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Genetic Characterization in Lentil (*Lens culinaris* Medik.) Using DNA-Based SSR Markers

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Abstract

Lentil (Lens culinaris Medik.), an economically significant legume known for its high nutritional content, remains a largely unexplored crop. This study was conducted on thirty-six lentil genotypes, including twenty exotic lines from ICARDA and several released varieties and local accessions. Simple sequence repeat (SSR) markers, a preferred tool in crop improvement, were employed to analyze the genetic diversity among these genotypes. Out of sixteen targeted markers, eleven SSR markers were found to be polymorphic, amplifying a total of 28 alleles, with an average of 1.75 alleles per locus. The Polymorphic Information Content (PIC) for all amplified loci ranged from 0.12 (LSSR 19) to 0.37 (LSSR 6-215, LSSR 107, and LSSR 184), with an average value of 0.29. The expected heterozygosity (He) ranged from 0.13 to 0.49. The UPGMA-based dendorogram classified all genotypes into three major clusters, with cluster II being the largest, comprising seventeen genotypes of different origins. This indicates that molecular diversity is minimally influenced by geographical boundaries. The results revealed a diverse set of lentil genotypes that can be utilized in future crossing programs to maximize yield potential in lentil.

Key words : *Lens culinaris, SSR markers, polymorphic information content (PIC), expected heterozygosity (He).*

Introduction

Lentil (*Lens culinaris* Medik.) is a self-pollinated diploid (2x=2n=14) rabi pulse crop with a relatively large genome size of 4063 Mbp (1). As a nutritious food legume with 28% protein content, it has various uses as food and fodder. It is one of the principal crops cultivated in semi-arid regions. Globally, lentil occupies only 5.38% of the total pulse area, with an annual production of 49.52 lakh tonnes and a productivity of 1140 kg/ha in 2013, compared to the productivity of 786 kg/ha in India during 2014-15. To bridge this gap in the Indian context, appropriate genotypes are needed for the genetic improvement of the crop. For this, the nature and magnitude of genetic divergence in the population should be known to identify genotypes for choosing diverse parents in meaningful hybridization (2).

In the context of quantifying the degree of molecular divergence, microsatellite SSR markers are the most potent markers, with advantages such as locus specificity, co-dominance, and high reproducibility. Microsatellites are short (1–6 bp) tandemly repeated DNA sequences (Litt and Luty, 1989) that are dispersed randomly and ubiquitously throughout the genome (3). They arise due to unequal crossing over or replication slippage and represent the hyper variable regions of the genome, which differ because of repeat motif number variation (4). With the conserved flanking regions of microsatellites, SSRs are locus-specific, which have proved their wide use for analyzing genetic diversity. The molecular level variation provides a clearer vision for genetic diversity analysis. The SSRs continue to be limited, those validated and demonstrated in lentil. Thus, in the present study, indigenous and exotic germplasm of lentil from different places were analyzed by sixteen SSR primers with the aim to know its genetic diversity for their further use in varietal development/improvement.

Materials and Methods

Genotypes : The aforementioned research was conducted in the laboratory at Bihar Agricultural University, Sabour, Bhagalpur, Bihar. Lentil lines were collected from different regions of Bihar, IIPR (Kanpur), GBPUAT (Pantnagar), ICARDA (Lebanon), and NBPGR (New Delhi). The details of the genotypes used for the present investigation are provided in Table-1.

DNA Extraction and PCR Amplification : The genomic DNA of each genotype was extracted from the 4-week-old seedling using the CTAB method and was analyzed on a 0.8% agarose gel in 1X TAE buffer. The PCR amplification

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Table-1 : List of 36 lentil genotypes and their source of origin.

