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GENETIC DIVERSITY OF BIO-FORTIFIED LENTILS (*LENS CULINARIS* MEDIK.) THROUGH SIMPLE SEQUENCE REPEAT MARKERS

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ABSTRACT

Nepalese lentils are comparatively rich in iron and zinc, making it a potential crop of whole food solution to aid in the global battle against the micronutrient malnutrition. Understanding the genetic basis for uptake of grain iron and zinc is required to increase their stable concentrations along with these minerals in lentils. This study aimed at characterizing genetic variation in micronutrient high grain iron and zinc concentrations and exploring the potential in lentil accessions. A set of 25 lentil accessions was evaluated in two seasons across the three locations and genotyped by using 40 simple sequence repeat (SSR) markers that are linked with lentil iron and zinc concentrations. Out of the 40 SSR markers, 23 markers were found polymorphic while 12 were monomorphic, and 5 markers were null. These 23 polymorphic markers produced a total of 584 alleles, of which 52 were polymorphic alleles, and average alleles per locus was 11.49. The linkage disequilibrium (LD) analysis was done using a mixed linear model (MLM) that identified three SSR markers, PBALC 13, PBALC 206, and GLLC 563, associated with grain Fe concentration, explaining 9% to 11% phenotypic variation, respectively, and four SSR markers (PBALC 353, SSR 317–1, PLC 62, and PBALC 217) associated with grain Zn concentration, explaining 14% to 21% phenotypic variation, respectively. The pairwise genetic similarity index among 25 lentil accessions varied from 0.16 to 0.83. The identified SSRs exhibited consistent performance across two seasons and have potential for utilization in lentil molecular breeding program.

Keywords: lentil, SSR marker, accessions, Genetic diversity, allele, molecular characterization

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INTRODUCTION

Lentil (*Lens culinaris* Medikus), is an autogamous diploid (2n = 2x = 14) species with haploid genome size of 4063 Mbp, and the most important pulse crop in Nepal often called the "poor man's meat" for its rich in protein content (28%) for human consumption and its straw is a valued animal feed consisted of minerals (2%) and carbohydrates (59%) (Frederick et al., 2006). Besides, it is low-fat food, i.e., low-glycemic carbohydrate, and helps to prevent chronic diseases such as diabetes and heart disease. Globally, it is cultivated for its protein-rich grains in as many as 52 countries on a 3.64-million-hectare area with an annual production of 3.60 million tons (FAOSTAT, 2011). However, about 95% of the global production comes from just ten countries- Canada, India, Turkey, Nepal, Australia, China, Iran, the USA, Syria, and Ethiopia. India accounts for 39% (1.47 million ha) of the global acreage with 0.90 million tons of production. Currently, annual world lentil production is approximately 4 million metric tons (MT), more than 85% of which is in five specific regions: India, Nepal, and Bangladesh (32%); western Canada (29%); Turkey and northern Syria (18%); and Australia (4%). In Nepal, lentil shares about 62% and 64% of the total legume acreage rea and production (MOAD, 2012), respectively. Nepalese lentil is highly preferred because of its quick cooking quality and tasty pink-red cotyledons, with high micronutrient contents and is popular in the international market (Dev et al., 2007). Bangladesh, Singapore, Sri Lanka, Germany, Korea, the UK, and Indonesia are the major export markets for Nepalese lentil (Gharti et al., 2014).

Nepalese lentil has potential for mineral biofortification as its nutritional profile is rich in Fe, Zn, and Se (Thavarajah et al., 2011; USDA National Nutrient Database, 2015). Alghamdi et al., (2014) evaluated 35 advanced ICARDA breeding lines in Saudi Arabia at one field location over two seasons and reported significant variation for Fe, Zn, Cu, Ca, Mg, P, K, and Mn concentrations. The mean iron concentration in both cultivated and wild lentils was reported 61 mg kg-1 across all 26 lentil genotypes. Among the 20 *L. culinaris* genotypes, Fe concentration ranged from 26 (IG72830) to 92 mg kg-1 (CDC Red Rider) with a mean of 58 mg kg^{-1}.

