Original Research Article

Identification of SSR markers in genomic regions contributing seed yield and nutritional traits in lentil lens culinaris medicus

Reena Mehra[1*], Rekha Khandia[2], Ashutosh Sarker[3], Muraleedhar Aski[4], Ashok Munjal[5]

[1]Department of Genetics, Barkatullah University, Bhopal, Madhya Pradesh, INDIA.
[2]International Center for Agricultural Research in the Dry Areas (ICARDA), 3rd floor, DPS Marg, New Delhi, INDIA.
[3]Division of Genetics, Indian Agriculture Research Institute, New Delhi, INDIA.
Email: reena_mehra@ymail.com

Abstract

Developing segregating populations derived from parents contrasting for target economic trait(s) as well as for larger number of chosen type of DNA markers is a preclude for identification of DNA markers linked to genomic regions controlling target traits. Most often than not, not all the pairs of parents contrasting for target traits are polymorphic for chosen type of DNA markers. A priori identification of appropriate parents to develop mapping populations is therefore essential for DNA marker-assisted discovery of genomic regions controlling the targeted trait(s). Seed yield is certainly an important trait that breeders and agronomists are looking for, and in the context of micro-nutrient malnutrition enrichment of lentil seed with high iron and zinc is a key objective of genetic enhancement in South Asia and Sub-Saharan Africa. Thus, an investigation was carried out during 2013-14 crop season to identify putative parents contrasting for seed yield, Zn and Fe content and a set of simple sequence repeat (SSR)-based markers for use in developing mapping populations in lentil. Higher polymorphism and allele richness indicates the presence of ample diversity at the 40 SSR loci among germplasm accessions. Based on *per se* performance, the pairs of accessions such as EC 267636 and EC 267678 vs EC 267569-A, EC 223397 and EC 225503, EC 267544-A and EC 267638 vs EC 78411, EC 78408 and EC 267604 and EC 267544-A and EC 267613vsEC 11371, EC 267563 and EC 267604 were identified as contrasting for seed yield plant^{-1}, Zn and Fe content, respectively. From among these, three pairs of contrasting genotypes namely, EC 225503 and EC 267636 for seed yield plant^{-1}, EC 78411 and EC 267544-A for Zn content and EC 11371 and EC 267544-A for Fe contentwere polymorphic to most number of SSR markers. These three pairs of genotype contrasting for both phenotypic traits as well as a set of SSR markers are suitable for use as most appropriate parents for developing mapping populations targeting or segregating these traits in lentil.

Key Words: Lens culinaris, Genetic diversity, Biofortification, Micronutrient, SSR markers.

***Address for Correspondence:**
Dr. Reena Mehra, Department of Genetics, Barkatullah University, Bhopal, Madhya Pradesh, INDIA.
Email: reena_mehra@ymail.com

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Accessed Date: 26 March 2018

INTRODUCTION

Lentil (*Lens culinaris* Medikus. Subspecies *culinaris*) is an important pulse crop for human food, animal feed and in farming systems of many countries globally. It is a good source of Protein and minerals, carbohydrates, vitamins and antioxidant compounds. Lentil has significant amounts of essential amino acids, which complement with amino acids in cereals and thus improves protein quality daily diet. Starch is the major storage carbohydrate followed by dietary fibre, oligosaccharides and simple sugars like glucose and sucrose. In lentil seed Calcium, magnesium, phosphorus, potassium and especially Iron, zinc present. Lentil is a good source of important vitamins such as riboflavin, niacin, thiamin, folate and the vitamin A, B-complex. However, like other pulses crop, lentil seeds also contain some anti-nutritional content which can reduced or eliminated by different cooking techniques. Lentil has so many potential health benefits and, in combination with other pulses and cereals, it could have beneficial effects

