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GENETIC DIVERSITY IRON AND ZINC CONTENT IN LENTIL (*LENS CULINARIS* MEDIKUS SUBSP.*CULINARIS*) AS ASSESSED BY SSR MARKER

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ABSTRACT: Iron and Zinc are very important to address 'hidden hunger',prevailing among pre-school children and pregnant women, which is rampant in South Asia and Sub-Saharan Africa. To address this issue, Lentil Breeders have a new challenge to develop varieties with high Fe and Zn contents. Screening of >2000 cultivated and wild germplasm shows that enormous variability exists for Fe content (42-168 ppm) and Zn (22-101 ppm), thus provides scope for genetic improvement for these two traits. New search is continuing and in this endeavor, an experiment with 181exotic lentil germplasm conducted in 2013/14 crop season to understand genetic variation for Iron (Fe) and Zinc (Zn) concentration and seed yield using SSR molecular markers. In this experiment the range of Fe was (11.2-110ppm), Zn was (18.1- 86.25ppm) and seed yield varied from 10.9 to 0.82 gm, Higher polymorphism and allele richness indicates the presence of ample diversity at the 40 SSR loci among 181 germplasm accessions.The accession, EC78933 was high Fe & EC 78414 was high in Zn and EC 223201 showed high yield. The accessions EC 223210, EC 78529, EC 223201, EC 78469, EC 267569-A, EC 267598, EC 267604, EC 223207, EC 223244, EC 225503, EC 78446, EC 78539, EC 267545-D, EC 223219, EC 267554 and EC 78541 were promising for multiple traits along with Zn and Fe content. To enhance the pace of lentil breeding, thesegermplasm will be used for marker-assisted breeding for the development of Fe and Zn fortified lentil varieties to address micro-nutrient malnutrition.

KEYWORDS: *Lens culinaris*, Genetic diversity, Biofortification, Micronutrient, SSR marker

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1.INTRODUCTION

Lentil, an important pulse crop, is predominantly grown and consumed in South Asia and West Asia, and North and East Africa. Despite its role in nutritional well-being, over two billion people mostly in South Asia and Sub-Saharan Africa suffer from micronutrient malnutrition termed as "hidden hunger". Women and children are worst affected. This can partially be addressed through development and consumption of Fe and Zn-rich varieties [1]. India produces about 1.29 million tons with its highest area in state of Madhya Pradesh 0.512 million/ha [2]. In the past, ICARDA in collaboration with national programs have developed biofortified lentils in Bangladesh, India, Ethiopia, Nepal, Syria and other countries. To address the issue and to enhance biofortification research an experiment was conducted with 181 exotic lentil germplasm to understand genetic diversity for Fe and Zn contents and seed yield through SSR markers. The promising Fe and Zn rich genotypes with high yield potential can be directly released as varieties after multilocation testing and can used by breeders to further create new variability [3]. The present study is a step forward towards biofortification research with a aim to develop micronutrient- dense high yielding lentil varieties to address micronutrient deficiencies through their consumption in traditional daily diet.

2. MATERIALS AND METHODS

The genetic materials consisted of 181 exotic germplasm accessions collected from ICARDA and are being maintained at NBPGR, India and All India Co-ordinated Research Project on MULLaRP at R.A.K. College of Agriculture, Sehore (M.P.).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 in six compact blocks during 2013-14 Rabi season at the experimental plot of RAK agriculture college Sehore, Madhya Pradesh. The experiment done in Augmented 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 plants were randomly selected from each row for seed yield plant-1 (dried seeds shelled taken by each dry pods harvested from 5 plants took weight & averaged), seed Zn & Fe contents (ppm) were analysed using AAS (atomic absorption spectrometry) method at division of Soil Science, IARI, New Delhi. 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 with some modifications suggested (17). The total genomic DNA isolated from all the germplasm accessionswas extracted from young & fresh seedlings using the C-Tab method (CetylTrimethyl Ammonium Bromide method [4]. 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 accessionswere 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 95^0C (hot start), 0.30 min at 95^0C (denaturation), 1 min at 50, 54 and 56^0C (annealing), 1 min at 72^0C (extension), 10 min at 72^0C (final extension) followed by infinite time at 4^0C 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. 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.

3. RESULTS AND DISCUSSION

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 [5].

