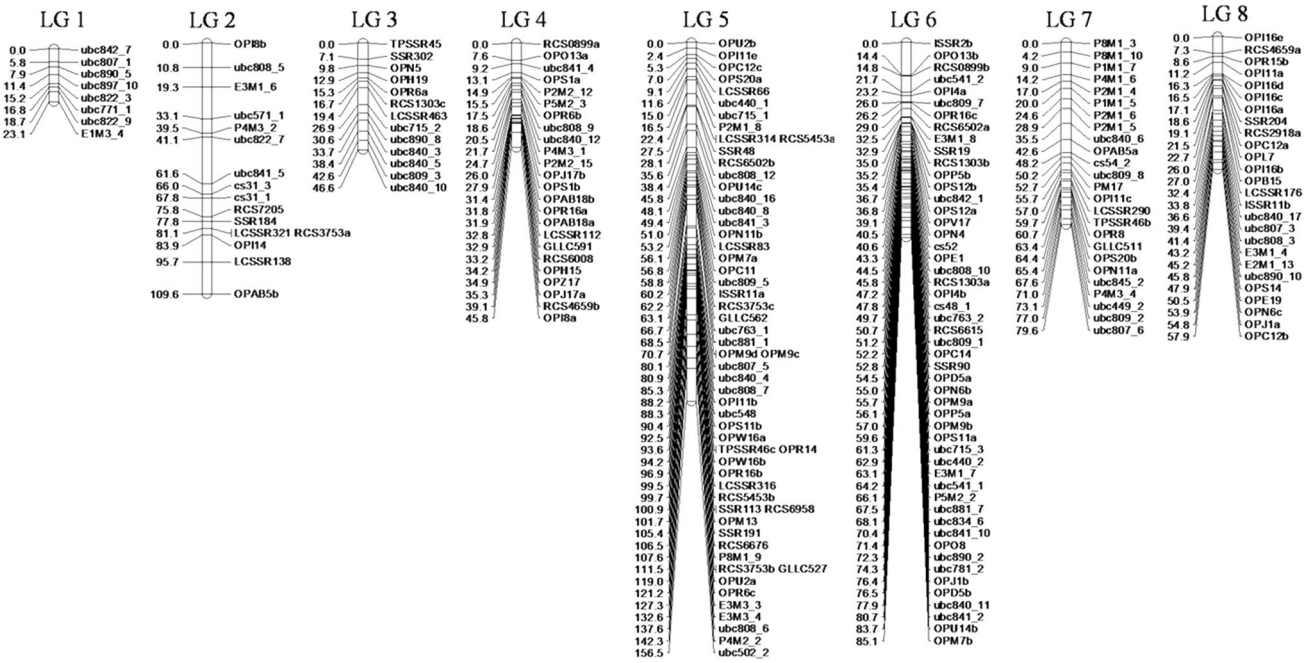

Fig. 2 Intraspecific genetic linkage map of lentil. A total of 220 loci were placed onto eight LGs. The map was developed from a RIL population (WA 8649090 × Precoz)

average marker spacing of 1.67 cM, whereas LG4 spanning a distance of 45.8 cM harboured 24 markers with average spacing of 1.91 cM.

Concerning the marker segregation pattern, of the 68 unlinked markers, nine RAPDs (OPA 10, OPAB6, ubc841_9, E2M1_9, cs54_2, ubc204_1, E2M1_14, ubc502_1, P8M1_8), seven SSRs (SSR 207, LcSSR70, SSR183, SSR309, TPSSR15, RCS5704, RCS7182) and one ISSR (ISSR2a) deviated significantly from the Mendelian segregation of 1:1 ($P < 0.01$). Similarly, one SSR (RCS6021a) and three RAPDs (P4M3_4, OPU14a, E3M1_3) deviated significantly at ($P < 0.05$). The

Table 1 Distribution of mapped loci on the linkage map

Linkage groups(LGs)	Marker			
	RAPD	ISSR	SSR	Total
LG1	8	–	–	8 (3.63%)
LG2	11	–	5	16 (7.27%)
LG3	9	–	4	13 (5.90%)
LG4	19	–	5	24 (10.90%)
LG5	39	1	17	57 (25.90%)
LG6	43	1	7	51 (23.18%)
LG7	22	–	3	25 (11.36%)
LG8	21	1	4	26 (11.81%)
Total	172 (78.18%)	03 (1.36%)	45 (20.45%)	220