on some of the important human diseases like Fe deficiency anemia, cognitive development, anorexia, depression, gastro-intestinal problems. Overall, we can say "Lentil is a House of Nutrients" a with a diverse array of potential nutritional and health benefits. Lentil is a self-pollinated crop (2n = 2x = 14) which belongs to the Vicieae tribe in Leguminosae family. The domestication of lentil occurred, together with that of emmer and einkorn wheat, barley, pea, chickpea, bitter vetch and flax, during the Neolithic Agricultural Revolution, which is believed to have taken place in the Eastern Mediterranean around the 8th and 7th millennia BC (Zohary and Hopf, 1973). Lentil spread rapidly with that of Neolithic agriculture to the Nile Valley, Europe and Central Asia. It was part of Harappen crop assemblage in the Indian subcontinent between 2250 and 1750 BC (Zohary and Hopf, 1993). After 1500 AD, the Spanish introduced lentil to South America via Chile (Solh and Erskine, 1984). More recently it has been cultivated in Mexico, Canada, the USA, New Zealand and Australia. The genus Lens consists of four species and seven taxa-*L. culinaris* Medikus {subsp. *culinaris*, subsp. *orientalis* (Boiss.) Ponert, subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, Slageren and Robertson and subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, Slageren and Robertson}, *L. ervoides* (Brign.) Grande, *L. nigricans* (M. Bieb.) Godron and *L. lamottei* Czefr (Ferguson *et al*., 2000). Among these subsp. *orientalis* is considered to be progenitor of the cultivated lentil. India has the first place in this field and its production is 43% and 37% of the world and 37% respectively with production. The highest productivity is recorded in New Zealand (2667 kg / ha), followed by China (2239 kg / ha). Compared to India, Canada (38%) is ranked first in production due to high levels of productivity (1971 kg / ha) compared to India (600 kg / ha) (Tiwari and Shivhare, 2016). During 2014-15, lentil production in India is 1.0 million tonnes in 1.52 million hectares. It is cultivated primarily in Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal under the conditions of rainfall. These states contribute 85% of the area together and 90% of pulses production. During the last three decades, area under pulses has increased by 85%, production is 151% and productivity is 39%. Despite all these positives, productivity of lentil is still low in India and even in South Asia, and the countries in the region import lentil from international markets at the expense of hard-earned foreign currency. The major bottleneck is the lack of genetic variability in local landraces that limit higher yield potential, susceptibility to biotic and abiotic stresses, poor harvest index, and lack of suitable ideo-types for different cropping systems niches. To broaden the genetic base, it is important that gene flow occurs between indigenous and exotic genetic resources. Thus, the germplasm used in this study are of exotic origin, introduced from ICARDA. On identifying valuable genes/allles/QTLs in exotic parents, marker-assisted breeding programs can initiate involving phenologically adapted landraces. In South Asian countries and from the developing world population also affected from micronutrient malnutrition. In Human is hidden hunger of micronutrient, which can be reducing from use of pulses. Lentils are rich in micronutrient and known as "House of nutrient". Fe and Zn plays an important role for human health. almost two billion people and almost 47% of women and children suffering from Iron and Zinc deficiency, which is affecting their mental and physical growth in child, its affect immune system, lead to anaemia, decreased learn ability. Germplasm assessment characterization helps identity different genotypes that can be used by breeders to create desired variation. Although the availability of limited morphological markers their poorly understand genetic control and agro-economical effect on the prototypical expression, it is inhibit to use it as a stable to use it as a stable marker in diversity analysis at different stages of development. DNA markers is the accurate characterization of genotype and measurement of genetic linkage, others markers. Microsatelite markers are currently using by researchers because it is the PCR based, specific to locus, polymorphic, genetically co-dominant. Microsatellite markers are DNA-based markers.

MATERIAL AND METHODS

Plant material and experimental design: The present investigation was carried out during the Rabi season of 2013-14 at the research field of RAK College of Agriculture, Sehore (M.P.). The material for the study consisted of 181germplasm accessions collected and are being maintained at ICARDA, Lebanon. The experimental material used and the method applied during the course of present investigation has been described below. The experimental material in a large number of lentil germplasm collected from local environments and from international genebank at ICARDA, Lebanon of All India Co-ordinate Research Project on MULLaRP at R.A.K. College of Agriculture, Sehore. The exotic germplasm, collected by ICARDA from Centre of Origin and primary diversity of Fertile Crescent of Near East, are reservoirs of precious genes to enhance yield, and address biotic and abiotic stresses will provide an opportunity to identify useful genotypes for direct use or utilization in Indian lentil breeding programs.

Assessment Of *Per Se* Performance Of Genotypes: The germplasm accessions, along with six check entries (L 4147, Precoz, L 830, L 4076, L 4594 and DPL 62), were

sown in an augmented design (Federer, 1956) in six compact blocks during 2013-14 rabi season at the experimental plot of RAK agriculture college Sehore, Madhya Pradesh. The experiment done in Agumented design with two replications and seeds were sown on 9 Nov 2013. The row length was 3 meter and row to row spacing was 30 cm. Standard agronomic practices were followed for raising and maintenance of the plants. Five random plants from each row were harvested and the seeds/grains were handled following the procedure suggested by ICARDA descriptor of Lentil. The space between plant to plant was maintained at 10 cm. Recommended management practices were followed during the crop-growing period to raise a healthy crop.

Plant Samples And Data Collection: Randomly five plants selected from each line for seed yield plant^{-1} (dried seeds shelled taken by each dry pods harvested from 5 plants took weight and average), seed Zn and Fe content (mg kg^{-1}) using method followed by AAS(atomic absorption spectrometry). Biochemical analysis for kernel Fe and Zn concentrations was carried out on triplicate ground samples of seeds by digestion with 9:4 diacid mixture (HNO3: HClO4) followed by atomic absorption spectrometry (AAS) method using ECIL AAS (Perkin Elmer) as per the protocol described by Zarcinas *et al*. (1987) with some modifications suggested by Singh *et al*. (2005).

SSR Marker Assay: The total genomic DNA isolated from all the germplasm accessions was extracted from young and fresh seedlings using the C-Tab method (Cetyl Trimethyl Ammonium Bromide method (Doyle and Doyle, 1987). The quality and quantity of extracted genomic DNA of all the germplasm accessionswere checked using 0.8% agarose gel. For all germplasm accession 40 lentil specific SSR primers (Table 2) were used for genotyping. The SSR priming regions of the germplasm accessions were amplified using PCR with *Taq* DNA polymerase. PCR mixtures contained approximately 2.0 µl of DNA (30ng per µl), 0.3µl *Taq* polymerase (1 unit per µl), 1.0 µl 10X TE buffer, 0.5 µl DNTPs (2mM) and 1.0 µl each of forward and reverse primers (1 µM) in a total of 10 µl solution. The PCR cycle consisted of 5 min at 95^{0}C (hot start), 0.30 min at 95^{0}C (denaturation), 1 min at 50, 54 and 56^{0}C (annealing), 1 min at 72^{0}C (extension), 10 min at 72^{0}C (final extension) followed by infinite time at 4^{0}C for holding. The denaturation, annealing and extension step were carried out for 40 cycles. The PCR products were loaded on 4 *per cent* hi-media agrose gel in 1X TAE buffer stained with ethidium bromide and bromophenol blue as loading dye. Amplicons were separated in an electrophoresis unit at 80 V for five hours using 1X TAE buffer.