Sr. No.	Entry Name	Source
1.	FLIP 2010-73L-2	ICARDA, Lebanon
2.	FLIP 2010-86L	ICARDA, Lebanon
3.	FLIP 2010 87 L	ICARDA, Lebanon
4.	FLIP 2010-90L	ICARDA, Lebanon
5.	FLIP 2010-90L-2	ICARDA, Lebanon
6.	FLIP 2011-17L	ICARDA, Lebanon
7.	FLIP 2011-17L-1	ICARDA, Lebanon
8.	FLIP 2011-17L-2	ICARDA, Lebanon
9.	FLIP 2011-62L	ICARDA, Lebanon
10.	X2011S-111-2	ICARDA, Lebanon
11.	X2011S-172-1	ICARDA, Lebanon
12.	X 2011S-188-1	ICARDA, Lebanon
13.	X 2011S-193-1	ICARDA, Lebanon
14.	X2011S-206-1	ICARDA, Lebanon
15.	X2011S-208	ICARDA, Lebanon
16.	X 2011S-210-1	ICARDA, Lebanon
17.	X 2011S-212-1	ICARDA, Lebanon
18.	X2011S-221	ICARDA, Lebanon
19.	X 2011S-221-1	ICARDA, Lebanon
20.	X 2011S-221-2	ICARDA, Lebanon
21.	LKH-1	Local selection, Lakhisarai
22.	LKH-2	Local selection, Lakhisarai
23.	BRL-1	Local selection, Sabour, Bhagalpur
24.	BRL-2	Local selection, Banka
25.	BRL-3	IIPR, Kanpur
26.	IPL-406 (Angoori)	IIPR Kanpur
27.	SHIVALIK (L-4076)	IARI, New Delhi
28.	Pusa Vaibhav	IARI, New Delhi
29.	Pusa Ageti(L-4717)	IARI, New Delhi
30.	PL-6	GBPUAT, Pantnagar
31.	PL-8	GBPUAT, Pantnagar
32.	NDL-1	NDUAT, Faizabaad
33.	KLS-218	CSAUAT, Kanpur
34.	Noori (IPL-81)	IIPR, Kanpur
35.	HUL-57	BHU, Varanasi
36.	Arun	RAU, Pusa

was carried out with paired SSR primers in an automated thermal cycler (Applied Biosystems) by preparing a 25 µl reaction volume, which contained 2µl (100 ng) of extracted genomic DNA, 12.5µl of PCR mix, 1.0 µl forward, 1.0 µl of reverse primer, and 8.5 µl of nuclease-free water. Template DNA was initially denatured at 94ºC for 5 minutes, followed by 30 cycles (30 sec denaturation at 94ºC, 1 minute at the respective annealing temperature, 40 sec of primer extension at 72ºC) of PCR amplification, and a final extension of 72ºC for 7 min, followed by a hold at 4ºC. The details of the primers with their respective annealing temperatures are provided in Table-2.

The amplified products were resolved on a 2% agarose gel, followed by gel documentation. The frequency of SSR polymorphism was calculated based on the presence or absence of common bands, where presence was denoted by 1 and absence was denoted by 0. Polymorphism Information Content (PIC) values were calculated by an online PIC calculator. Coefficients of similarity were calculated using Jaccard's similarity coefficient, and a dendorogram was generated with UPGMA with the help of the DARwin-6.0 program.

Results and Discussion

The study encompassed 36 lentil genotypes, which were analyzed for allelic diversity using a set of sixteen SSR markers. Out of these, 11 markers exhibited polymorphism, while 5 markers (LSSR 48, LSSR 99,

Table-2 : List of primers used for molecular diversity analysis.

