

Research paper

Phenotypic variability and SNP markers for root and shoot traits in lentil

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ABSTRACT

Two hundred recombinant inbred lines of lentil derived from a cross IPL 98/193 (long root) × EC208362 (short root) was phenotyped in PVC tubes for root traits over two years. The observations were recorded after 60 days of sowing. Significant phenotypic variability for root and shoot traits under controlled conditions and for agronomic traits under normal sown conditions was observed in the present investigation among the RILs. The genotype and environment interactions were significant for all studied traits in the present investigation. No or poor association of root traits was observed with agronomic conditions in the present investigation. Bulk segregant analysis with SNP markers showed association of 15 SNP markers with root traits. Thus phenotypic variability present in the present RIL population can be used for identifying the QTLs for root traits and their use in breeding programs for the development of drought tolerant cultivars.

Key words: Bulk segregant analysis, Recombinant inbred lines, Root traits, SNP markers, Variability

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an important cool season pulse crop. Globally, lentil is cultivated on 5.0 m ha area with an annual production of 5.86 mt and an average productivity of 1305 kg/ha in the year 2020 across 52 countries (FAO 2017, 2022). India is one of the major lentil producing countries in the world having its 1.30 m ha area and 1.58 mt production with average productivity of 1216 kg/ha (AICRP Report 2021-22). The biotic and abiotic stresses highly affect the productivity of lentil crop. Intermittent drought and terminal moisture stress are one of the main abiotic stresses that lead to forced maturity and lower yield in lentil (Silim *et al.*, 1993). Limited water availability causes several morphological, physiological, biochemical, and molecular level changes in plants leading to poor growth and development of lentil crop. The ability of plants to sustain these changes depends on the growth stage, genetic potential, stress intensity, and duration (Zhu *et al.*, 2002). However, the seedling and reproductive stages are extremely sensitive to drought and may impact the yield significantly (Salehi *et al.*, 2012). Therefore, the development of improved cultivars with better water-use efficiency (WUE) and root traits is the economical approach

for managing water-limited conditions. Although no direct selection has been done for (WUE) by breeders in legumes, indirect selection for yield traits under stress may have an association with the desired root traits for the target environment (Wasson *et al.*, 2015). Studies showed an association between root system architecture (RSA) especially primary root length (PRL) and yield under drought conditions in several legume crops including soybean (Fried *et al.*, 2019), common bean (Sofi *et al.*, 2018), chickpea (Ramamoorthy *et al.*, 2017) and lentil (Kumar *et al.*, 2012). The long roots along with dense lateral root development (i.e. high dry root weight) are known to enhance the capacity of the plant to extract water from the lower soil strata (Kumar *et al.*, 2012). In lentil, plant shoot and root attributes observed at an early stage of growth are related to drought tolerance through a dehydration avoidance mechanism that allows crop plants to thrive in water-limited environments (Sarker *et al.*, 2005). Therefore knowledge of genetic variability for root traits can help to breed improved drought-tolerant cultivars in lentil. Earlier significant variation for different root traits was observed among several lentil germplasm (Kumar *et al.*, 2012; Priya *et al.*, 2021). However, root traits are complex in genetic architecture, and many genes/

QTLs along with high genotype \times environment interactions are involved to control the root trait inheritance. Thus genetic dissection of root traits through molecular marker-based mapping and tagging can help to identify tightly linked markers with genes/QTLs responsible for these traits, which can be further utilized in breeding for developing drought tolerant cultivars through marker-assisted selection. In an earlier study, 18 QTLs controlling 14 root and shoot traits were identified and among these a QTL-hotspot genomic region controlling several root and shoot characteristics associated with drought tolerance was identified (Idrissi *et al.*, 2015). Besides, polygenic inheritance has been observed for root traits and quantitative trait loci (QTLs) have been identified in other legume crops such as common bean (Cichy *et al.*, 2009; Aswaf and Blair, 2012), soybean (Brensha *et al.*, 2012) and chickpea (Kashiwagi *et al.*, 2014). The aim of this study was (i) to phenotype the RILs for root traits and other agronomic traits and (ii) to identify the polymorphic SNP markers between parents and their association with root traits through bulk segregant analysis.