SSR Marker Data Data Scoring: The different sized amplicons of SSR priming regions of genomic DNA at defined product size range (the amplicons in the same row) scored as different alleles at each of the SSR marker locus. The variation in amplicon intensity does not taken into consideration to avoid confusion in scoring.

Statistical Analysis

Estimation of population genetic parameters: Various population genetic parameters such as polymorphic SSR loci, polymorphic information content (PIC), average number of alleles per locus, effective number of alleles per locus and major and minor allele frequency were estimated using the software, Power Marker V3.25 (Liu and Muse, 2005).

Arranging lines and testers into different cluster

Estimate of joints of germplasm accessions of the coefficients of simple matching inequality (Sokal and Michener 1958) were used to arrange them in different groups (Cladogram) in the diagram. To accomplish this, the unmated pair group method was implemented with arithmetic instrument (UPGMA) using the Darwin version 2.02 software package

Assessment of *per se* performance of genotypes: Analysis of variance was performed to decompose total variability among germplasm accessionsinto sources attributable to differences in germplasm accessions, checks and error.Significance of differences among the 181 germplasm accessions were assessed using theFisher 't' test.Based on *per se* performance, the pairs of accessions most contrasting for seed yield plant^{-1}, Zn and Fe content were identified.

Identification of putative parents for development of mapping population parents: The number of polymorphic SSR markers between phenotypically contrasting pairs of the accessions were counted and per cent polymorphism was calculated as kp/k (Blair *et al*., 1999); where, 'kp' is the number of polymorphic SSR loci and 'k' is the total number of SSR loci assayed. Similarly, the total number of detected alleles across all SSR markers between them were counted and average simple matching dissimilarity coefficients (Sokal and Michener, 1958) were estimated.

RESULTS

Quantitative traits-based differences among the germplasm accessions: ANOVA revealed highly significant mean squares attributable to 'germplasm accessions' for all traits (Table 4). Mean squares due to 'accessions vs check varieties' were also found significant for all traits. These results suggested significant differences among the accessions and they differed from the checks. Significant differences were detected among germplasm accessions for all the traits investigated (Table

5). For seed yield plant^{-1}, EC 267569-A out-yielded significantly than other accessions followed by EC 223397 and EC 225503 (Table 5). Similarly, for Zn content, EC 78411, EC 78408 and EC 267604, for Fe contentEC 11371, EC 267563 and EC 267604 were better than the others. The accessions, EC 267636 and EC 267678, EC 267544-A and EC 267638 and, EC 267544-A and EC 267613 were significantly lower performing ones compared to all the other genotypes for seed yield plant^{-1}, Zn and Fe content, respectively. The pairs of genotypes such as EC 267636 and EC 267678 vs EC 267569-A, EC 223397 and EC 225503, EC 267544-A and EC 267638 vs EC 78411, EC 78408 and EC 267604 and EC 267544-A and EC 267613vsEC 11371, EC 267563 and EC 267604 were contrasting for seed yield plant^{-1}, Zn and Fe content, respectively. The germplasm accessions were grouped into five clusters based on neighbor-joining cluster analysis (Fig. 1). The cluster 5 consisted of highest number of accessions followed by cluster 3 and cluster 2.

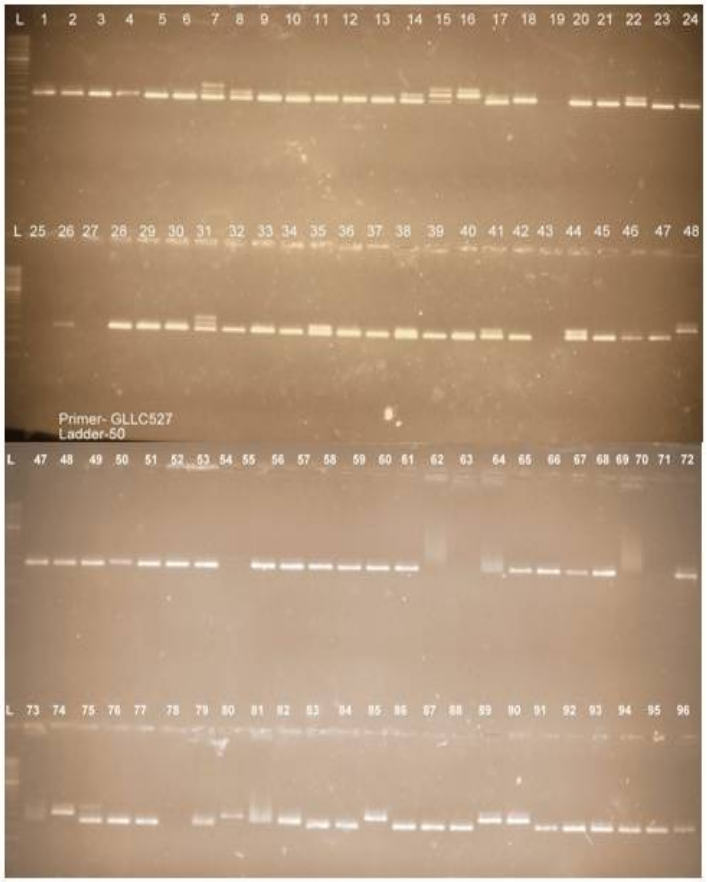

Figure 1: PCR amplification products obtained with GLLC 527 primer for lentil genotypes (label number represents genotype number as given in Table)