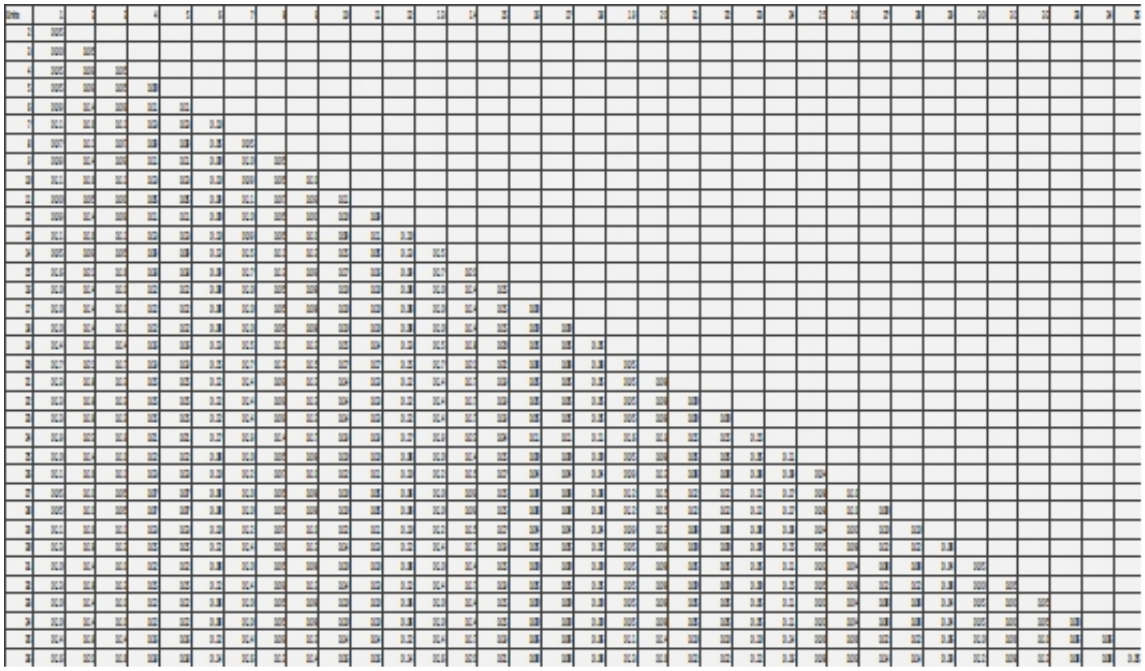
S. No.	Markers Name	Primer sequence (5'- 3')	Annealing Temperature (°C)	Band Size Range
1.	LSSR 6-215	F: CATTATATTTCTTTGGTGC R: CTTTTCTTCTCTTCCCC	53	400-500
2.	LSSR 13	F: GAAACAACACCGAAATACAC R: CGAAGTCAGATGAAGTTTG	45.5	300-350
3.	LSSR 19	F: GACTCATACTTTGTTCTTAGCAG R: GAACGGAGCGGTCACATTAG	53	150-200
4.	LSSR 33	F: CAAGCATGACGCCTATGAAG R: CTTTCACTCACTCAACTCTC	49	150-200
5.	LSSR 48	F: CATGGTGGAATAGTGATGGC R: CTCCATACACCACTCATTCAC	53	-
6.	LSSR 80	F: CCATGCATACGTGACTGC R: GTTGACTGTTGGTGTAAGTG	51	100-200
7.	LSSR 99	F: GGGAATTTGTGGAGGGAAG R: CCTCAGAATGTCCCTGTC	51	-
8.	LSSR 107	F: GCGGCGAGCAAATAAAT R: GGAGAATAAGAGTGAAATG	48.5	175-225
9.	LSSR 113	F: CCGTAAGAATTAGGTGTC R: GGAAAATAGGGTGGAAAG	46.5	250-300
10.	LSSR 130	F: CCACGTATGTGACTGTATG R: GAAAGAGAGGCTGAAACTTG	50	-
11.	LSSR 151	F: GGTATTTGAGATAGTTG R: GGAGCAAGAAGAAGCAG	46.5	100-150
12.	LSSR 154	F: GGAATTTATCACACTATCTC R: GACTCCCAACTTGTATG	46.5	-
13.	LSSR 156	F: GTACATTGAACAGCATCATC R: CAAATGGGCATGAAAGGAG	48.5	175-225
14.	LSSR 167	F: CACATATGAAGATTGGTCAC R: CATTTATGTCTCACACACAC	48.5	150-200
15.	LSSR 184	F: GTGTGTACCTAAAGCCTTG R: GTAAGTTGATCAAACGCCC	53	150-250
16.	LSSR 199	F: GTGTGCATGGTGTGTG R: CCATCCCCCTCTATC	46.5	-

Table-3 : Details of eleven polymorphic SSR markers under study.

S. No.	Marker Name	Major allelic frequency	Heterozygosity (He) value	PIC value
1.	LSSR 6-215	0.58.58	0.490.49	0.37
2.	LSSR 13	0.83	0.28	0.24
3.	LSSR 19	0.07	0.13	0.12
4.	LSSR 33	0.15	0.26	0.23
5.	LSSR 80	0.11	0.20	0.18
6.	LSSR 107	0.42	0.49	0.37
7.	LSSR 113	0.36	0.46	0.35
8.	LSSR 151	0.39	0.48	0.36
9.	LSSR 156	0.32	0.43	0.34
10.	LSSR 167	0.18	0.30	0.25
11.	LSSR 184	0.43	0.49	0.37
	AVERAGE	0.35	0.36	0.29