Assessment of genetic diversity in germplasm is prerequisite for any breeding program so that genetic gain is not limited because of narrow genetic base of parental lines (Kumar et al., 2004). In earlier studies, molecular markers such as Simple Sequence Repeat (SSR), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD) have been preferred for genetic diversity analysis in lentil (Havey and Muehlbauer, 1989; Abo-Elwafa et al., 1995; Sharma et al., 1995, 1996; Ahmad et al., 1996; Ford et al., 1997; Udupa et al., 1999; Abe et al., 2003; Hamwieh et al., 2005, 2009; Reddy et al., 2010) and gene mapping (Eujayl et al., 1998; Tullu et al., 2003; Duran et al., 2004; Kahraman et al., 2004; Hamwieh et al., 2005). SNPs were identified across Palestinian lentil accessions for development of cost-effective and robust

genotyping assays (Basheer-Salimia et al., 2015). Kaur et al. (2014) identified SSR and SNP markers from transcriptome and EST for construction of gene-based genetic linkage map in lentil. Several reports from our lab have established transferability of SSR markers from one legume genera/species to other (Datta et al., 2010a, 2010b; 2011; 2012; 2013a, 2013b; 2015), and observed high level of sequence conservation of microsatellite markers in legumes.

Simple sequence repeat markers are inexpensive and readily adaptable technique for routine germplasm fingerprinting and evaluation of genetic relationship between accessions or genotypes (Sardana et al., 1998; Dixit et al., 2004, Edossa et al., 2007) and construction of genetic linkage maps (Abo-Elwafa et al., 1995). This technique has been used to assess genetic diversity in germplasm collection (Gilbert et al., 1999; Salimath et al., 1995), to identify cultivars (Prevost et al., 1999). Till date, in Nepal, there has been very little study on molecular breeding in lentil breeding improvement, which takes a longer breeding process and mostly follows classical breeding. There is a big gap in the breeding improvement process, and for fast-track breeding, the present investigation was undertaken to assess the diversity and genetic relatedness of Nepalese, Indian, and exotic lentil genotypes with the objectives of studying the polymorphism and genetic relationship among them and identifying genotype-specific markers. Fig. 1 below shows different marker systems used in plant breeding in Nepal.

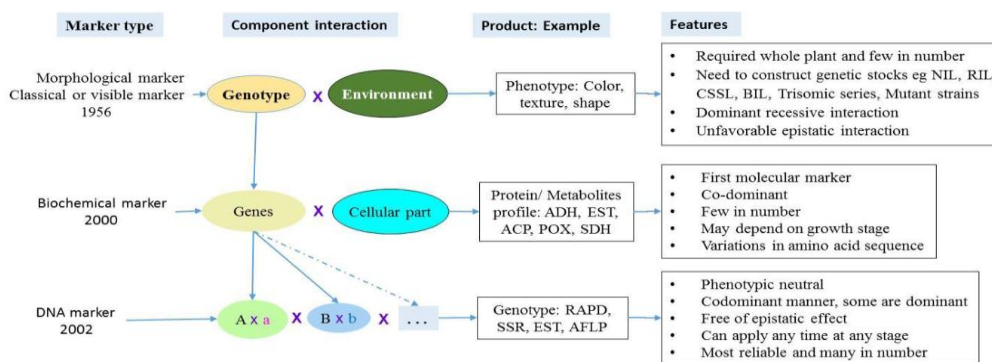

Figure 1. Different Marker Systems used in Nepal

MATERIALS AND METHODS

Plant material

A core collection of lentil germplasm available at GLRP was developed by selecting a small set of Fe and Zn rich accessions representing the diversity of the entire collection, based on passport available in Gene bank or ICARDA website, laboratory and morphological data. This collection consists of 25 genotypes (Table 1). These accessions were from the SAARC region, and for this study, 11 ICARDA breeding lines and 14 lines (Nepal 7, India 6, Bangladesh 1) were employed.