Table 2 Salient features of the intraspecific genetic linkage map of lentil

Linkage groups (LGs)	Length (cM)	Markers mapped	Average marker spacing (cM)	Largest inter-marker distance (cM)	Smallest inter-marker distance (cM)
LG1	23.1	8	2.89	4.4	1.6
LG2	109.6	16	6.85	13.9	1.6
LG3	46.6	13	3.58	4	1.4
LG4	45.8	24	1.91	6.7	0.1
LG5	156.5	57	2.75	14.2	0.2
LG6	85.1	51	1.67	2.8	0.1
LG7	79.6	25	3.18	2.6	1
LG8	57.9	26	2.23	3.1	0.2
Total	604.2	220	2.74		

remaining 47 unlinked markers (39 RAPDs, one ISSR and 7 SSRs) segregated in Mendelian fashion. On the other hand, 42 (20 RAPD, 21 SSR and 1 ISSR) distorted markers could be successfully placed onto the linkage map.

QTL mapping

The two parents (WA 8649090 and Precoz) differed significantly with regards to the quantitative traits measured here. Precoz showed higher SD of 5.2 mm compared to 3.7 mm for WA 8649090 (Fig. 3). The RIL had a minimum SD of 3.2 mm and a maximum of 5.6 mm with an average value of 4.40 mm (Table 3). The parents were highly contrasting for DF with Precoz showing average DF value of 88 days in two locations, whereas WA 8649090 was late maturing with average DF value of 172. DF in RIL ranged from 83 to 145.25 with average of 114.12 in two locations. Meager difference was observed for ST (mm) with WA 8649090 and Precoz showing the ST values of 2.3 and 2.2 mm, respectively. In RIL, ST ranged from 2.0 to 3.0 mm with an average of 2.50 mm. Due to variations in both SD and ST, difference in SP score was observed. The genotype WA 8649090 exhibited SP value of 6.2, whereas Precoz with more seed diameter had a plumpness score of 4.2. SP values in RIL varied from 3.6 to 7.8 with an average of 5.7. A significant difference was recorded for 100-SW (g) between the two parents (WA 8649090: 2.6 g and Precoz: 4.63 g), while 100-SW in RIL ranged from 1.82 to 5.50 g with an average of 3.66 g.

Analysis of different genetic parameters also showed a significant difference in the RIL for these traits (Table 4). For all traits, phenotypic values greater than that of the higher parent and lower than that of the lower parent were observed. We observed a higher level of heritability for traits viz. DF, SD, ST, SW and SP with percent heritability of 97.56, 98.23, 99.28, 70.27 and 88.31, respectively. Concerning correlations among different traits, significant positive correlations were recorded of ST with DF and SP with that of SW. By contrast, significant but negative correlations were observed for SD with DF, and of SW with ST.

QTL analysis uncovered a total of nine QTL (QTL 2, 3, 4, 5, 6, 8, 9, 11 and 15) for seed size related traits that explained more than 10% of the phenotypic variation (PV)