Table 1: Distribution of plant material with accession No

No. Of Genotypes	Exotic Collection- EC No./ Checks					
187	EC 11371	EC 27120	EC 78397	EC 78422	EC 78432	EC 78386
	EC 78387	EC 78389	EC 78390	EC 78391	EC 78393	EC 78394
	EC 78396	EC 78401	EC 78403	EC 78405	EC 78142	EC 78143
	EC 78145	EC 78416	EC 78419	EC 78421	EC 78423	EC 78425
	EC 78429	EC 78436	EC 78473	EC 78475	EC 78476	EC 78477-A
	EC 78503	EC 78540	EC 78542-A	EC 78552-A	EC 78461	EC 223188
	EC 223235	EC 223242	EC 223244	EC 223294	EC 225501	EC 225503
	EC 241476	EC 255489	EC 255491	EC 267514	EC 267526	EC 267529
	EC 267533	EC 267536	EC 267539	EC 267540	EC 223397	EC 267544-A
	EC 267545-D	EC 267554	EC 267555	EC 267557-D	EC 267563	EC 267569-A
	EC 267569-B	EC 2675471	EC 267573	EC 2675770	EC 267591	EC 267598
	EC 267603-A	EC 267604	EC 267609	EC 267613	EC 267620	EC 267625-C
	EC 267628-A	EC 267636	EC 267638	EC 267641	EC 267657	EC 267676
	EC 267677	EC 267678	EC 267687	EC 267692	EC 267696	EC 267709
	EC 267710	EC 299587	EC 329166	EC 267567	EC 267595-C	EC 267605

	EC 267634	EC 78388	EC 78395	EC 78402	EC 78406	EC 78408
	EC 78411	EC 78414	EC 78424	EC 78426	EC 78430	EC 78434
	EC 78437	EC 78438	EC 78439	EC 78441-B	EC 78442	EC 78446
	EC 78447	EC 78453	EC 78459	EC 78468	EC 78469	EC 78470
	EC 78472	EC 78474	EC 78477	EC 78483	EC 78488	EC 78490
	EC 78491	EC 78495	EC 78497	EC 78498	EC 78499	EC 78504
	EC 78505	EC 78506-D	EC 78508	EC 78509	EC 78510	EC 78511
	EC 78513	EC 78515	EC 78516	EC 78517	EC 78518	EC 78519
	EC 78520	EC 78521	EC 78524-A	EC 78525 A	EC 78593	EC 78598
	EC 78526C	EC 78528	EC 78529	EC 78532	EC 78533	EC 78534
	EC 78536	EC 78539	EC 78541-A	EC 78543	EC 78545	EC 78554
	EC 78933	EC 95634	EC 139824-A	EC 223150	EC 223191	EC 223197-A
	EC 223199-B	EC 223201	EC 223205B	EC 223207	EC 223209-B	EC 223210
	EC 223211	EC 223212-A	EC 223212-B	EC 223215	EC 223219	EC 223220
	EC 223221	EC 223222	EC 223223	EC 223226	EC	EC 223229-B
	EC 223230					
6 Checks	**C1_L4147**	**C2_PRECOZ**	**C3_L830**	**C4_L4076**	**C5_L4594**	**C6_DPL62**