DNA extraction and PCR amplification

Total DNA was extracted according to the Cetyltrimethyl Ammonium Bromide Method (CTAB method), described by Rogers and Bendich (1985). Bulk genomic DNA of each lentil accession were isolated from 100mg of young fresh leaf tissue of 5 individual seedlings of 2-week-old using CTAB method (50mM Tris-HCL, 25mM EDTA, 1M NaCl, 1% CTAB, 0.15% 2-mercaptoethanol). The DNA extract in the form of pellet was suspended in 100µl of TE buffer and prepared the 5% working DNA solution with deionized water and used for Polymerase Chain Reaction (PCR) amplification of SSRs. Forty-four SSR markers that are linked to lentil iron and zinc concentration reported by Hamweieh et al. (2005), fifty-eight SSR markers reported by Kaur et al. (2011), and 18 EST SSR markers developed in IARI's laboratory exhibiting polymorphosis across Lens species were assayed for identification of polymorphic SSR markers. The SSR markers developed by Creganetal (1999) were used in the present study. A total of 40 SSRs were initially screened for their ability to produce polymorphic patterns across the 25 lentil accessions. The details of SSR markers, their sequences and motifs are given in supplementary (Table 2). This genotyping was performed in Seed Science and Technology Division, National Agriculture Research Institute, Khumaltar, Lalitpur. PCR was carried out using a PE 9600 thermo cycler (Perkin-Elmer, Foster City, CA). After initial denaturation of 3 min at 94^{0}C, followed by 30 cycles performed for 30 s at 94°C, annealing for 30 s at either 52°C, 53°C, 54°C or 55°C (depending on the locus) and elongation for 1 min at 72°C, followed by final extension step of 5 min at 72°C. Amplified products were detected on a Mega BACE 500 Capillary System (Amersham Pharmacia Biotech, Piscataway, NJ). Samples were prepared by adding 1 µl of diluted PCR products to 9 µl formamide and samples included 1% (v/v) ET-Rox 900 bp DNA size standard (Amersham Bioscience). Microsatellite fragment sizes were estimated using the Mega BACE Genetic Profiler Version 2.0 (Amersham Pharmacia Biotech). The amplified SSR products were measured using a UV illuminator as bands on visualization gel. The analysis only used the trustworthy bands.

SSR allele scoring and data analysis

The presence or absence of SSR fragments in each accession was recorded for all the polymorphic SSR markers. The SSR bands appearing without ambiguity were scored as 1 (present) and 0 (absent) for each primer. The size of the amplified product was calculated on the basis of its mobility relative to the molecular mass of the marker., Thermo Scientific, USA). The polymorphism information content (PIC) a measure of the allelic diversity at a locus, was determined by using the formula described by Botstein et al. (1980).

$$PIC = 1-\Sigma Pi2$$

Where, Pi is the frequency of the ith allele for its marker in the set of accessions analyzed, calculated for SSR locus. The genetic similarity among accessions was estimated based on Jaccard's similarity coefficient. The resulting similarity matrix was

further analyzed using the unweighted pair-group method arithmetic average (UPGMA) clustering algorithm for construction of dendogram; the computations were carried out using MINITAB Inc. File version 14.13.0.0.

Table 1. Details of 25 lentil accessions used for molecular characterization and genetic diversity analysis based on the source of origin

Accession Code no	Accessions	Pedigree	Source/Origin
1	ILL-8006	ILL5888xILL5782	Bangladesh
2	RL-6	ILL8008xILL5888	Nepal
3	RL-12	Sindur x Khajura-2	Nepal
4	ILL-7715	FLIP95-59L	ICARDA
5	ILL-7164	PAK86591	ICARDA
6	ILL-3490		ICARDA
7	Khajura-2(PL639)	L9-12xType 8	GPPUAT, Pantanagar
8	Simal(LG7)	Land race from India	India
9	Shital(ILL2580)	L1278	ICARDA/India
10	Sagun(ILL6829)	ILL4907(Pakistan)x ILL4605 (Argentine)	ICARDA
11	HUL-57	Mutant of HUL1	BHU, Banaras, India
12	LG-12	Local landrace from India	India
13	PL-4	UPL175x(PL184xP228)	GPPUAT, Pantanagar
14	RL-11		Nepal
15	RL-4	ILL6037xILL8007	Nepal
16	ILL-2712		ICARDA
17	Black Masuro	Local landrace from Rasuwa, Nepal	Nepal
18	RL-79	99S95-2-1	Nepal
19	ILL-6467	ILL4605xILL2582	ICARDA
20	ILL-7979	FLIP1996-47L	ICARDA
21	ILL-6819		ICARDA
22	ILL-7723	Sel89503	ICARDA/Pakistan
23	WBL-77	Mutant of BR25	RAU, Dholi, India
24	ILL-4605	ILL5888x ILL5782	USA
25	RL-49	NR9901-1-17	Nepal

Table 2. Forward and reverse primer sequences and annealing temperatures used for amplifications microsatellite loci