Arranging lines and testers into different cluster: Estimate of joints of germplasm accessions of the coefficients of simple matching inequality [6] 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 the Fisher‘t’ test. Based on *per se* performance, the pairs of accessions most contrasting for seed yield plant^{-1}, Zn and Fe content had 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 [7]; 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 [6] were estimated.

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 content EC 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.

1. High performing accession for seed yield plant-1 EC 223210 (248.4 gm/plot), followed by EC 78529 (198.8) and EC 225503 (133.2). Similarly, for Zn content, EC 78411 (86.22 ppm), EC 223219 (82.83 ppm) and for Fe content EC 78536 (111.4 ppm), EC 267625-C (110.26) and EC 267604 (111.18) were better than the others.
2. The 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.
3. 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 (Table 3.) of SSR markers, number of alleles detected and average dissimilarity coefficient.
4. The germplasm accessions were grouped into five clusters based on neighbour-joining cluster analysis (Fig. 2). The cluster 5 consisted of highest number of accessions followed by cluster 3 and cluster 2.

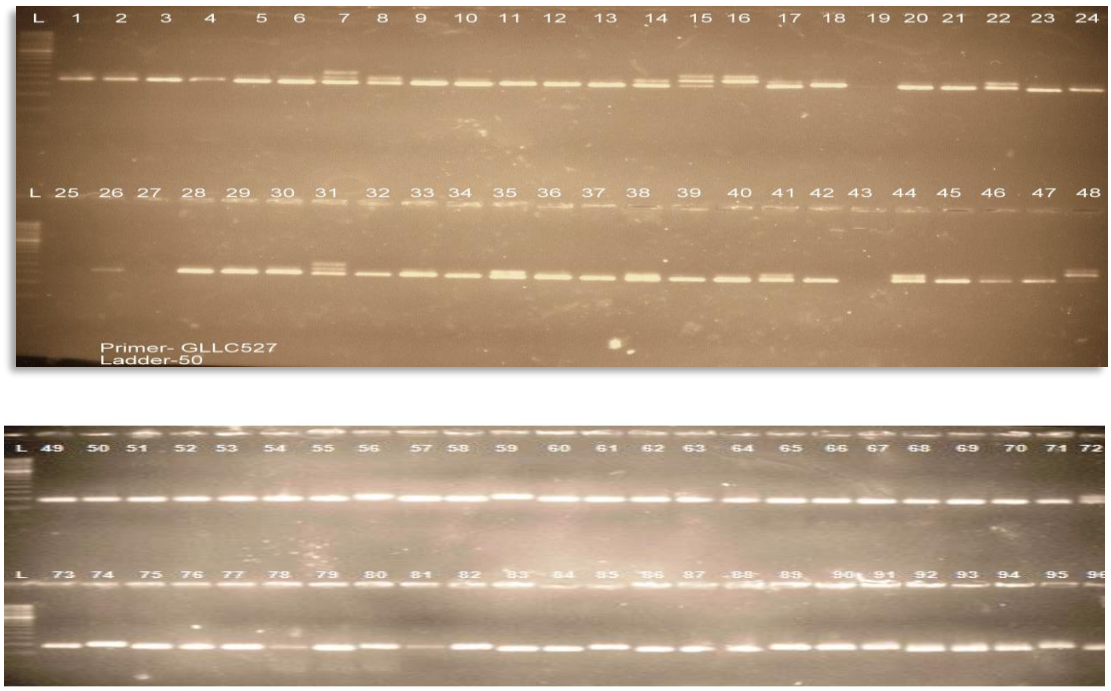

Fig.1.PCR amplification products obtained with GLLC 527 primer for lentil genotypes