Table 2: List of SSR primers used to characterize germplasm accessions in lentil

Sl. No.	Primer name	Forward primer (5' – 3')	Reverse primer (5' – 3')	Annealing temp. (^{0}C)
1	GLLC614	AACCCCAGCCAGATCTTACA	AAGGGTGGTTTTGGTCCTATG	56^{0}C
2	GLLC527	GTGGGACGGTTTGAATTTGA	GAACATAAAATGGGAGTGTCACAA	56^{0}C
3	PBALC90	AAGCTGCCGGTGATCTTCTA	AAGTCCCACCTGATCCTCCT	56^{0}C
4	PBALC233	AGTTGAAGACGGTGCAAA	CGAGAATGATGACCTTTAAGA	56^{0}C
5	PBALC556	CTTACACGTAATTCGAACACC	AGACGAAGAGAAGAAAGAGGA	56^{0}C
6	PBALC205	TTGAGTTTGAGGATGAGGATA	CATAAAACCCCAAACATTACA	56^{0}C
7	PBALC213	AAGTTTGGGATAAACCTTTTG	CATCATGCTAAAATCAAAACC	56^{0}C
8	PBALC216	AAATAGAAGTGGAGAGGCAAT	TTCGTTCTTGAGTGATATCGT	56^{0}C
9	PBALC203	CATAGTCAACACTTGGTCGTT	GTCCACAATGAAACTCATCAC	56^{0}C
10	PBALC207	ATGGAACACAAACCAATACAC	TGTGGTGTCCTTTGTAGAAGT	56^{0}C
11	PBALC250	TGCATTTACCATCATCTCTAAC	TGATTGATTCGGTACTTTTTG	56^{0}C
12	PBALC254	ATGTTAATAAGCAGCAGCAAC	AAGTTGCATGTAACCACAAAC	56^{0}C
13	PBALC260	GTGAACTACCTCTGTGAATGC	AGGCGAAATTTCATCTTCTA	56^{0}C
14	SSR13	GAAACAACACCGAAATACAC	CGAAGTCAGATGAAGTTTG	56^{0}C
15	PBALC353	CCATAACAGACAAAACCCTACT	ATTCTCAAAGCCCATTTAGTT	56^{0}C
16	LENT4	AACCAATCATGGCTTCTGCT	TTTCACCGTCTTTATGAACCA	56^{0}C
17	LENT8	CAAACTGGAAGATGCTGCTG	TGACCCATCCTCATCCTTAAA	56^{0}C
18	PBALC18	CGTTGGTGGTGCAGTATTTG	CCATAAACAAGTGCAATCCAG	56^{0}C
19	GLLC106	ACGACAATCCTCCACCTGAC	AACAAGGAAGGGGAGAGGAG	56^{0}C
20	GLLC108	CGACAATCCTCCACCTGAC	ACAAGGAAGGGGAGAGGAAG	56^{0}C
21	GLLC 614	AACCCCAGCCAGATCTTACA	AAGGGTGGTTTTGGTCCTATG	56^{0}C
22	GLLC527	GTGGGACGGTTTGAATTTGA	GAACATAAAATGGGAGTGTCACAA	56^{0}C
23	GLLC541	TGGGCTCATTGAACCAAAAG	CCCCCTTTTAAGTGATTTTCC	56^{0}C
24	GLLC 559	CATGGATCCAAATGCAAAAA	GCTTCTTCAAGAGCACGTTTC	56^{0}C
25	GLLC562	TGTGTAGGCACATCAACAAAA	GGTGGGCATGAGAGGTGTTA	56^{0}C
26	SSR19	GACTCATACTTTGTTCTTAGCAG	GAACGGAGCGGTCACATTAG	56^{0}C
27	PBALC364	GACTGCTTCTATGGTTGTTTG	GACAATGGAAGTATCCAACAC	56^{0}C
28	SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	56^{0}C
29	SSR233	CTTGGAGCTGTTGGTC	GCCGCCTACATTATGG	56^{0}C
30	GLLC559	CATGGATCCAAATGCAAAAA	GCTTCTTCAAGAGCACGTTTC	56^{0}C
31	GLLC595	TTGTCTGGTGGTGTTTTTGG	CACAAAGTTTCTCACCTCACG	56^{0}C

32	GLLC607	AAGTTGTGGCCAAGAGGATT	CCAAAACCCCCACTACTTTA	56^{0}C
33	GLLC563	ATGGGCTCATTGAACAAAAG	CCCCCTCTAAGAGATTTTCCTC	56^{0}C
34	GLLC598	TGGGCTCATTGAACCAAAAG	CCCCCTTCTAAGTGATTTTCC	56^{0}C
35	GLLC 548	CTGTTGTGGCCAAGAGGATT	CCAAAACCCCCACTACTTCA	56^{0}C
36	SSR 230	F:CCAACAACAATTCACCATAC	R:AACATTGTACTGAGAGGTG	56^{0}C
37	PBALC 273	TGAAACCTTTTTGAAGACAAG	TCCATCTTCTAGATTCTTCCA	56^{0}C
38	SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	56^{0}C
39	SSR 99	GGGAATTTGTGGAGGGAAG	CCTCAGAATGTCCCTGTC	56^{0}C
40	GLLC 563	ATGGGCTCATTGAACAAAAG	CCCCCTCTAAGAGATTTTCCTC	56^{0}C