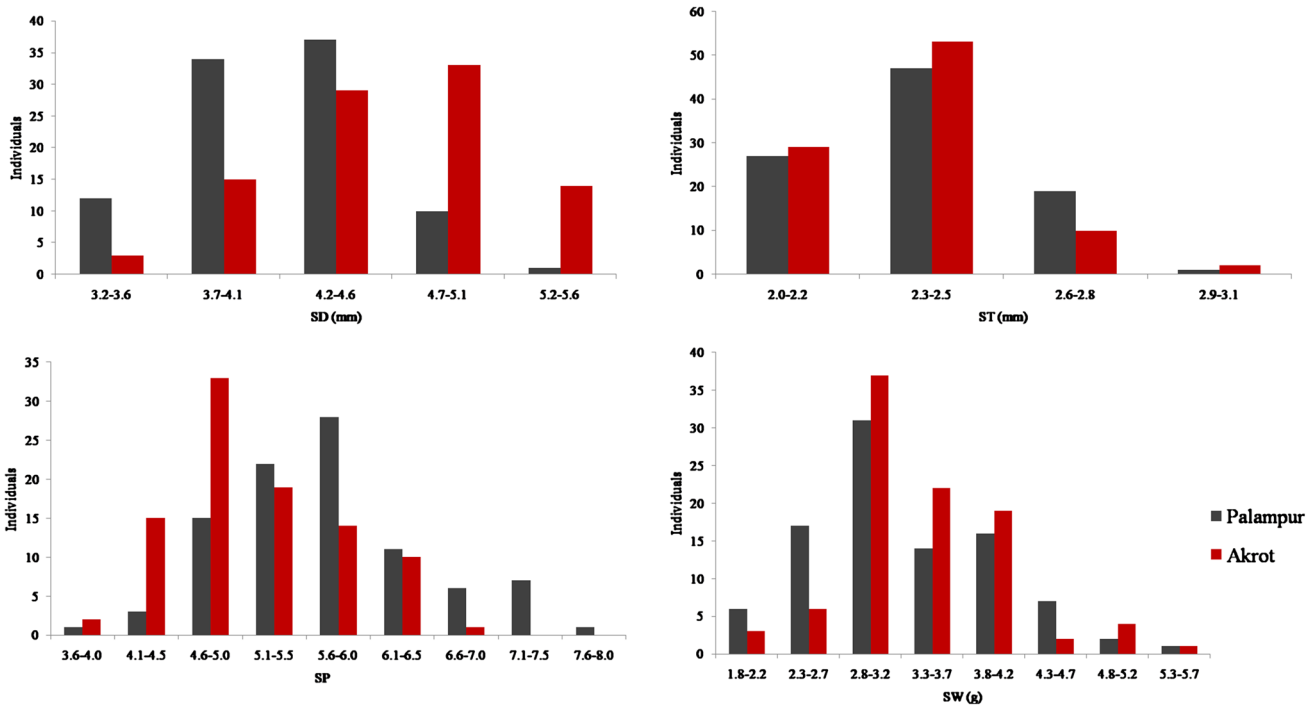

Fig. 3 Frequency distribution of different seed related traits in parents and RIL in two locations

Table 3 Trait mean and range of parents and RI individuals

S. no	Traits	P1	P2	RILs		
		(WA 8649090)	(Precoz)	Minimum	Maximum	Average
1	DF	172	88	83	145	114.12
2	SD (mm)	3.7	5.2	3.2	5.6	4.40
3	ST (mm)	2.3	2.2	2	3.0	2.50
4	SP	6.2	4.2	3.6	7.8	5.7
5	SW (g)	2.6	4.63	1.82	5.5	3.66

Table 4 Genetic parameters in RIL for different traits

Traits	Heritability (%)	GA	% GA	PCV	GCV	GM	CV
DF	97.56	42.42	33.3	16.57	16.36	127.99	2.59
SD (mm)	98.23	113.52	120.18	59.39	58.86	94.46	7.91
ST (mm)	99.28	337.11	149.25	72.98	72.71	225.87	6.2
SW (g)	70.27	0.08	19.26	13.3	11.15	0.42	7.25
SP	88.31	0.09	19.92	10.95	10.29	0.46	3.74

(Fig. 4). Remaining nine QTL with PVs ranging between 2.5 and 9.8% were obtained for traits SD, SW, SP, ST, PHT, and SPP. No significant QTL could be detected for DF, DM and PB. Concerning major QTL, two QTL for SD i.e. QTL 2 in L1 and L2 and QTL 3 (in L2) accounted for 35.3, 14.6 and 12.6% PV, respectively. Similarly, three major QTL for ST i.e. QTL 4 (in L2 with 11.7% PV), QTL 5 (in L1 and L2 with 24.0 and 20.3%, PV respectively) and QTL 6 (in L1 and L2 with 18.6 and 17.3% PV, respectively) were mapped on three different LGs (Table 5). Three major QTL were detected for SP on two LGs viz. on LG5, QTL 8 with 18.3 and 17.0% PV in L1 and L2, respectively and QTL 9 (in L2) with 15% PV, and QTL 11 (L2) explaining 10.2% PV on LG7. Similarly, one major QTL for SW on LG5 (QTL 15) explained 50.0% and with 34.2% PV in L1 and L2, respectively.