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	EC78477-A
	EC 78503	EC 78540	EC78542-A	EC78552-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	EC267544-A
	EC267545-D	EC 267554	EC 267555	EC267557-D	EC 267563	EC267569-A
	EC267569-B	EC2675471	EC 267573	EC 2675770	EC 267591	EC 267598
	EC267603-A	EC 267604	EC 267609	EC 267613	EC 267620	EC267625-C
	EC267628-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	EC267595-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	EC78506-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	EC78524-A	EC 78525 A	EC 78593	EC 78598
	EC 78526C	EC 78528	EC 78529	EC 78532	EC 78533	EC 78534
	EC 78536	EC 78539	EC78541-A	EC 78543	EC 78545	EC 78554
	EC 78933	EC 95634	EC139824-A	EC 223150	EC 223191	EC223197-A
	EC223199-B	EC 223201	EC 223205B	EC 223207	EC223209-B	EC 223210
	EC 223211	EC223212-A	EC223212-B	EC 223215	EC 223219	EC 223220
	EC 223221	EC 223222	EC 223223	EC 223226		EC223229-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	56^0C
17	LENT8	CAAACTGGAAGATGCTGCTG	TGACCCATCCTCATCCTTAAA	56^0C
18	PBALC18	CGTTGGTGGTGCAGTATTTG	CCATAAACAAGTGCAATCCAG	56^0C
19	GLLC106	ACGACAATCCTCCACCTGAC	AACAAGGAAGGGGAGAGGAG	56^0C
20	GLLC108	CGACAATCCTCCACCTGAC	ACAAGGAAGGGGAGAGGAAG	56^0C
21	GLLC 614	AACCCCAGCCAGATCTTACA	AAGGGTGGTTTTGGTCCTATG	56^0C
22	GLLC527	GTGGGACGGTTTGAATTTGA	GAACATAAAATGGGAGTGTCACAA	56^0C
23	GLLC541	TGGGCTCATTGAACCAAAAG	CCCCCTTTTAAGTGATTTTCC	56^0C
24	GLLC 559	CATGGATCCAAATGCAAAAA	GCTTCTTCAAGAGCACGTTTC	56^0C
25	GLLC562	TGTGTAGGCACATCAACAAAA	GGTGGGCATGAGAGGTGTTA	56^0C
26	SSR19	GACTCATACTTTGTTCTTAGCAG	GAACGGAGCGGTCACATTAG	56^0C
27	PBALC364	GACTGCTTCTATGGTTGTTTG	GACAATGGAAGTATCCAACAC	56^0C
28	SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	56^0C
29	SSR233	CTTGGAGCTGTTGGTC	GCCGCCTACATTATGG	56^0C
30	GLLC559	CATGGATCCAAATGCAAAAA	GCTTCTTCAAGAGCACGTTTC	56^0C
31	GLLC595	TTGTCTGGTGGTGTTTTTGG	CACAAAGTTTCTCACCTCACG	56^0C
32	GLLC607	AAGTTGTGGCCAAGAGGATT	CCAAAACCCCCACTACTTTA	56^0C
33	GLLC563	ATGGGCTCATTGAACAAAAG	CCCCCTCTAAGAGATTTTCCTC	56^0C
34	GLLC598	TGGGCTCATTGAACCAAAAG	CCCCCTTCTAAGTGATTTTCC	56^0C
35	GLLC 548	CTGTTGTGGCCAAGAGGATT	CCAAAACCCCCACTACTTCA	56^0C
36	SSR 230	F:CCAACAACAATTCACCATAC	R:AACATTGTACTGAGAGGTG	56^0C
37	PBALC 273	TGAAACCTTTTTGAAGACAAG	TCCATCTTCTAGATTCTTCCA	56^0C
38	SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	56^0C
39	SSR 99	GGGAATTTGTGGAGGGAAG	CCTCAGAATGTCCCTGTC	56^0C
40	GLLC 563	ATGGGCTCATTGAACAAAAG	CCCCCTCTAAGAGATTTTCCTC	56^0C