S/N	Name of Primers	Forward primer sequences	Reverse primer sequences	Annealing temperature (°C)
1.	PLC10	TGCAACAAAGGACACTAGAGGTT	ATTTCTTTCTCCCTAACCAGCC	59
2.	PLC16	CGTTTGATCTTCTAAGCCCCTA	AAGGGAAAGGATGTTTGACTTG	59
3.	PLC17	AAGCTGAAGGAAATCAAAGTGGT	CAACACACTCCATGTTTAGAGC	59
4.	PLC21	AACTCGCATCCTCTTCACAACT	GGACCTTTCCCTTGTAGTCACC	59
5.	PLC22	TACACTGAAGGAGATGCACTGG	TAACAACAAAACACAGCTTCGC	60
6.	PLC5	CATTGCAGCTTATTCTCACAGC	TGACCCATCCTCATCCTTAAAT	60
7.	PLC35	TTGCTTCCTCCTCTTCTCACTC	AGCCTCAGTACCCTCCTCTTTT	60
8.	SSR 124	GAACATATCCAATTATCATC	GTATGTGACTGTATGCTTC	52
9.	SSR 154	GGAGCAAGAAGAAGCAG	GGAATTTATCACACTATCTC	51
10.	SSR 66	GGTAGTGGTGAGGAATGAC	GCATCACTGCAACAGACC	55
11.	SSR 90	CCGTGTACACCCCTAC	CGTCTTAAAGAGAGTGACAC	55
12.	SSR 207	GAGAGATACGTCAGAGTAG	GATTGTGCTTCGGTGGTTC	55
13.	SSR 72	CAAACAGTACAAGGAAAGGAG	CTGACTGAGCTGCTTGAAC	55
14.	SSR 132RN	CCAGAACAAACGTAAACC	CTATCGCATATGAGTGAAC	52
15.	SSR 107	GCGGCGAGCAAATAAAT	GGAGAATAAGAGTGAAATG	51
16.	SSR 113	CCGTAAGAATTAGGTGTC	GGAAAATAGGGTGGAAAG	51
17.	SSR 33	CAAGCATGACGCCTATGAAG	CTTTCACTCACTCAACTCTC	56
18.	SSR 19	GACTCATACTTTGTTCTTAGCAG	GAACGGAGCGGTCACATTAG	58
19.	SSR 48	CATGGTGGAATAGTGATGGC	CTCCATACACCACTCATTCAC	57
20.	SSR 46-2	CACTAAACATGGAAAATAGG	CTTATCTTTGTTTGAAGCAA	50
21.	SSR 28	GAGGGCATAAATTCAGATTC	GGACAACGCACATTTGATG	53
22.	SSR 183	GCTCGCATTGGTGAAAC	CATATATAGCAGACCGTG	52
23.	SSR 253	GAAGAAGCATTCACGGTG	GAGGGACTACTATATCAG	53
24.	SSR 34-2	CGGCGGATGAAACTAAAG	CATTTCCTTCACAAACCAAC	53
25.	SSR 191	GCAAATTTCTTGGTCTACAC	GGGCACAGATTCATAAGG	53
26.	SSR 230	CCAACAACAATTCACCATAC	AACATTGTACTGAGAGGTG	53
27.	SSR 202	CAACCTCACTTACCTTAC	GCTCTTTATCATCATTCTAC	52
28.	SSR 197	CACCAATCACCAACACAC	GAGCTGTGAAGTCTTATCTG	54
29.	SSR 99	GGGAATTTGTGGAGGGAAG	CCTCAGAATGTCCCTGTC	57
30.	SSR 130	CCACGTATGTGACTGTATG	GAAAGAGAGGCTGAAACTTG	55
31.	SSR 323	AGTGACAACAAAATGTGAGT	GTACCTAGTTTCATCATTG	51
32.	SSR 156	GTACATTGAACAGCATCATC	CAAATGGGCATGAAAGGAG	53
33.	PBALC0353	CCATAACAGACAAAACCCTACT	ATTCTCAAAGCCCATTTAGTT	59
34.	GLLC 106	ACGACAATCCTCCACCTGAC	AACAAGGAAGGGGAGAGGAG	56
35.	GLLC 511	ATTGAGAGGAGGCGGAGAA	CGCGTGTCTCTCTCTCTCAC	56
36.	GLLC 563	ATGGGCTCATTGAACAAAAG	CCCCCTCTAAGAGATTTTCCTC	56
37.	GLLC 598	TGGGCTCATTGAACCAAAAG	CCCCCTTCTAAGTGATTTTCC	56
38.	PLC60	TGCTTGGACCCTAAATTTGC	AAGAAAAGGGCAACCACTGA	60
39.	PBALC13	GCAGCAGCATGAGAAAATGA	ATTACTCGACGCCCCCTAGT	60
40.	PLC64	ATTGGTGGGGAGTTTGAGTG	AAACAACTCATGATGTGCCCT	61

