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Microsatellite Markers-Based Genetic Diversity and Population Structure Analysis of Lentil (*Lens culinaris* Medik.) Accessions

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Abstract

Lentil is a nutritious, protein-rich winter-season pulse crop acting as a boon for mitigating malnutrition and maintaining global food security. Genetic variation and selection is the basis for any crop improvement programme. To explore the pattern of genetic diversity among lentil accessions under salt stress, microsatellite (SSR) markers were used. The development of long-lasting, stable cultivars requires the utilisation of diverse germplasm. This study was conducted at the ICAR-Central Soil Salinity Research Institute, Karnal, using 100 lentil accessions. In this study, 12 SSR (simple sequence repeat) markers were used. Minor allele frequency (MAF) ranged from 0.07 to 0.49, with a mean value of 0.27. Genetic diversity (GD) for SSRs ranged from 0.11 to 0.50. The polymorphic information content (PIC), which ranged from 0 to 1, was 0.12 to 0.38 in the present study. Three sub-groups based on population structure analysis, denoted as the red, green and blue clusters, encompassed of 35, 48 and 17 lentil accessions, respectively. A UPGMA-based phylogenetic tree was also constructed, which grouped all genotypes into three main clusters. The information generated in our study may have significant implications for molecular characterisation, fingerprinting, and documentation of accessions in the lentil improvement programme.

Key words: Lentil, *Lens culinaris*, Germplasm, Genetic diversity, Minor allele frequency, Simple sequence repeat (SSR) markers, Population structure

Introduction

Lentil (*Lens culinaris* Medikus.) is a self-pollinating, annual, winter season crop with a genome size of approximately 4 Gb. It belongs to the family Papilionaceae and is an important dietary source of energy, protein, carbohydrates, fibre, minerals, vitamins, and antioxidant compounds, as well as diverse non-nutritional components such as protease inhibitors, tannins, α-galactoside oligosaccharides, and phytic acid (Urbano *et al*., 2007). Lentils also possess important medicinal value, i.e., they prevent homocysteine accumulation, improve cholesterol levels, control blood pressure levels, and help with colon cancer, constipation, ulcers, and smallpox. Lentil also improves soil fertility through its leguminous nature, which enables high nitrogen fixation, making it suitable for crop rotation with cereal crops and promoting sustainable agriculture. Genetic variation and selection form the basis of any plant breeding programme. Understanding genetic diversity, genetic relationships among accessions, and the association between yield and different attributes play an important role in any crop improvement programme. The most direct approach to improving lentil productivity under salt stress is to identify and increase the abundance of novel genes and alleles associated with salt stress in commercially relevant lentil germplasm (Singh *et al.,* 2017; 2020). In recent years, significant focus has been placed on implementing NGS (next-generation sequencing) techniques for genetic improvement, including DNA markers, advanced sequencing technologies, and bioinformatics tools. Molecular studies of salt tolerance have been conducted in many crops, including cereals, oilseeds and pulses. Quantitative trait loci (QTLs), significant single-nucleotide polymorphism (SNP) markers, and candidate genes have already been identified for salt

tolerance in other crops. Various bioinformatics approaches, such as GBS (genotyping by sequencing), association mapping, and omics approaches like transcriptomics, have been studied in other crops, but limited information is available on salt tolerance in lentils. To breed varieties with stable grain yield under salt stress conditions, deep insight into the genetic basis of yield, its attributing traits, nutrient content, and associated salt-tolerance mechanisms is necessary. Integration of classical breeding approaches with next-generation genomics and phenomics tools, along with generation-acceleration protocols, can hasten progress in the pulse crop improvement programme.

As with other crops, some studies have been conducted on pulses to evaluate genetic diversity and other biotechnological applications, but limited information is available for lentils. Zaccardelli *et al*. (2012) found that microsatellite (SSR) molecular markers were highly effective in discerning genetic variations and relationships among different lentil landraces, with these polymorphic bands successfully differentiating each accession. Verma *et al*. (2014) successfully developed 122 new SSR markers for lentil by constructing a GA/CT motif-enriched genomic library and clustering them into two groups, indicating genetic relationships within and between lentil species. Kushwaha *et al*. (2015) examined 96 lentil accessions from Nepalese research centres using 33 polymorphic SSR markers for genetic profiling and classified them into four clusters across two main groups, revealing significant genetic diversity, with the largest cluster representing 58.33 per cent of genotypes. Wong *et al*. (2015) evaluated 60 germplasm collections of lentil using the genotype by sequencing technique, where different taxa were formed under phylogenetic tree construction, followed by the identification of four gene pools named as *L. culinaris*/*L. orientalis*/*L. tomentosus*, *L. lamottei*/*L. odemensis*, *L. ervoides* and *L. nigricans* that represent primary, secondary, tertiary and quaternary gene pools, respectively. Idrissi *et al.* (2016) conducted a diversity analysis of 70 Mediterranean lentil landraces using SSR and amplified length polymorphism markers under drought stress. Yadav *et al*. (2016) evaluated the genetic diversity of 185 diverse lentil germplasm by using 30 polymorphic SSR markers and revealed that ten major clusters underlie divergence in lentil accessions, recommending their utility in future lentil breeding programme.