MATERIALS AND METHODS

Development of RILs

Parental lines IPL 98/193 having long root length (80.2 cm) and EC208362 (41.9 cm) for short root lengths were identified earlier at ICAR-Indian Institute of Pulses Research, Kanpur after the screening of forty-three (43) lentil genotypes of active germplasm including varieties, breeding lines, and exotic germplasm lines (Kumar *et al.*, 2012). These lines were used to develop the RIL mapping population. For this, a cross between these two parents was made in 2010-11 and F_1 s were grown in 2011-12. Subsequently, segregating the F_2 population of this cross was advanced to F_6 using the single seed decent method at ICAR-Indian Institute of Pulses Research, Kanpur. This resulted in the development of 200 RILs, which were used in the present study.

Phenotypic evaluation of RILs

Pot experiments: The parents and RIL population were grown in a complete block design with two replications in PVC tubes (Rabi 2017-18 to 2018-19). PVC tubes were filled with 10:1 soil and vermiculate mixture. Pots were watered as and when needed to obtain good seedling growth.

Measurements of stem length (cm), taproot length (cm), and dry root weight (g) were made on 65-day-old seedlings. All measurements were taken manually using one-meter steel scale. For dry root weight, the root of four plants was dried in the oven for 24 h and measured their weight in grams (g).

Field experiment: Two hundred RILs along with parents were evaluated for agronomic traits during the Rabi season (2017-18 to 2018-19) in a randomized complete block design with two replications. Single-row plot of 3 m length with row to row distance of 30 cm was used and data was recorded on days to 50 % flowering (no.), plant height (cm), pods/plant (no.), grain yield/plant (g), days to maturity (no.) and 100-seeds weight (g).

Analysis of statistical parameters

Mean, range, and analysis of variance were calculated using the statistical data analysis program of Excel software.

Construction of bulks for double digest restriction-site associated DNA sequencing (ddRADseq)

The genomic DNA of 10 individual plants of RILs having the longest and shortest root length was bulked separately and used along with the parental lines DNA for sequencing. Illumina TrueSeq chemistry on the Illumina HiSeq 2000 platform used for ddRAD sequencing. The reads based were clustered on the sequence similarity $\geq 90\%$ (Uclust version 1.2). All clusters with singleton reads are filtered out from further processing. The centroid sequence of the cluster has been considered as a reference/representative sequence for that respective cluster reads. The reads are then aligned to the reference sequence selected from the clusters using the Bowtie 2 (version 2.2.9) program. The aligned samples are processed using in-house Perl script and variant calling is performed using the Samtools program (Samtools version 0.1.18).

Identification of SNP marker associated with root traits

Sequencing reads having polymorphic SNPs between parents were aligned with the same sequence reads of bulks. At each SNP locus, the common nucleotide between the sequencing reads of parent and bulk having a higher value and the common nucleotide between parent and bulk having the lower value was considered to be associated with the targeted trait as given below.

---NNNNANNNNNN--- Parent (Higher Value)
 ---NNNNANNNNNN--- Bulk (High Value)
 ---NNNNGNNNNNNN--- Parent (Lower Value)
 ---NNNNGNNNNNNN--- Bulk (Lower Value)

RESULTS AND DISCUSSION

Intermittent drought and terminal moisture stress are important abiotic stresses that cause forced maturity and lower yield in lentil due to several morphological, physiological, biochemical, and molecular level changes in plants (Silim *et al.*, 1993; Zhu *et al.*, 2002). The studies showed positive association of root system architecture (RSA) especially primary root length (PRL) with higher yield under drought conditions in several legume crops including soybean (Fried *et al.*, 2019), common bean (Sofi *et al.*, 2018), chickpea (Ramamoorthy *et al.*, 2017) and lentil (Kumar *et al.*, 2012). The long roots along with dense lateral root development (i.e. high dry root weight) are known to enhance the capacity of the plant to extract water from the lower soil strata leading to tolerance under drought conditions (Kumar *et al.*, 2012). Therefore genetic knowledge of root traits can help to develop genotypes with better root systems. However, root traits are complex in genetic architecture, and many genes/QTLs along with high genotype x environment interactions are involved to control the root trait inheritance. Thus genetic dissection of root traits through molecular marker-based mapping and tagging can help to