The QTL associated with seed related traits were flanked by different markers, viz. QTL 2 (in L1 and L2) for SD was flanked by the DNA markers OPW16b and LcSSR316, while QTL 3 for the same trait (L2)

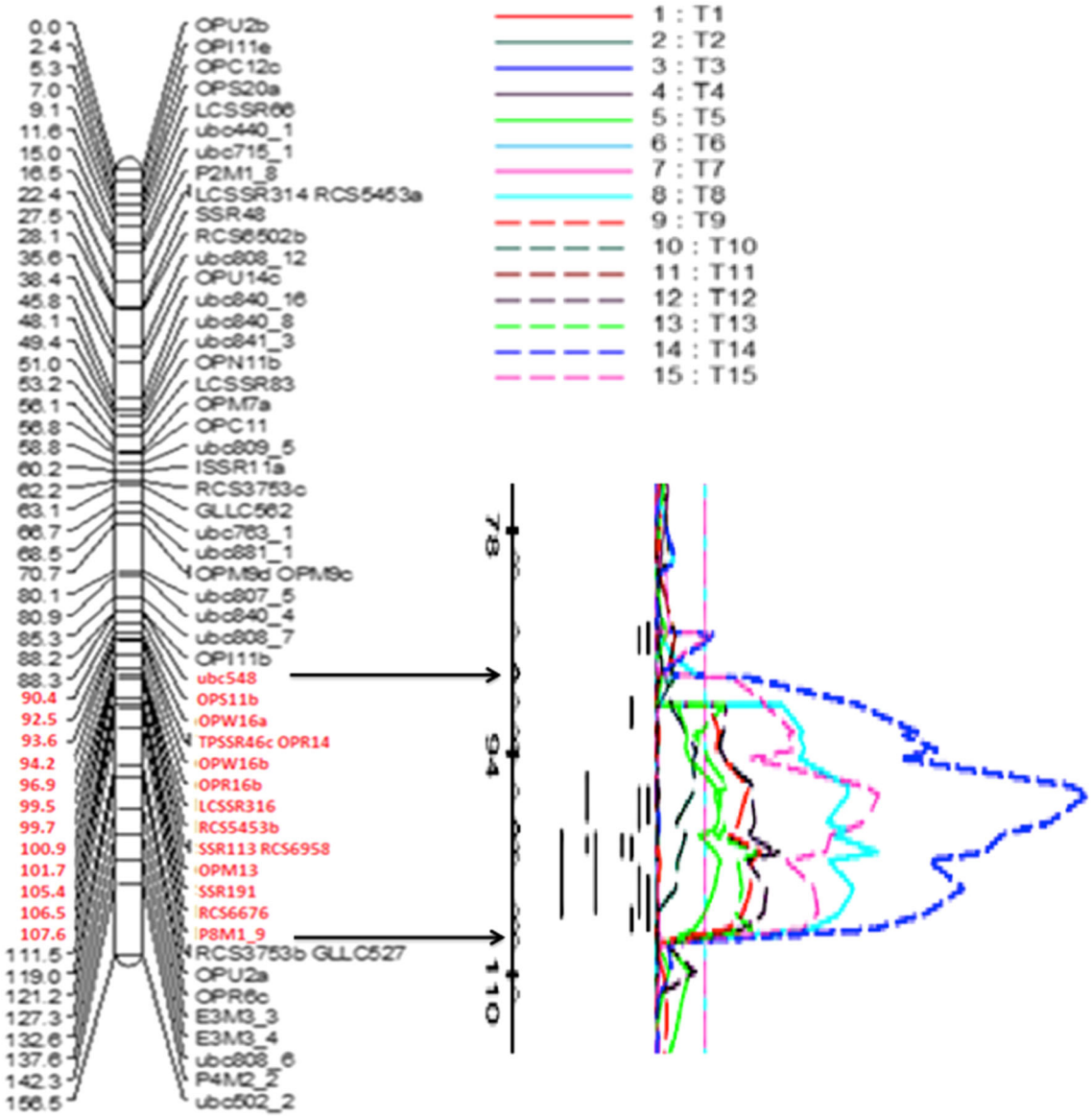

Fig. 4 Genomic region on LG5 harbouring multiple QTL. Five major effect QTL controlling seed related traits were discovered on LG5, of which three QTL were mapped within a genomic region of 90-107 cM. Codes of the traits used for QTL analysis from T1-T15 are as follows: T1 (DF, L1), T2 (DF, L2), T3 (PB, L1), T4 (DM, L1), T5 (PHT, L1), T6 (SPP, L1), T7 (SPP, L2), T8 (SD, L1), T9 (SD, L2), T10 (ST, L1), T11 (ST, L2), T12 (SP, L1), T13 (SP, L2), T14 (SW, L1), T15 (SW, L2)