Table 3: Estimates of population genetic diversity parameters of SSR loci

Sl. No.	Primer	# of alleles	# of effective alleles	Major allele frequency	Minor allele frequency	Polymorphic information content	Resolving power
1	GLLC614	5	2.50	0.35	0.06	0.73	2.13
2	GLLC527	4	1.56	0.56	0.06	0.56	2.21
3	PBALC90	4	1.41	0.66	0.04	0.51	2.04
4	PBALC233	4	1.57	0.44	0.07	0.65	2.00
5	PBALC556	3	1.71	0.48	0.11	0.62	1.96
6	PBALC205	3	1.57	0.54	0.01	0.62	2.48
7	PBALC213	4	1.54	0.40	0.05	0.72	1.85
8	PBALC216	5	2.40	0.42	0.04	0.74	1.77
9	PBALC203	4	1.48	0.28	0.03	0.80	1.90
10	PBALC207	4	1.48	0.41	0.07	0.76	1.99
11	PBALC250	5	1.46	0.29	0.09	0.77	1.96
12	PBALC254	4	1.56	0.33	0.17	0.76	1.88
13	PBALC260	3	1.69	0.42	0.09	0.65	1.96
14	SSR13	5	1.50	0.39	0.01	0.66	2.13
15	PBALC353	5	1.39	0.51	0.08	0.69	1.88
16	LENT4	5	1.46	0.38	0.02	0.72	1.98
17	LENT8	4	1.63	0.49	0.06	0.55	2.31
18	PBALC18	3	1.37	0.33	0.06	0.88	1.38
19	GLLC106	4	1.55	0.40	0.04	0.71	1.88
20	GLLC108	4	1.65	0.44	0.09	0.60	2.27
21	GLLC 614	5	3.49	0.41	0.02	0.70	2.10
22	GLLC527	5	3.48	0.40	0.05	0.71	2.06
23	GLLC541	4	1.58	0.35	0.14	0.73	1.96
24	GLLC 559	4	1.61	0.36	0.14	0.69	2.08
25	GLLC562	6	3.53	0.33	0.17	0.68	2.35
26	SSR19	4	1.58	0.36	0.15	0.73	1.96
27	PBALC364	3	1.65	0.45	0.11	0.69	2.06
28	SSR 212-1	4	1.53	0.51	0.10	0.66	1.98
29	SSR233	4	1.56	0.41	0.09	0.71	1.94
30	GLLC559	5	2.41	0.35	0.06	0.77	1.77
31	GLLC595	3	1.74	0.41	0.18	0.66	2.08
32	GLLC607	4	1.57	0.49	0.07	0.57	2.15
33	GLLC563	4	2.60	0.35	0.07	0.70	2.02
34	GLLC598	3	1.64	0.57	0.20	0.59	2.31
35	GLLC 548	4	1.57	0.34	0.17	0.60	1.92
36	SSR 230	3	1.30	0.79	0.05	0.35	2.02
37	PBALC 273	4	1.02	0.03	0.01	0.53	0.08
38	SSR 212-1	4	1.57	0.40	0.05	0.68	1.96
39	SSR 99	4	2.22	0.14	0.04	0.96	0.79
40	GLLC 563	4	1.63	0.61	0.08	0.44	2.58
	Mean	4.08	1.79	0.41	0.08	0.67	1.95

Table 4: Analysis of variance for seed yield plant^{-1} in lentil germplasm accessions

Source of variation	Df	Seed yield

		plant^{-1} (g)
Blocks	05	13.90
Entries	186	6504.16**
Checks	05	12101.26**
Accessions	180	6384.81**
Checks vs. accessions	01	1.77
Error	25	6.66

Table 5: Germplasm accessions contrasting for seed yield plant^{-1}, Zn and Fe content in lentil

Sl. No.	Seed yield plant^{-1} (g)		Zn (mg kg^{-1})		Fe (mg kg^{-1})	
	Accessions	Mean	Accessions	Mean	Accessions	Mean
			High performing accessions			
1	EC 223210	248.4	EC 78411	86.22	EC 78536	111.40
2	EC 78529	198.8	EC 223219	82.83	EC 267604	111.18
3	EC 223201	176.4	EC 78408	79.42	EC 267625-C	110.26
4	EC 78499	153.0	EC 78541-A	75.03	EC 267709	109.18
5	EC 95634	152.2	EC 267604	74.56	EC 267563	109.02
6	EC 78518	148.8	EC 223229-A	72.28	EC 223294	108.85
7	EC 78526C	143.0	EC 223226	71.74	EC 11371	108.13
8	EC 78142	139.0	EC 223210	71.72	EC 267657	108.13
9	EC 223223	137.0	EC 267514	71.07	EC 267539	108.04
10	EC 267569-A	137.0	EC 223201	69.84	EC 78554	107.98
11	EC 223209-B	134.9	EC 78406	69.81	EC 267677	107.75
12	EC 223397	138.8	EC 78421	69.72	EC 299587	107.07
13	EC 223207	133.4	EC 267677	69.38	EC 267636	106.09
14	EC 225503	133.2	EC 78483	69.07	EC 139824-A	105.87
15	EC 78539	119.6	EC 78529	68.81	EC 267634	105.60
			Low performing accessions			
1	EC 267678	16.07	EC 223212-B	9.82	EC 223212-B	10.40
2	EC 78393	18.05	EC 267544-A	18.05	EC 267613	11.12
3	EC 267709	18.27	EC 267638	23.49	EC 78145	12.39
4	EC 267636	19.47	EC 267657	35.11	EC 267544-A	18.43
5	EC 329166	21.07	EC 223197-A	36.01	EC 78393	23.72
CD @ 5%		8.12				

Table 6: SSR marker assay-based polymorphism between phenotypically contrasting pairs of genotypes in lentil

Parents contrasting for	No. of polymorphic SSR loci	*Per cent* polymorphism	No. of detected alleles	Average dissimilarity coefficient
		Seed yield plant^{-1} (g)		
B/w EC 267569-A and EC 267636	26	65.00	39	0.34
B/w EC 223397 and EC 267636	21	52.50	38	0.28
B/w EC 225503 and EC 267636	29	72.50	41	0.36
B/w EC 267569-A and EC 267678	27	67.50	37	0.35
B/w EC 223397 and EC 267678	22	55.00	37	0.31
B/w EC 225503 and EC 267678	25	62.50	39	0.33
		Zn content (mg kg^{-1})		
B/w EC 78411 and EC 267544-A	29	72.50	41	0.39
B/w EC 78408 and EC 267544-A	23	57.50	39	0.33
B/w EC 267604 and EC 267544-A	16	40.00	35	0.20
B/w EC 78411 and EC 267638	23	57.50	40	0.29
B/w EC 78408 and EC 267638	19	47.50	39	0.27
B/w EC 267604 and EC 267638	23	57.50	38	0.31
		Fe content (mg kg^{-1})		
B/w EC 11371 and EC 267544-A	25	62.50	37	0.34
B/w EC 267563 and EC 267544-A	23	57.50	37	0.30
B/w EC 267604 and EC 267544-A	14	35.00	35	0.20
B/w EC 11371 and EC 267613	22	55.00	37	0.36