identify tightly linked markers with genes/QTLs responsible for these traits, which can be further utilized in breeding for developing drought tolerant cultivars through marker-assisted selection. In the present investigation, a RIL mapping population derived from a cross having better and poor root systems has been evaluated for root traits and other agronomic traits to study the genetic variability among the RILs compared to parental lines, which can be further utilized in the dissection of the complex genetics of root traits through QTL mapping. The mean root length between contrasting parents (IPL 98/193 and EC 208362) over two years ranged from 17.3±2.34 cm to 42.0±4.35 cm. The mean root length among the 200 RILs derived from these parental lines ranged from 17.0 cm to 50 cm, while S.E. of mean in these lines ranged from 0.03 cm to 19.7 cm over two years. For shoot length, contrasting parents (IPL 98/193 and EC 208362) had 11.67±1.67 cm to 37.83±0.50 cm shoot length over two years. The mean shoot length among the 200 RILs derived from these parental lines ranged from 8.58 cm to 37.83 cm, while S.E. of mean among these lines ranged from 0.02 cm to 9.38 cm over two years. The mean dry root weight between contrasting parents (IPL 98/193 and EC 208362) over two years was 0.10 g to 0.44 g. However, it ranged from 0.05 g to 0.58 g among RILs. The S.E of mean among RILs was 0.00 g - 0.35 g. Thus a wider range of variability was observed among the RILS for these

Table 1. Mean, range and S.E of mean for root length (cm), shoot length (cm) and dry root weight (g) among parental lines and RILs over the years.

Parents/ RILs	Root length (cm)				Shoot length (cm)				Dry root weight (g)			
	2018	2019	Mean	S.E of Mean	2018	2019	Mean	S.E of Mean	2018	2019	Mean	S.E of Mean
IPL98/193	46.3	57.7	52.0	4.35	37.33	38.33	37.83	0.50	0.48	0.39	0.44	0.05
EC208362	15.0	19.7	17.3	2.34	13.0	10.0	11.67	1.67	0.12	0.07	0.10	0.02
RILs (Range)	11.8- 67.6	7.8-55.3	17.0 50.0	0.03- 19.7	4.70- 37.33	8.00- 38.33	8.58- 37.83	0.02- 9.38	0.03-0.82	0.04- 0.87	0.05- 0.58	0.00- 0.35

Table 2. Combined analysis of variance for agronomic traits among RILs over two years under normal shown condition

Source of variation	DF	Mean of Squares					
		50% flowering (days)	Plant height (cm)	Pods/plant (no.)	Yield/Plant (g)	Maturity (days)	100-seed weight (g)
Replication	1	102.25	469.12	175.91	3.05	0.32	3.55
RILs	199	35.71**	16.17**	884.23**	2.02*	33.33**	0.16**
Years	1	2422.08**	1161.94**	7377.11**	58.11*	36153.61**	11.31**
RILs × Year	199	32.29**	20.69**	1,070.82	2.037*	29.24**	0.15**
Error	399	4.165	11.79	680.424	1.628	6.70	0.12

* and ** significant at <0.05 and <0.01 probabilities, respectively

root and shoot traits. However, S.E. of the mean also showed a wider range indicating instability among RILs for root and shoot traits of the present study. The range of variability was found wider in RILs compared to the parental lines from which these RILs were derived through crossing for root traits. The recombinant inbred lines of the present study were also evaluated for other agronomic conditions under normal sown conditions over two years. The combined analysis of variance showed significant genetic variability at $p = < 0.01$ or $p = < 0.05$ among RILs for these agronomic traits. Similarly environmental conditions of each year and RILs \times Year interaction also significantly contributed to the phenotypic variability of these traits. Present study showed a wide range of genetic variability among RILs for root and shoot length and dry root weight. This variability was comparatively higher in RILs compared to parental lines indicating transgressive segregation for these traits in RILs. Thus genotypes with better root systems than parental lines have been provided in this mapping population which can be useful for mapping the QTLs for root traits. However, instability among RILs for root and shoot traits was also observed as a very higher S.E of mean

was found for many RILs over the year. Similarly environmental conditions of each year and RILs \times Year interaction also significantly contributed to the phenotypic variability of these traits indicating the role of environments in contributing to the variability for these quantitatively inherited traits. Earlier several studies also demonstrated that root traits are complex in genetic architecture and many genes/QTLs along with high genotype \times environment interactions are involved to control the root traits inheritance (Kumar *et al.*, 2012; Priya *et al.*, 2021).