RESULTS AND DISCUSSION

Molecular Characterization and Genetic Diversity

The 40 SSR markers listed by Harsh Kumar Dikshit et al. (2015), Aladdin Hamwieh et al. (2009), and Harish Kumar et al. (2014) were used for this study. Out of the 40 SSRs, 23 were polymorphic, and the 25 accessions of lentil were profiled with these 23 polymorphic SSR markers which produced 584 alleles. The details of SSR loci, their allele number, PIC values and allele size range is provided in table 3. The allele number for each SSR locus varied from 2-4 with an average of 2.26 indicating a greater magnitude of diversity among the studied accessions. The fragment size of these 584 alleles was ranged from 100bp to 600bp. Fig. 1 showed an example of DNA profiles at the SSR 107 loci with 4 distinct alleles among different lentil accessions. Out of the 584 alleles, unique alleles were amplified by four different SSR loci in 25 accessions (Table 3). PBALC0353 SSR alleles were found rare with a frequency of 0.14 in the whole sample studied (Table 3). The three accessions RL-79, ILL4605 and RL-49 amplified unique alleles as well as rare alleles in SSR 107 marker. These three accessions may serve as good sources for identification of new alleles of important genes. Three SSR markers (PBALC 13, PBALC 206, and GLLC 563) were associated with grain Fe concentration explaining 9% to 11% of phenotypic variation and four SSRs (PBALC 353, SSR 317–1, PLC 62, and PBALC 217) were associated with grain Zn concentration explaining 14%, to 21% of phenotypic variation reported by Singh et al. 2017.

PIC values, a measure of the allelic diversity of SSRs ranged from 0.14 in PBALC0353 to 0.57 in SSR 28 with an average PIC value of 0.46. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus. Varietal profiling based on SSR markers will be more reliable as compared to other markers, since SSR markers detect finer levels of variations among closely related lines. Babayeva et al. (2009) found 33 alleles determined ranging from 3-8 per locus estimated gene diversity value for 33 loci was 0.66 in lentil. Hamweih et al. (2009) reported large variation among microsatellite markers for both allele numbers and gene diversity among lentil species. Highly informative and detectable polymorphic markers for this study were found in SSR 28, SSR 124, SSR 107, SSR 113, SSR154, SSR 156, PLC16 and GLLC511 which indicated the power and higher resolution of those marker systems in detecting molecular diversity. Similarly, markers SSR 33, PLC 22, PLC 10, PLC 17, SSR 132 RN, GLLC 563, PLC 21, SSR 19, SSR 46, SSR 34, PLC 35, SSR 48, SSR 130 had comparatively lower PIC value and hence were less informative. This study revealed the divergence among lentil accessions which can be further used in lentil breeding programs. All accessions involved in this study exhibited wide range of genetic variability due to different center of origin, different genetic constitution. The genetic relatedness detected in this study might be the foundation base for future systematic lentil breeding programs. Similar results have

been reported by several authors Yadav, NK et al. 2016, Hamwieh et.al.(2005, 2009) and Dikshit et al., 2014.