B/w EC 267563 and EC 267613	15	37.50	34	0.23
B/w EC 267604 and EC 267613	14	35.00	36	0.23

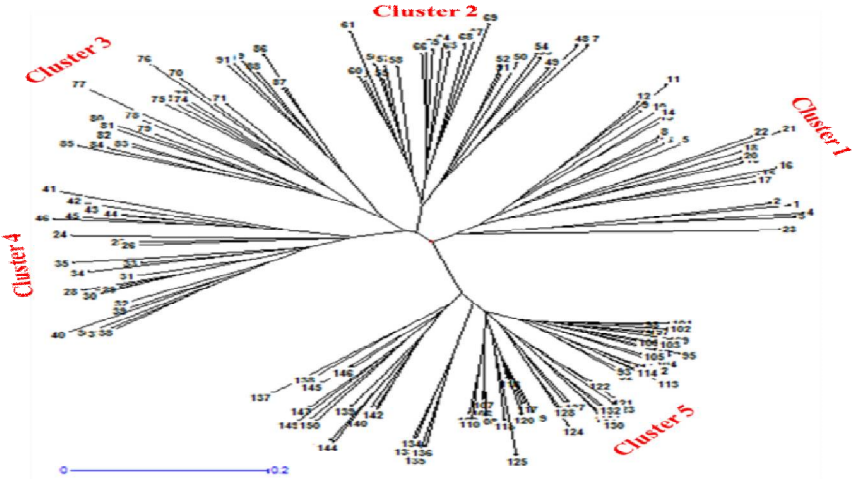

Figure 1: Alleles-based cladogram showing grouping of germplasm accessions of SSR marker

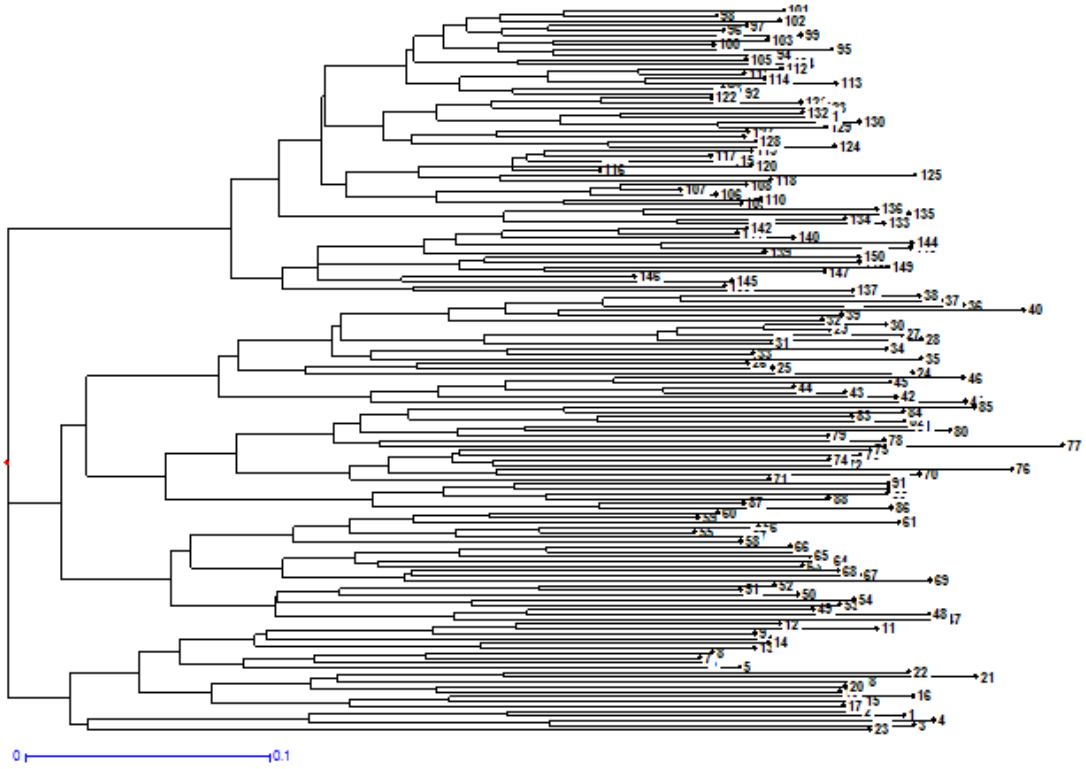

Figure 2: Neighuour joining dendrogram of 161 genotype of lentil with 40 SSRs Marker.

DISCUSSION

Lentil is highly nutrious plant of legume family. India is the largest producer of lentil, India produced about 1.0 million tons from 1.52 million ha area and average yield was 600kg/ha. Madhya Pradesh state counted in first position in lentil acreage with respect 5.50 lakh/ha. Micronutrient malnutrion is current issue of developing countries. Mostly women and children are deficit from Iron and Zinc mineral. Information about level and extent of polymorphism of protected material is therefore of great value for the use of protected genetic material. In this study the firstly lentil genotype was described for

Iron and Zinc content which could be helpful for construct new genotypes with high Iron, high Zinc, high yield. Secondly analyse genetic diversity present in the germplasm with the using molecular markers.