The association of root and shoot length and root dry weight of RILs estimated in controlled conditions was established with agronomically important traits evaluated under normal shown conditions. The correlation among these traits calculated for 200 RILs is presented in Table 3. These results showed no positive correlation between root length and root dry weight with most of agronomically traits studied in the present investigation. However a poor but significantly positive correlation of root length was observed with days to maturity ($r = 0.17$) at $p = 0.05$ and a poor but significantly negative correlation of root

Table 3. Correlation of root and shoot traits with agronomic traits in RILs

Traits	Root length (cm)	Shoot length (cm)	Root dry weigh (g)	50% flowering (days)	Plant height (cm)	Pods/plant (no.)	Yield/Plant (g)	Maturity (days)	100-seed weight (g)
Root length (cm)	1.00								
Shoot length (cm)	0.08	1.00							
Root dry weigh (g)	-0.08	-0.01	1.00						
50% flowering (days)	0.10	0.10	0.05	1.00					
Plant height (cm)	0.04	0.05	0.01	0.29*	1.00				
Pods/plant (no.)	-0.14*	0.09	0.09	-0.03	0.05	1.00			
Yield /plant (g)	0.04	0.01	0.06	0.04	0.35*	0.35*	1.00		
Maturity (days)	0.17*	0.07	0.05	0.72*	0.33*	0.05	0.13	1.00	
100 -seed weight (g)	-0.16*	-0.06	0.01	-0.11	-0.10	-0.09	0.04	-0.20*	1.00

*Significant at 0.05 probabilities and 198 D.F.

Table 4. Raw reads, clean reads, % passed reads, SNPs and Idel obtained through ddRADseq in parental genotypes and bulks derived from a RILs population for root length and dry root weight in lentil.

Parents/Bulks	Raw reads	Clean reads	% passed reads	SNP (2X)	Indel (2X)	SNP (5X)	Indel (5X)	SNP (10 x)	Indel (10X)
IPL 98/193	2,571,328	2,525,698	98.23	34	0	15	0	4	0
EC208362	2,285,474	2,246,350	98.29	22	0	16	0	1	0
Bulk 1 (high)	1,753,882	1,712,528	97.64	27	0	19	0	2	0
Bulk 2 (low)	2,239,804	2,193,590	97.94	41	0	24	0	2	0

Table 5. SNPs associated with root traits in lentil identified through BSA-ddRADseq in the present study

Read ID	SNPs in parents and corresponding bulks			
	Long root parent IPL 98/193	Bulk of long root RILs	Short root parent EC 208362	Bulk of Short Root RILs
U130578_65	G/T	G/T	G/G	G/G
U496110_85	T/T	T/T	G/G	G/G
U520704_33	T/T	T/T	G/G	G/G
U520704_34	G/G	G/G	T/T	T/T
U557798_35	A/A	A/A	--	--
U1027770_37	T/T	T/T	T/G	T/G
U1027770_74	C/C	C/C	C/A	C/A
U1145114_33	T/T	T/T	C/C	C/C
U1396189_67	A/G	A/G	A/A	A/A
U1396189_70	G/T	G/T	G/G	G/G
U1396189_75	G/T	G/T	G/G	G/G
U1396189_82	C/T	C/T	C/C	C/C
U1491880_65	C/C	C/C	C/T	C/T
U2434730_23	A/G	A/G	G/G	G/G
U2434730_76	C/A	C/A	A/A	A/A

length with pods per plant ($r = -0.14$) and 100-seed weight ($r = -0.16$) at $p = 0.05$. A significantly high and positive correlation was observed between days to 50% flowering and days to maturity ($r = 0.72$) (Table 3). Although no correlation of root traits with agronomic traits was observed in the present study, RILRT 139, RILRT 178, and RILRT 178 RILs had long root lengths (31.9 cm – 45.8 cm) and showed good performance under drought conditions. Thus, in the present investigation, no or poor correlation of root traits was observed with agronomic traits indicating that these traits are independent and combined in one background through conventional and molecular breeding.

The association of SNP markers was also studied in the presented study through bulk segregant analysis. The ddRADseq was used to sequence the parental line (i.e IPL 98/193 and EC 208362) involved in the RIL mapping population. The number of raw reads in parent IPL 98/193 was 2,571,328 that covered 257 Mb bases while the number of raw reads in another parental line EC 208362 was 2,285,474 that covered 228 Mb of lentil genome (Table 4). The percentage of GC content in these reads was 42.64 and 42.89, respectively. SNPs were identified at different read depths. The clean reads are used to identify the SNP markers associated with these traits. Although a large

number of sequencing reads were identified in parents and bulks for each trait, a limited number of sequencing reads (436) contained SNPs. Ninety-four (94) SNP markers were identified as polymorphic between the parental lines (IPL 98/193 and EC 208362). Among these SNPs, 15 SNPs showed their association with root length based on bulk segregant analysis (Table 5). These SNPs are useful for further mapping the root traits of QTLs in this mapping population. Earlier many QTLs controlling root and shoot traits were identified in lentil and a QTL-hotspot genomic region controlling several root and shoot characteristics associated with drought tolerance were also identified in lentil (Idrissi *et al.*, 2016). Thus present investigation showed genetic variability for root and shoot traits along with other agronomically important traits. Hence present RIL mapping population is suitable for better understanding the genetic architecture of these complex traits through QTL mapping.