LSSR 130, LSSR 154, and LSSR 199) were monomorphic. The amplification profiles of representative markers for each genotype were resolved on a 2.0% agarose gel, with band sizes ranging from 150bp to 450bp (Fig.-1 to Fig.-11). Collectively, a total of 28 alleles were amplified from the sixteen SSR markers. The polymorphic features of each SSR marker are detailed in Table 3. Among the eleven polymorphic SSR markers, the number of alleles detected per locus ranged from 1 (LSSR 19, LSSR 33, LSSR 80, LSSR 107, LSSR 113, and LSSR 167) to 2 (LSSR 13, LSSR 156, and LSSR 184), with an average of 1.75 alleles per locus. The Polymorphic Information Content (PIC) value varied from 0.12 (LSSR 19) to 0.37 (LSSR 6-215, LSSR 107, and LSSR 184), with

Table-4 : Dissimilarity matrix of 36 lentil genotypes.



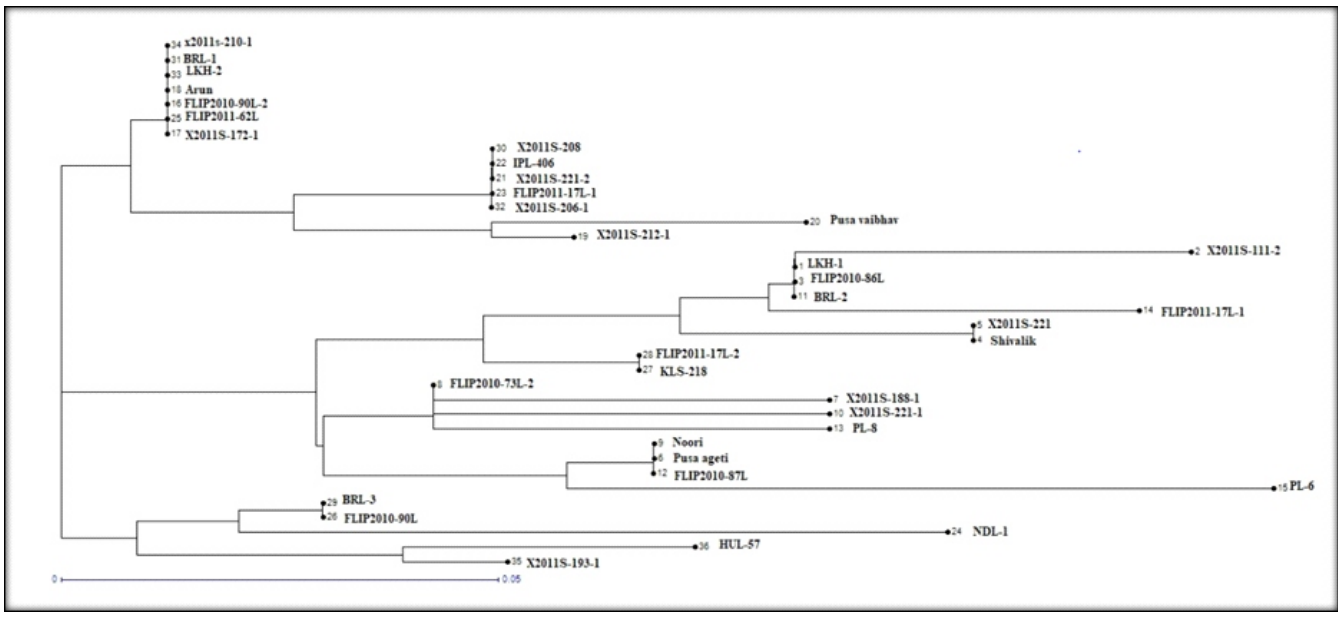


Fig.-1 : Dendorogram depicting genetic relationship among 36 lentil genotypes by UPGMA based on simple matching dissimilarity indices.

an average value of 0.29. The expected heterozygosity (He) ranged from 0.13 (LSSR 19) to 0.49 (LSSR 6-215, LSSR 107, and LSSR 184), with an average of 0.36 per locus (Table-3).