Table: 3. Analysis of the DNA profiling (fingerprinting) among various lentil accessions

S/N	Primer code	Molecular wt. range (bp)	Total no. of alleles	No. of polymorphic alleles	Alleles per locus	Polymorphism information content (PIC)
	PLC 10	300-400	24	2	12	0.45
2	PLC 16	200-300	25	3	8.33	0.5
3	PLC 17	300-400	25	2	12.5	0.46
4	PLC 21	300-400	25	2	12.5	0.48
5	PLC 22	300-400	23	2	11.5	0.43
6	PLC 35	200-300	25	2	12.5	0.49
8	SSR 124	400-600	38	3	12.66	0.55
9	SSR 154	400-600	19	2	9.5	0.5
14	SSR 132 RN	400-500	25	2	12.5	0.46
15	SSR 107	100-200	25	3	8.33	0.54
16	SSR 113	200-300	25	3	8.33	0.53
17	SSR 33	200-230	25	2	12.5	0.4
18	SSR 19	200-300	25	2	12.5	0.48
19	SSR 48	200-300	24	2	12	0.49
20	SSR 46	100-200	25	2	12	0.48
21	SSR 28	400-500	25	3	8.33	0.57
24	SSR 34	300-400	25	2	12.5	0.48
30	SSR 130	200-300	24	2	12	0.49
32	SSR 156	100-200	37	2	18.5	0.5
33	PBALC0353	100-200	25	2	12.5	0.14
34	GLLC 106	100-200	25	3	8.33	0.33
35	GLLC 511	100-200	23	2	11.5	0.5
36	GLLC 563	100-200	22	2	11	0.47
			Total alleles =584	Total no. of polymorphic alleles=52	Average alleles per locus=11.49	Average PIC value= 0.46

Molecular diversity analysis (Cluster Analysis)

The choice of parental genotypes for the formation of segregated populations and varieties benefits from knowledge of the genetic diversity of germplasm. Genetic variety based on molecular markers is unaffected by environmental variables, making it highly repeatable and dispersed across the genome. Understanding molecular diversity is crucial to expand the genetic basis of lentil accessions in an effective

manner. In the present study, to assess the genetic resemblances among the accessions, Jaccard's similarity coefficients were calculated for all 40 SSR alleles detected 25 lentil accessions. The pairwise genetic similarity among 25 accessions varied from 0.16 to 0.83. The similarity coefficients matrix was used for UPGMA cluster analysis. The dendogram or cladogram constructed based on the genetic similarities between accessions showed that the 25 accessions formed five major clusters (Fig. 2). The cluster I contains four accessions namely 1.ILL-8006,2. RL-6, 3. RL-12, 4.ILL-7715 representing from Bangladesh, Nepal and ICARDA origin. The cluster II contains largest seven accessions namely 5. ILL-7164, 6.ILL-3490, 7. Khajura-2(PL639), 8. Simal(LG7), 9.Shital(ILL2580), 1.Sagun(ILL6829), 12.LG-12 represent ICARDA and Indian origin. The cluster III contains the second-largest group of six accessions, namely 11.HUL-57, 13.PL-4, 14.RL-11, 15.RL-4, 16.ILL-2712, and 17.BlackMasuro, representing India, Nepal, and ICARDA. Cluster IV contains three accessions. 18. RL79, 24.ILL-4605, 25. RL-49 represent USA and Nepal origin. Cluster V contains five accessions, namely 19. ILL-6467, 20. ILL-7979, 21. ILL-6819, 22. ILL-7723, and 23. WBL-77, from ICARDA, Pakistan, and Indian origin. Likely lentil accessions were grouped into five clusters based on the neighbor-joining cluster analysis, with a dissimilarity min value of 0.028 and a dissimilarity max value of 0.55 (Fig. 2). In the cladogram, cluster II also contains the highest number of lentil accessions, followed by cluster III and cluster V. The closest related accessions in Cluster I were RL-6 and RL-12 at a similarity coefficient of 0.83, followed by ILL8006 at a similarity coefficient of 0.79. Lentil accessions. ILL-3490 was closely related to Khajura-2 (PL639) at a similarity coefficient of 0.72, while the popular and released variety Simal (LG7) was closely related to Shital (ILL2580) at a similarity coefficient of 0.70. Cluster IV and Cluster V had high genetic distances (4.66-4.74) from the centroids that determined the possible candidates with Cluster I or other clusters for the hybridization program (Table 4). This cluster pictorial indicated that there was genetic diversity among the high-grain Fe and Zn concentration lentil accessions due to the different sources of origin and diverse genetic formation. These diverse genetic materials may be used for genetic improvements in lentil accessions.