Estimation of population genetic parameters: DNA marker allele-based variation present in germplasm would be useful for determining whether morpho-metric traits-based variation reflect variations at DNA sequence level as well. It would also provide information on the population structure, allelic richness, and parameters that specify diversity among germplasm to help breeders to choose appropriate genetic resources for cultivar development more effectively. Of late, germplasm characterization based on DNA markers has gained importance due to the speed and quality of data generated. Several DNA-based markers are available for genetic diversity analysis. The SSR markers are now the markers of choice in various applications of plant breeding research as they are codominant, multi-allelic, highly polymorphic even between closely related lines, require low quantity of DNA, can be easily automated for high throughput genotyping, can be exchanged between laboratories and are highly transferable between populations. SSR marker assay help understand genetic relationship among germplasm accessions/ breeding lines, selection of parents for hybridization, organization of variation in germplasm accessions and identification of cultivars (Benabdelmouna *et al*., 2001). The SSR markers were used to study the genetic diversity in germplasm accessions of lentil, chickpea (Upadhyaya *et al*., 2008), common beans (Blair *et al*., 2009), etc. The number of alleles needed to provide same heterozygosis if all the alleles are equally frequent (Hartl and Clark, 1997) as quantified by effective number of alleles (Ne) were more for penta-allelic SSR markers than the tri and tetra-allelic markers with an average of 1.79 alleles per marker. When allelic frequencies are similar, the estimate of effective number of alleles is close to the observed number of alleles in a locus. Therefore, large differences between observed and the effective number of alleles indicate low frequencies of a few alleles because they are present in only one or a few genotypes. For this reason, estimate of effective number of alleles could be useful in indicating rare alleles (Laurentin, 2009). In the present study, large differences between the estimates of observed and the effective number of alleles indicate relatively low frequencies of a few alleles, which could be considered as rare alleles. In general, these results suggest the presence of ample diversity at the 40 SSR loci among germplasm accessions.

Identification of mapping population parents: The significant quantitative traits-based differences among the contrasting genotypes amply reflected in SSR marker loci as well (Table 6). The pairs of contrasting genotypes differed for number of polymorphic SSR loci (consequently *per cent* polymorphism), number of detected alleles and average dissimilarity coefficient. From among the contrasting pairs of accessions, EC 225503 and EC 267636 followed by EC 267569-A and EC 267678 for seed yield plant^{-1}, EC 78411 and EC 267544-A and EC 78408 and EC 267544-A for Zn content and EC 11371 and EC 267544-A and EC 267563 and EC 267544-A for Fe content were polymorphic to most number of SSR markers, number of alleles detected and average dissimilarity coefficient. These quantitative traits and SSR marker alleles-based pairs of contrasting genotypes could be used as putative parents for developing mapping population to identify SSR markers linked to genomic regions controlling economic traits and/or could be used to effect crosses to derive superior pure-lines for use as varieties for commercial cultivation. In this study Iron and zinc synthesis in lentil are positively correlation. Those germplasm who shown high level in iron, high in zinc content and high in yield can be used in breeding/crossing programme.

ACKNOWLEDGEMENT

Authors are extremely thankful to Professor, Head Division of Genetics, Barkatullah University, Bhopal (M.P.) for providing the necessary facilities for the research work. Wild germplasm/species provided by ICARDA, Lebanon through NBPGR, New delhi, India. The first author is thankful to the DBT-Builder Program, Barkatullah University, Bhopal (Department of Biotechnology, New Delhi, India) for the grant of Junior Research Fellowship/Senior Research Fellowship for the PhD. The study was partially supported by RAK Agriculture college of Sehore, Madhya Pradesh, India and Division of Genetics, IARI, New Delhi. Sincere thanks to Division of Genetics, IARI, New Delhi. The authors express their sincere thanks to ICARDA.

Key message of the manuscript: Our findings can applied in the plant breeding and genetics right away, In this study we used exotic material of Lentil and some material performed very well. It can be use in breeding program as some entry having high Iron and Zinc contain so it can reduce malnutrition in womens and children which is biggest challenge in South Asian countries.

Author contribution: Conception or design of the experiment, Germplasm arrangements: Ashutosh Sarker. Drafting the article and supervised: Rekha Khandia. Analyzed the data, contributed reagents/analysis tools : Dr Murleedhar Aski, Critical revision of the article and final approval of the version to be published by Dr Ashok Munjal, Head of the department of Genetics, Barkatullah University, Madhya Pradesh, India. Perfomed the

experiment, wrote the paper, Data collection, data analysis, and interpretation done by Reena Mehra,

Conflict of interest statement

The authors declare that they have no conflict of interest.

We have a competing interest to declare that

1. This is to certify that all authors have seen and approved the manuscript being submitted.
2. We warrant that the article is the Authors original work. We warrant that the article has not received prior publication and is not under consideration for publication elsewhere.
3. On the behalf of all Co-authors, the corresponding author shall bear full responsibility for the submission.
4. This research has not been submitted for publication nor has it published in whole or in part elsewhere.
5. We attest to the fact that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity, data and its interpretation and agree to its submission to the Theoretical and Applied Genetics (TAAG) International journal of plant breeding.

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Source of Support: None Declared
Conflict of Interest: None Declared