The genetic dissimilarity index among the thirty-six lentil genotypes, computed as the weighted mean for all pair wise comparisons of the simple matching dissimilarity indices based on all SSR markers, ranged from 0.042 to 0.240. The frequency distribution analysis of all pair wise comparisons showed a concentration of dissimilarity values in the classes 0.206 to 0.240, indicating maximum divergence among the studied lentil genotypes. Conversely, the lowest class limit values of 0.042 to 0.045 indicated maximum similarity and least diversity (Table-4).

The dendorogram from the neighbor-joining UPGMA (DARwin Program 6.0) cluster analysis of the pair wise simple matching dissimilarity coefficients matrix grouped

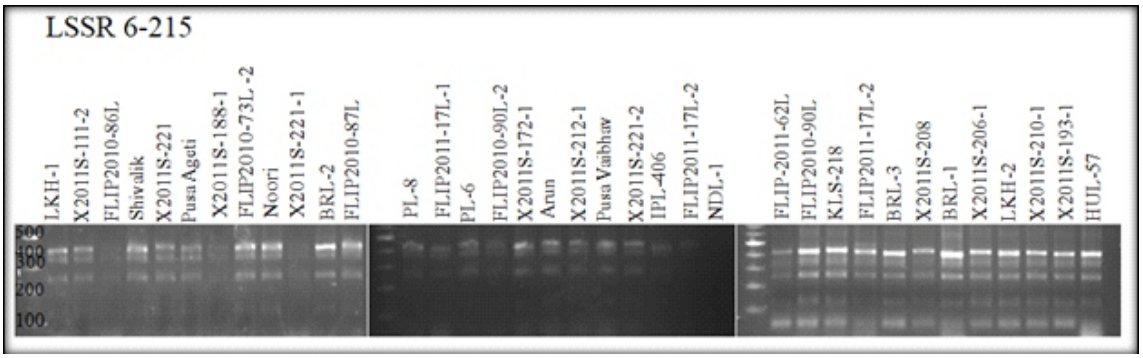


Fig.-2 : SSR profiling using SSR marker LSSR 6-215.

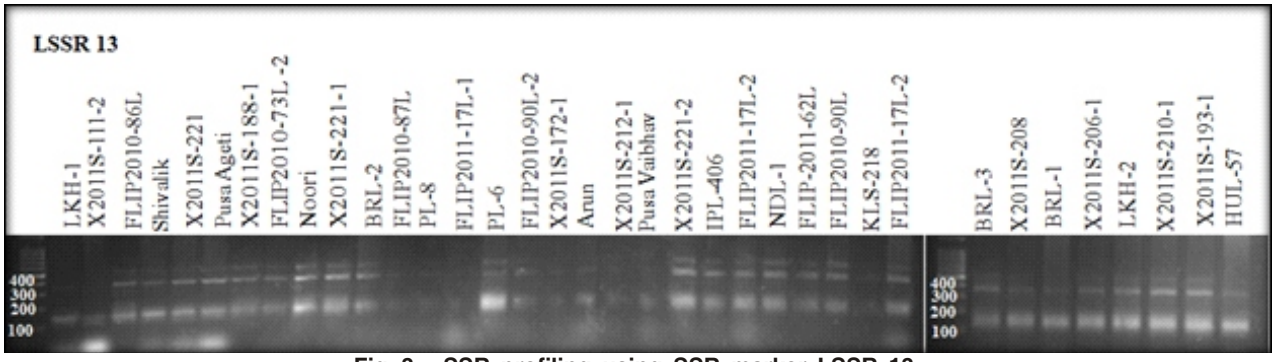


Fig.-3 : SSR profiling using SSR marker LSSR 13.