contained within the marker interval ubc808_6-ubc502_2 on LG5. Three QTL for ST, QTL 4 (in L2) flanked by the markers LcSSR138 and OPAB5b, QTL 5 (in L1 and L2) flanked by the markers SSR90 and OPN6b, and QTL 6 (in L1 and L2) flanked by the markers LcSSR176 and ubc840_17 were detected on LG2, LG6 and LG8, respectively. Three QTL for the trait SP were detected on two different LGs viz. LG5 and LG7, of which QTL 8 (in L1 and L2) and QTL 9 (in L2) were located within the marker intervals RCS5453b-OPM13 and ubc808_6-ubc502_2, respectively, whereas QTL 11 (in L2) on LG7 was flanked by the markers ubc840_6 and OPAB5a. For the trait SW, QTL 15 was detected on LG5 in both locations (L1 and L2) within the marker interval OPR16b-RCS5453b.

Of the total five major-effect QTL detected on LG5, three were located with a 17-cM region, which included QTL 2 for SD, QTL 8 for SP and QTL 15 for SW. Importantly all these QTL were detected in both locations L1 and L2 (Fig. 4). On the other hand, QTL 3 for SD and QTL 9 for SP located at 148 cM and 150 cM, respectively were obtained only in one location (L2) (Table 5). In total, nine QTL were mapped on LG5, with the QTL for SW (L1) showing the highest PV of 50%.

Table 5 QTL for seed-related traits in RIL (WA8649090 × Precoz) across two locations viz. Palampur (L1) and Akrot (L2), Himachal Pradesh, India

Traits	LG	[a]QTL	Position (cM)	Marker interval	Maximum LOD	Additive effect	%PV
SD(mm)(L2)	LG5	QTL 1	9.16	OPS20a–ubc440_1	2.6	−0.0138	7.2
SD(mm)(L1)	LG5	QTL 2	96.2	OPW16b–LcSSR316	12	−0.031	35.3
SD(mm)(L2)	LG5	QTL 2	96.2	OPW16b–LcSSR316	6.5	−0.019	14.6
SD(mm)(L2)	LG5	QTL 3	148	ubc808_6–ubc502_2	2.6	0.018	12.6
ST(mm)(L2)	LG2	QTL 4	97.71	LcSSR138–OPAB5b	3.8	−0.009	11.75
ST(mm)(L1)	LG6	QTL 5	54	SSR90–OPN6b	7	0.0107	24
ST(mm)(L2)	LG6	QTL 5	54	SSR90–OPN6b	6	0.0094	20.3
ST(mm)(L1)	LG8	QTL 6	33.84	LcSSR176–ubc840_17	6.5	0.0093	18.62
ST(mm)(L2)	LG8	QTL 6	33.84	LcSSR176–ubc840_17	5.6	0.0098	17.36
ST(mm)(L1)	LG8	QTL 7	41.38	ubc807_3–E3M1_4	2.6	0.0053	6.2
SP(L1)	LG5	QTL 8	100.9	RCS5453b–OPM13	7	0.361	18.3
SP(L2)	LG5	QTL 8	100.9	RCS5453b–OPM13	6.5	0.0314	17
SP(L2)	LG5	QTL 9	150	ubc808_6–ubc502_2	3.5	−0.029	15
SP(L2)	LG5	QTL 10	156.33	P4M2_2–ubc502_2	2.6	−0.0236	9.8
SP(L2)	LG7	QTL 11	39.53	ubc840_6–OPAB5a	2.6	0.0249	10.22
SP(L1)	LG8	QTL 12	33.84	LcSSR176–ubc840_17	3	2.38	8
SW(g)(L1)	LG4	QTL 13	23.71	P4M3_1–OPJ17b	5.5	−0.1964	6.4
SW(g)(L1)	LG5	QTL 14	58.8	OPC11–ISSR11a	2.6	−0.1333	2.5
SW(g)(L1)	LG5	QTL 15	98.8	OPR16b–RCS5453b	22	−0.55	50
SW(g)(L2)	LG5	QTL 15	98.8	OPR16b–RCS5453b	12	−0.4	34.18
SPP(L1)	LG5	QTL 16	63.12	ISSR11a–GLLC562	2.6	−47.28	7.7
SPP(L1)	LG8	QTL 17	26.03	OPL7–OPB15	3.2	−64.13	9.35
PHT(cm)(L1)	LG3	QTL 18	14.87	OPN5–OPR6a	2.8	−2.94	7.48

[a] In each location, QTL with the highest PV is shown in cases where more than one QTL is detected within the same marker interval