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REFERENCES

- AICRP (2021-22). Project Coordinator's Report on MULLarP Rabi Pluses 2021-22. ICAR-Indian Institute of Pulses Research, Kanpur, India.
- Brensha, W, Kantartzi, SK, Meksem, K, Grier, IV RL, Barakat, A, Lightfoot, DA, Kassem, MA. 2012. Genetic analysis of root and shoot traits in the 'Essex' by 'Forrest' recombinant inbred line (RIL) population of soybean [*Glycine max* (L.) Merr.]. *Plant Genetics, Genomics, and Biotechnology* **1(1)**: 1-9.
- Cichy, KA, Snapp, SS, Blair MW. 2009. Plant growth habit, root architecture traits and tolerance to low soil phosphorus in an Andean bean population. *Euphytica* **165**: 257-68.
- FAO (2017) FAOSTATE database. Food and Agriculture Organization of the United Nations. Rome, URL: <http://faostat.fao.org>.
- FAO (2022) FAOSTATE database. Food and Agriculture Organization of the United Nations. Rome, URL: <http://faostat.fao.org>.
- Fried HG, Narayanan S, Fallen B. 2019. Evaluation of soybean [*Glycine max* (L.) Merr.] genotypes for yield, water use efficiency, and root traits. *PloS One* **14(2)**: e0212700
- Idrissi O, Udupa SM, De Keyser E, McGee RJ, Coyne CJ, Saha GC, Muehlbauer FJ, Van Damme P, De Riek J. 2016. Identification of quantitative trait loci controlling root and shoot traits associated with drought tolerance in a lentil (*Lens culinaris* Medik.) recombinant inbred line population. *Frontiers in Plant Science* **7**: 1174.
- Kumar J, Basu PS, Srivastava E, Chaturvedi SK, Nadarajan N, Kumar S. 2012. Phenotyping of traits imparting drought tolerance in lentil. *Crop and Pasture Science* **63(6)**: 547-54.
- Kashiwagi, J, Krishnamurthy, L, Purushothaman, R, Upadhyaya, HD, Gaur, PM, Gowda, CLL, Ito, O, Varshney, RK. 2015. Scope for improvement of yield under drought through the root traits in chickpea (*Cicer arietinum* L.). *Field Crops Research* **170**: 47-54
- Priya S, Bansal R, Kumar G, Dikshit HK, Kumari J, Pandey R, Singh AK, Tripathi K, Singh N, Kumari NP, Kumar S. 2021. Root trait variation in lentil (*Lens culinaris* Medikus) germplasm under drought stress. *Plants* **10(11)**: 2410.
- Ramamoorthy P, Lakshmanan K, Upadhyaya HD, Vadez V, Varshney RK. 2017. Root traits confer grain yield advantages under terminal drought in chickpea (*Cicer arietinum* L.). *Field Crops Research* **201**: 146-61.
- Salehi, M. 2012. The study of drought tolerance of lentil (*Lens culinaris* Medik) in seedling growth stages. *International Journal of Plant Production* **3**: 38-41
- Sarker A, Erskine W and Singh M. (2005). Variation in shoot and root characteristics and their association with drought tolerance in lentil landraces. *Genetic Resources and Crop Evolution* **52**: 89-97.
- Silim, SN, Saxena MC, Erskine W. 1993. Adaptation of lentil to the Mediterranean environment. I. Factors affecting yield under drought conditions. *Experimental Agriculture* **27**: 155-175
- Sofi PA, Djanaguiraman M, Siddique KH, Prasad PV. 2018. Reproductive fitness in common bean (*Phaseolus vulgaris* L.) under drought stress is associated with root length and volume. *Indian Journal of Plant Physiology* **23**: 796-809.
- Wasson, AP, Richards, R, Chatrath, R, Misra, SC, Prasad, SS, Rebetzke, GJ, Kirkegaard, JA, Wong, MML, Gujaria-Verma, N, Ramsay, L, *et al.* Classification and characterization of species within the genus lens using genotyping-by-sequencing (GBS). *PLoS ONE* **10**: e0122025
- Zhu JK. 2002. Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology* **53**: 247-2734.