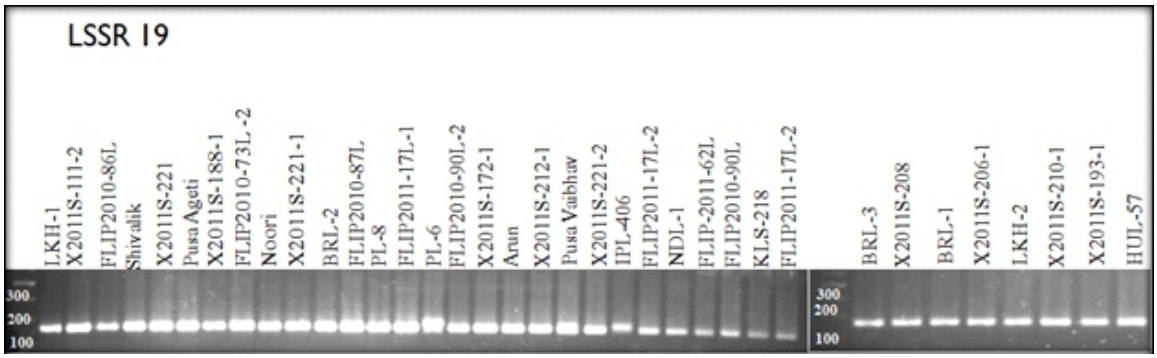


Fig.-4 : SSR profiling using SSR marker LSSR 19.

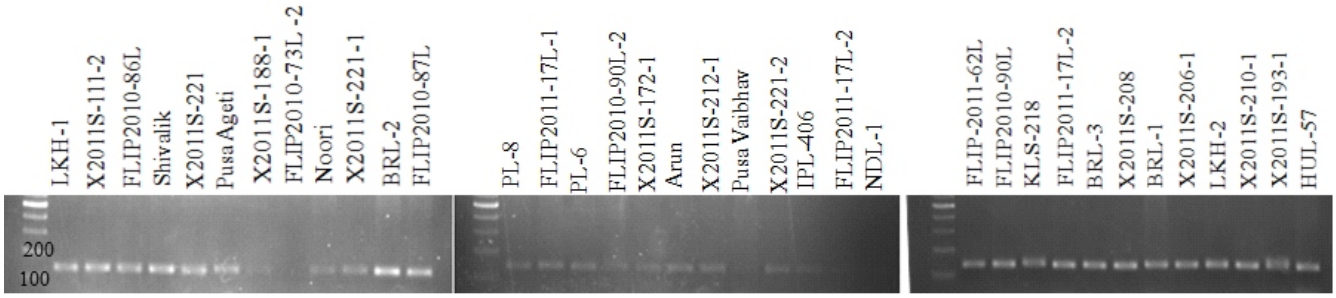


Fig.-5 : SSR profiling using SSR marker LSSR 80.

lentil genotypes into three major clusters. Cluster II was the largest, comprising 17 genotypes, followed by 14 genotypes in Cluster I, and 5 genotypes in the smaller Cluster III (Fig.-1).

The dendorogram revealed that all major clusters comprised a mixture of genotypes released from different locations, indicating that molecular diversity is minimally influenced by geographical locations. These results, based on SSR profiling, are in accordance with earlier reports (5, 6, 7, 8).

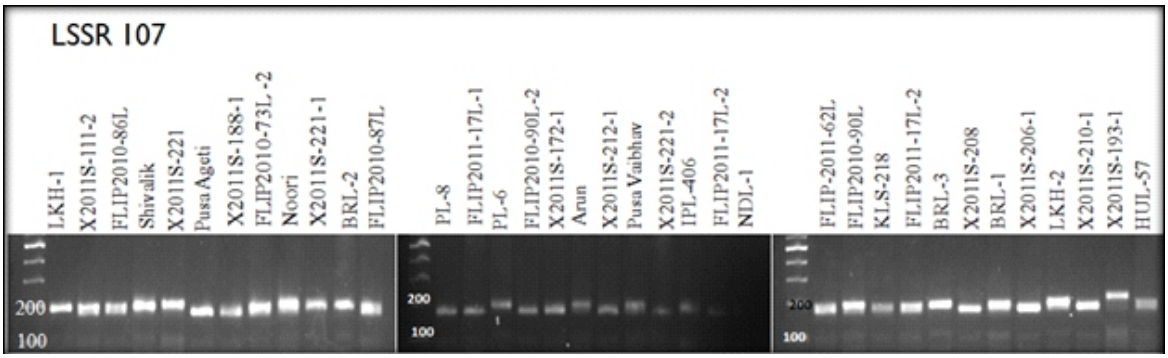


Fig.-6 : SSR profiling using SSR marker LSSR 107.