Discussion

Biparental QTL mapping is a standard method to discover the genomic regions that are tightly associated with economically significant traits. DNA markers and linkage maps are essential prerequisite for the identification of these candidate genomic segments that harbour the genes/QTL influencing the traits of interest. To date, various linkage maps have been constructed in lentil using inter-specific as well as intra-specific experimental populations (see Bohra et al. 2014). Different kinds of mapping populations like F_2 as well as RIL have been employed for the development of genetic linkage maps by exploiting DNA marker systems such as RAPD, AFLP, ISSR (Eujayl et al. 1998; Rubeena and Taylor 2003; Duran et al. 2004; Kahraman et al. 2004, 2010; Tullu et al. 2008), SSR (Hamwieh et al. 2005;Verma et al. 2015) and SNP (Kaur et al. 2013). In the present investigation, we could map 220 loci (45 SSRs, 3 ISSRs and 172 RAPDs) onto eight LGs, varying in lengths from 23.1 cM to 156.5 cM. The map spanned a total of 604.2 cM of the lentil genome with an average marker density of 2.74 cM. The genetic map allowed assignment of 23 new cross-genera SSR markers from *T. pratense* (reported by Sato et al.2005) out of total 45 SSR markers placed onto the linkage map. We could not compare mapping positions/order of the loci in the current linkage map with that of earlier published maps (Hamwieh et al. 2005; Phan et al. 2007;Saha et al. 2010; Gupta et al.2012). The positive and negative additive effects of a QTL controlling a particular trait imply towards an increase in the phenotypic value of the trait by the alleles contributed respectively by Precoz and WA 8649090.

With regard to marker segregation pattern, several DNA markers used in the current analysis showed deviation from Mendelian inheritance. Sixty-six markers showed segregation distortion of the total 288 DNA markers assayed in the population. Though more pronounced in case of inter-specific crosses (Eujayl et al. 1998), instances of segregation distortion were also observed in cultivated crosses in lentil. For instance, Rubeena and Taylor (2003) observed a segregation distortion of about 14% (17 out of 118 markers) in an intraspecific F_2 population of lentil. Similarly,

segregation distortions up to varying degrees were reported in lentil mapping populations (Duran et al. 2004; Hamwieh et al. 2005; Phan et al. 2007; Tullu et al. 2008; Saha et al. 2010; Gupta et al. 2012; Verma et al. 2015).

Detection of genetic determinants underlying seed related traits via QTL mapping is a promising way to facilitate targeted trait improvement in lentil. Given the context, we analyzed the phenotypic and genotypic data recorded on a RIL population in order to map important traits such as seed related characters (SD, ST, SW and SP). It becomes imperative to mention here that a set of five major-effect QTL was mapped on LG5 across two locations. Most importantly, the genomic region on LG5 contained multiple QTL associated with different traits. The QTL contained within this genomic region control important traits like SW (QTL 15 in L1 and L2), SD (QTL 2 in L1 and L2) and SP (QTL 8 in L1 and L2). For SW, QTL 15 at L1 explained the highest PV of 50%. Also, the QTL located within this 17 cM region explained considerable PV ranging between 14.60 and 50%. More recently, Verma et al. (2015) reported QTL for SW on LG4. Occurrence of such genomic regions that contain a range of QTL controlling multiple traits has been reported in case of other grain legume crops. For example, Varshney et al. (2014) reported a hot spot QTL in chickpea through analyzing data on two RIL populations, and notably, the QTL detected within this region explained PV up to 58%.

Likewise, Tahir and Muehlbauer (1995) also obtained three QTL for seed weight on LG1, 4 and 5. As reported in chickpea by Varshney et al. (2013), 100-seed weight QTL was found to be consistent across environments out of the QTL for 12 traits contained within the *QTL hotspot* region. Knowledge of genomic segments that harbour QTL for multiple traits offers exciting opportunities for genomics assisted crop improvement. The recent example includes the introgression of QTL hotspot into an elite yet drought susceptible chickpea cultivar (Varshney et al. 2013), thereby demonstrating the relevance of such genomic regions in crop breeding.

In summary, the current study offers valuable supplements to genomic toolkit of lentil breeder. A 220-loci genetic linkage map with 604.2 cM length was built. Most importantly, we could identify a set of large-effect QTL within a 17-cM genomic segment on LG5. This genomic region, after confirmation in independent experiments, could serve as a promising candidate for future lentil genomics and breeding.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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