The efficiency of markers and their utility in terms of polymorphism and quantitative estimation could be expressed in mean heterozygosity and marker index (Choudhury et al., 2007). The average Hav, (Hav)p, and MI were found to be 0.579, 0.124, and 0.640, respectively. PIC values, a measure of the allelic diversity of SSRs, ranged from 0.14 in PBALC0353 to 0.57 in SSR 28, with an average PIC value of 0.46. Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus. Babayeva et al. (2009) found 33 alleles determined, ranging from 3–8 per locus. The estimated gene diversity value for 33 loci was 0.66 in lentil. Hamweih et al. (2009) reported large variation among microsatellite markers for both allele numbers and gene diversity among lentil species. Highly informative and detectable

polymorphic markers for this study were found in SSR 28, SSR 124, SSR 107, SSR 113, SSR 154, SSR 156, PLC 16, and GLLC 511, which indicated the power and higher resolution of those marker systems in detecting molecular diversity. Similarly, markers SSR 33, PLC 22, PLC 10, PLC 17, SSR 132 RN, GLLC 563, PLC 21, SSR 19, SSR 46, SSR 34, PLC 35, SSR 48, and SSR 130 had comparatively the lowest PIC value, which was less informative. This study revealed the divergence among lentil accessions, which can be further used in lentil breeding programs. The information on genetic diversity among these lentil accessions will be helpful to lentil breeders in selection of appropriate hybridizing parents in developing superior accessions.

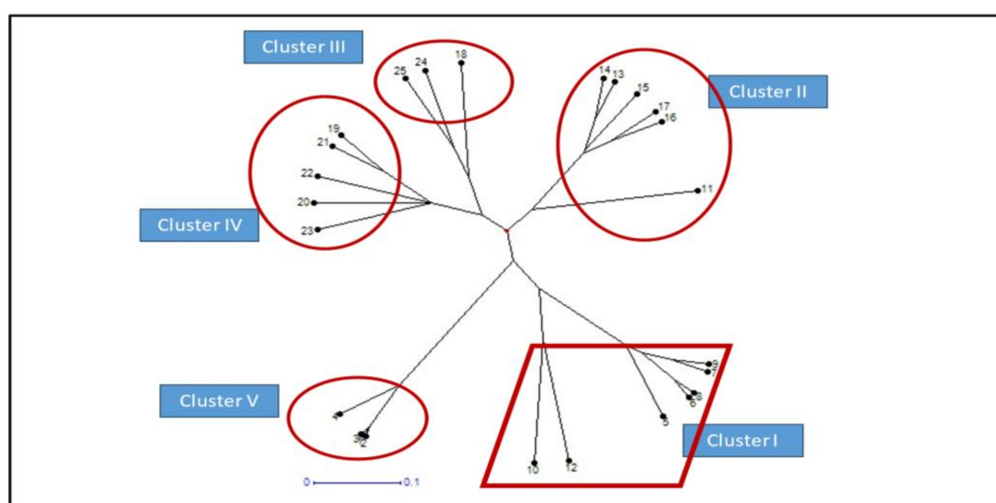

Figure 2: Genetic relationship among 25 lens accessions

(1.ILL-8006,2. RL-6,3. RL-12,4.ILL-7715,5. ILL-7164,6.ILL-3490, 7.Khajura-2(PL639), 8.Simal(LG7), 9.Shital(ILL2580) ,10.Sagun (ILL6829),11.HUL-57,12.LG-12,13. PL-4, 14.RL-11, 15.RL-4, 16.ILL-2712,17.Black Masuro, 18.RL-79,19. ILL-6467,20.ILL-7979, 21.ILL-6819,22.ILL-7723, 23.WBL-77,24. ILL-4605, 25.RL-49)using Unbiased neighboring joining dendrogram of 23 SSR markers developed by Darwin software.

CONCLUSION

Genetic variations in lentil have continued to narrow down due to continuous selection pressure for specific traits like high yield, disease and insect resistance and has jeopardized the potential for long term genetic improvement. Therefore, it is extremely important to study the genetic relationship of the existing modern-day genotypes in comparison with their ancestors and related species. In this study, genomic diversity was studied in 25 varieties. Out of 40 SSR markers tested, 23 produced unambiguous polymorphisms. Amplified markers produced easily scorable bands ranging from 100-600 bp in length. A total of 584 SSR fragments were amplified, with an average of 11.49 alleles per marker. The three accessions RL-79,

ILL4605 and RL-49 amplified unique alleles as well as rare alleles in SSR 107 marker. These three accessions may serve as good sources for identification of new alleles of important genes.

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