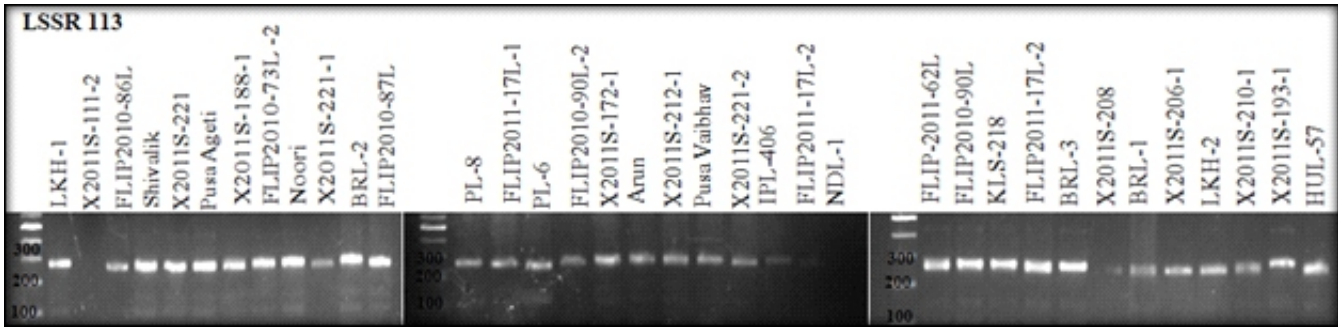


Fig.-7 : SSR profiling using SSR marker LSSR 113.

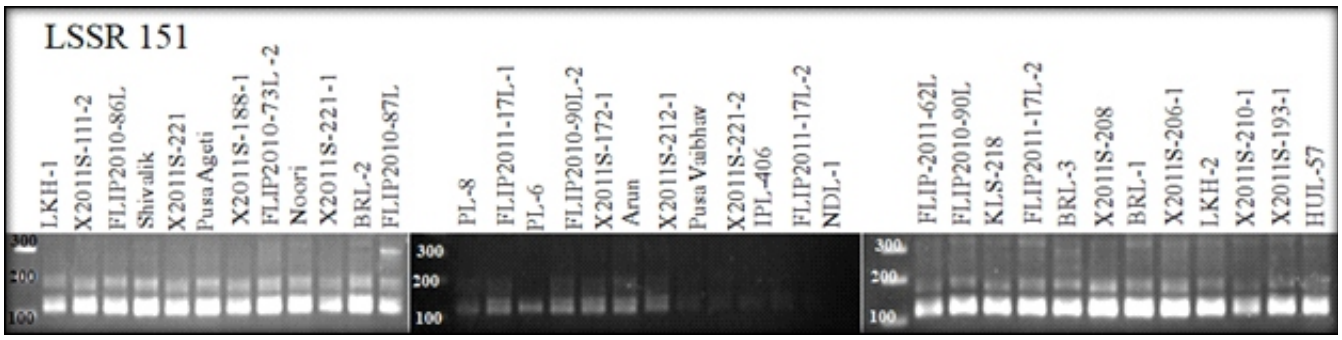


Fig.-8 : SSR profiling using SSR marker LSSR 151.

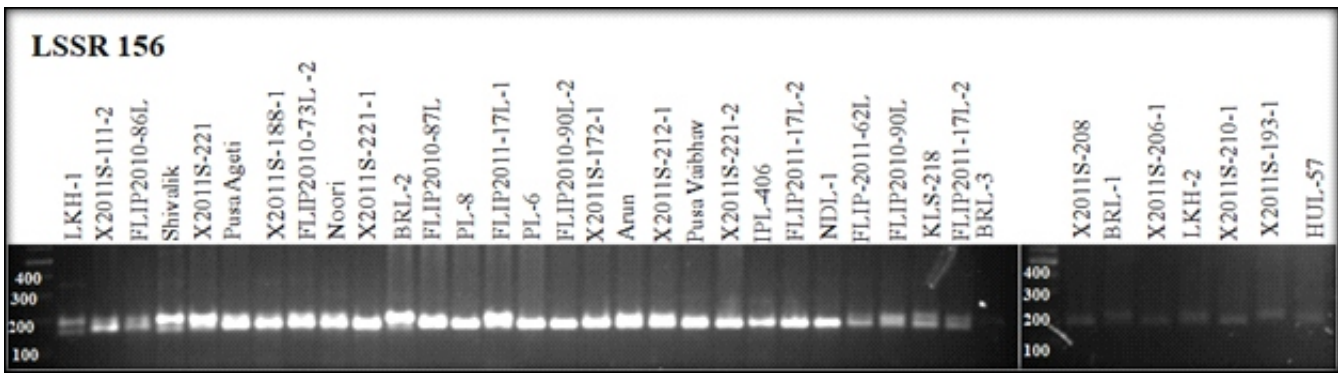


Fig.-9 : SSR profiling using SSR marker LSSR 156.