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

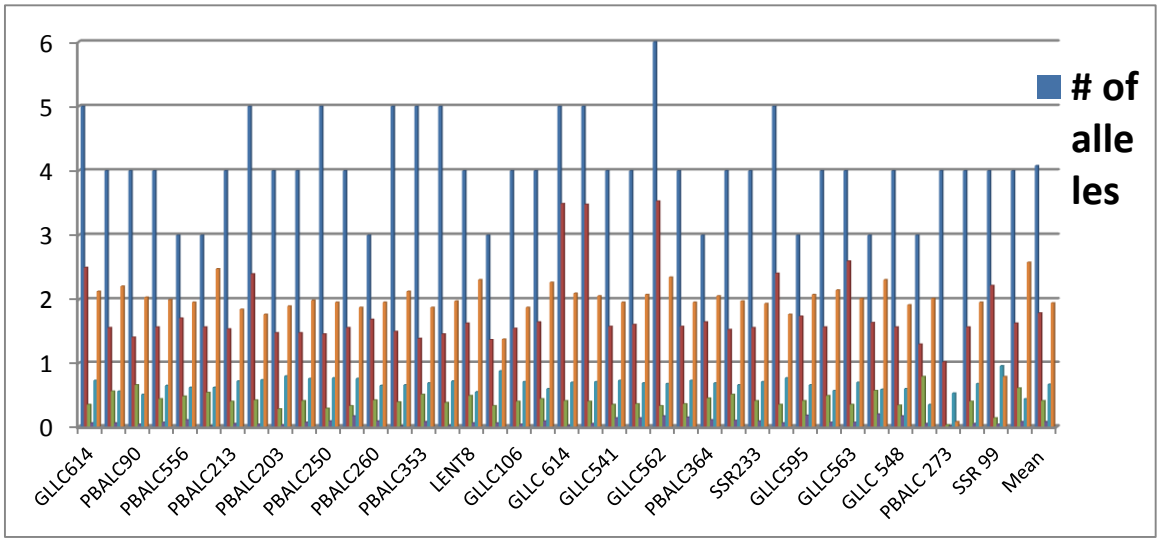

Table 4. Analysis of variance for seed yield plant^{-1}in lentil germplasm accessions

Source ofvariation	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 (ppm)		Fe (ppm)	
	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				

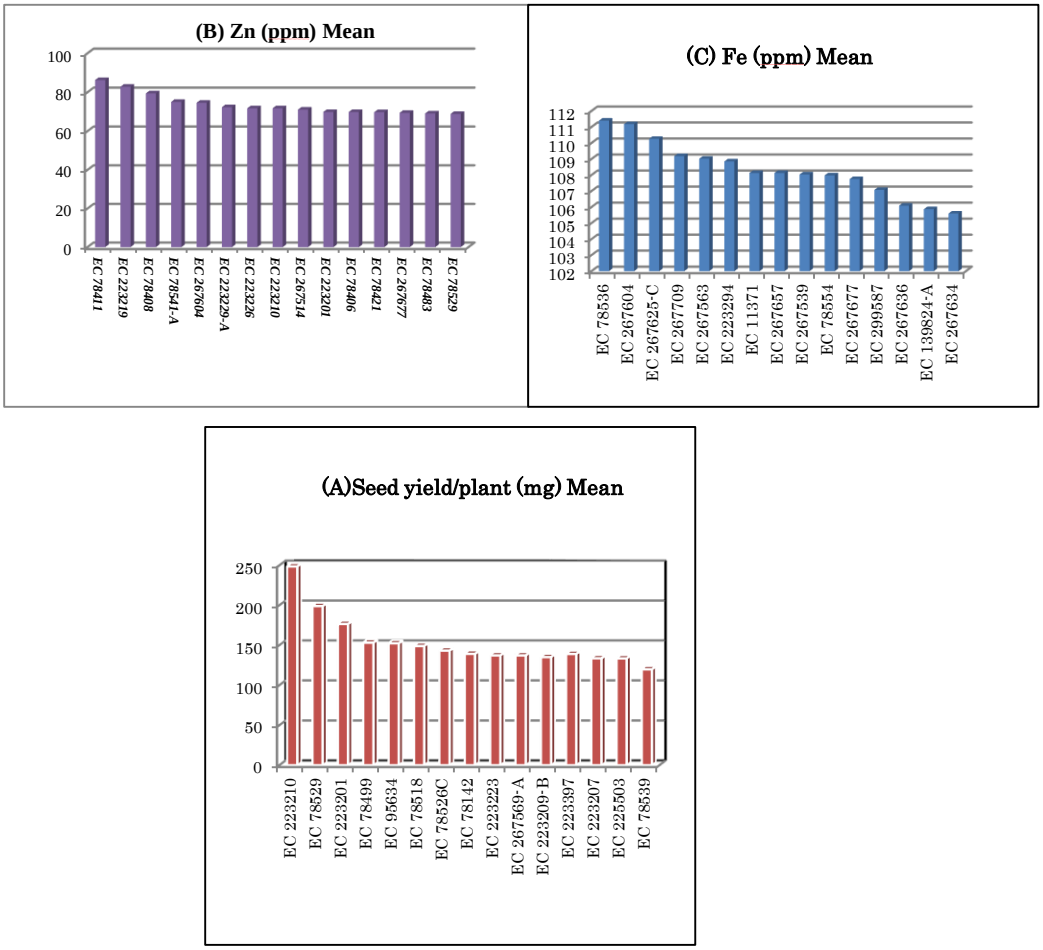

Fig: Germplasm accessions contrasting for seed yield plant^{-1}, Zn and Fe content in lentil