Conclusion

Genetic characterization in lentil has recently gained popularity to study genetic diversity and expedite current breeding programs. All 36 lentil accessions, consisting of indigenous and exotic germplasm lines, exhibited a considerable level of divergence by selected 16 SSR markers that could be exploited in future breeding

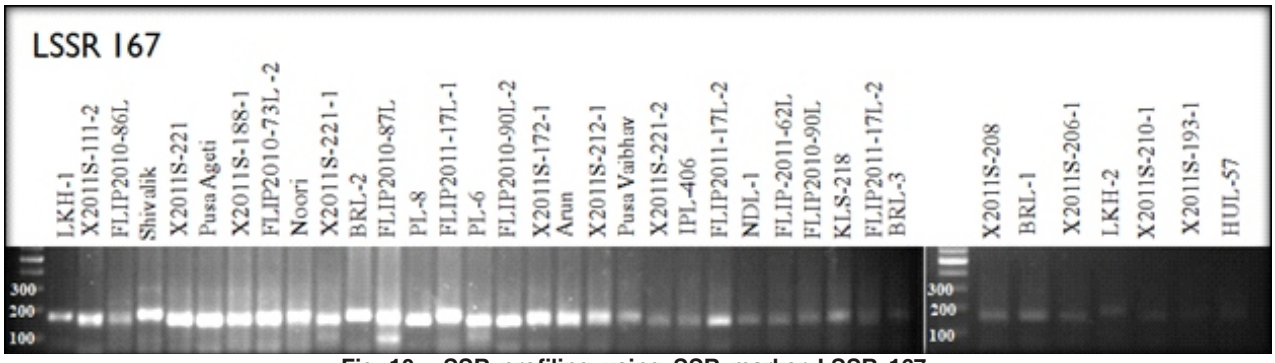


Fig.-10 : SSR profiling using SSR marker LSSR 167.

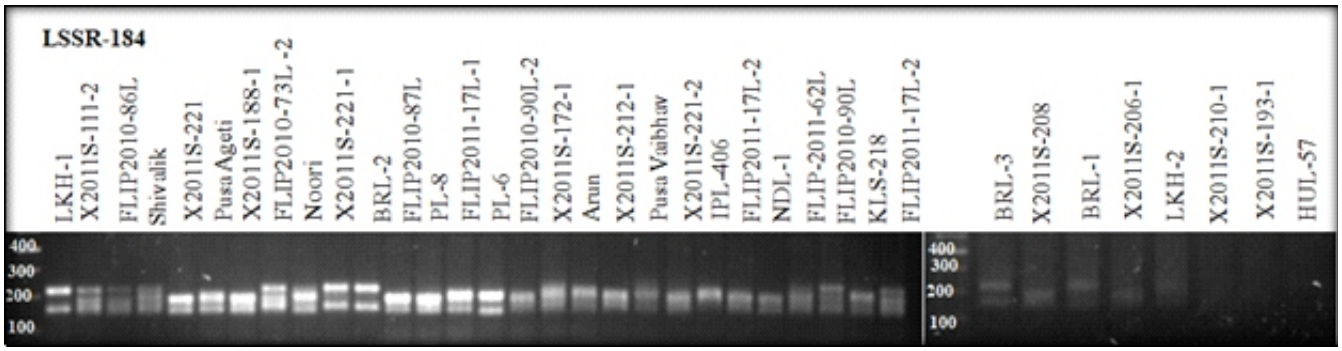


Fig.-11 : SSR profiling using SSR marker LSSR 184.

programs. The UPGMA-based dendorogram grouped all the genotypes into three major clusters, revealing that molecular diversity is not significantly influenced by geographical location in the present study. These results suggest the fidelity of SSR markers for DNA fingerprinting and genetic diversity analysis of lentil cultivars. This work will also be beneficial for targeted crossing in lentil breeding programs.

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