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

Parents contrasting for	No. of polymorphic SSR loci	Percent 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

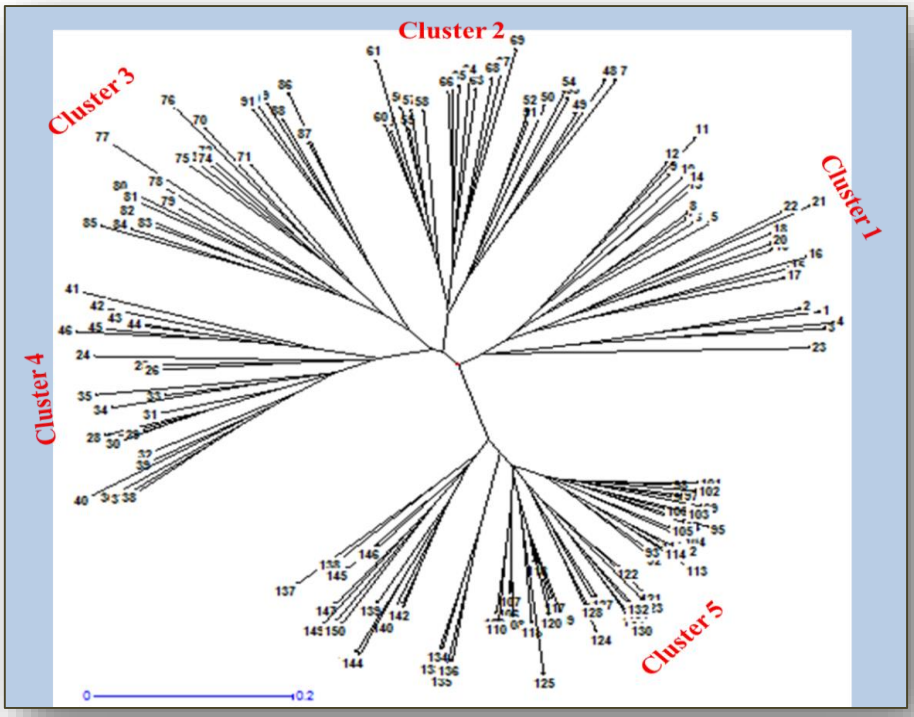

Fig.2. Alleles-based cladogram showing grouping of germplasm accessions of SSR marker

Discussion

Lentil is a highly nutritious plant of the legume family. India is the largest producer of lentil, producing about 1.0 million tons from 1.52 million ha area with an average yield of 600kg/ha. The state of Madhya Pradesh ranks first in lentil acreage with respect 5.50 lakh/ha. Micronutrient malnutrition is current issue of developing countries. Mostly women & children are deficit from Iron & 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 & 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 co-dominant, multi-allelic, highly polymorphic even between closely related lines,

require low quantity of DNA, could 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. The SSR markers were used to study the genetic diversity in germplasm accessions of lentil, chickpea [2], common beans [8] etc. The number of alleles needed to provide same heterozygosis if all the alleles are equally frequent (23) 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 [9]. 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 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 all 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.

4. CONCLUSION

Micronutrient malnutrition can be addressed through food diversification, consuming fortified foods and supplementation, but these measures are beyond reach of the poor. Lentil is traditionally in the food system and consuming Fe and Zn-rich lentils in daily diet will provide health support without extra-cost. Thus, the result of this research will contribute to lentil biofortification programs in India and elsewhere. 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 & zinc synthesis in lentil are positively correlation. Those germplasm who shown high level in iron, high in zinc

content & high in yield can be used in breeding/crossing programme.

5.ACKNOWLEDGEMENT

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6. CONFLICT OF INTEREST

We have no conflict of interest to declare.

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