Singh *et al*. (2017) analysed 50 lentil accessions utilising 20 genomic and 54 EST-SSR markers and revealed that these accessions exhibited lower polymorphism in EST-SSRs compared to genomic SSRs. Pandey *et al*. (2018) assessed genetic diversity in 10 lentil genotypes using microsatellite markers. Skliros *et al*. (2018) utilised omics technologies to study the metabolic responses of lentil (Lens culinaris) to salinity, examining both acclimated and non-acclimated plants. Tsanakas *et al*. (2018) assessed genetic diversity within the 'Eglouvis' lentil landrace with comparison to the 'Samos' and 'Demetra' varieties using both morphological and molecular markers, where divergence distance among individuals and PCoA (principal coordinate analysis) indicated that the 'Eglouvis' possesses a distinct genetic base, significantly differing from both 'Samos' and 'Demetra'. Chowdhury *et al*. (2020) investigated genetic diversity among 20 lentil genotypes and formed three clusters using Ward's method. Singh *et al*. (2020) assessed salt stress tolerance, screened 495 SSR markers across the population, and identified 11 polymorphic markers between the parents, with a QTL genomic region on LG-1. Dissanayake *et al*. (2021) evaluated 276 genotypes of lentil using targeted genotyping by sequencing (t-GBS) and transcriptome genotyping by sequencing (GBS-t). Johnson *et al*. (2021) phenotyped 143 accessions to evaluate their responses to prebiotic carbohydrates and conducted GWAS to identify associated markers. Tomar *et al*. (2023) used SSR markers to analyse the diversity among 37 lentil genotypes, resulting in the formation of two clusters with PIC values (up to 0.77). Among a variety of molecular markers, microsatellite markers, or simple sequence repeats (SSRs), are the most used and preferred for genetic diversity studies. SSRs are cost-effective, easy to score, quick, reliable, and require minimal DNA quantities (Gupta and Varshney, 2000). They are highly polymorphic, co-dominant, and widely distributed across the genome, so they are preferentially favoured for

genetic diversity analysis (Hoshino *et al*., 2012; Mondini *et al*., 2009).

Our present study explores patterns of genetic diversity among lentil accessions, population structure, and genotypic relationships using microsatellite markers to facilitate the conservation and utilisation of the germplasm resources studied.

Material and Methods

Experimental site and planting material

The experiment was conducted at ICAR- Central Soil Salinity Research Institute (ICAR-CSSRI), Karnal. ICAR-CSSRI is situated at a geo-sensing of 29°43′ N latitude, 76°58′ E longitude and 245 meters elevation. The climate is subtropical to tropical (hot subhumid), and the average annual rainfall at the experimental site is 828 mm, mostly received during the monsoon season (July-August). The planting material for the present investigation consisted of a 100-plant diversity set of lentil accessions collected from NBPGR, New Delhi; SKNAU, Jobner (Rajasthan); PAU, Ludhiana (Punjab); and two released salt-tolerant varieties. All crop management practices were performed as per the standard package of practices for lentil to grow a healthy crop.

Genomic DNA isolation

Approximately 5g of leaf sample of 15-day-old seedling ground with a sterilised mortar pestle in 1ml DNA extraction buffer (DEB with â-mercaptoethanol). After hot-water bath incubation, 1 ml of chloroform and isoamyl alcohol (24:1) were added to each Eppendorf tube, then mixed thoroughly using a Hula Mixer (Invitrogen by Thermo Fisher Scientific) for 15-20 minutes. The chilled iso-propanol was added to each tube (1:1 ratio, 600-1000µl) and mixed just by two inversions and kept overnight at 40 °C. 70 per cent ethanol and sodium acetate (3:1) were added for washing (200 il), and the mixture was centrifuged again at 10,000 rpm for 5 minutes at 40 °C. Supernatant was discarded, and the pallets were air-dried. The DNA was subsequently dissolved in an appropriate volume of nuclease-free water, and samples were stored at -200 °C until further use. RNA contamination was removed by adding 1 µl of 10 mg/ml RNase to the dissolved DNA samples. The quality of the completely dissolved genomic DNA was assessed using the NanoDrop™ Spectrophotometer (DS-11 Series, DeNovix Inc., USA).

Genetic diversity analysis

A total of 12 amplified SSR markers from diverse lentil accessions were used for diversity analysis. PCR amplification was performed using a thermocycler (Mastercycler® nexus-Eppendorf). The PCR products were examined using agarose gel electrophoresis. Agarose (3.5% by volume in 500 ml) was melted in 1xTBE buffer, and once it had warmed, ethidium bromide (4 µl/ml) was added. The amplified PCR products were viewed under UV light fluorescence using Omega Lum™G Imaging System (Aplegen-Gel Company, USA). To perform the unrooted neighbour-joining (NJ) - based phylogenetic tree (with 1000 bootstrap replicates) construction, the generated SSR data of 100 genotypes were analyzed using Power Marker v3.51 (Liu and Muse, 2005) software. A dendrogram was created using the UPGMA method in Darwin 6.

Population structure analysis

The population structure among the one hundred diverse accessions was determined by analysing the SSR data using STRUCTUREv2.3.4 with the model-based programme following the method of Pritchard et al. (2000). Further, to determine the optimum population number (K) value, the ad hoc, delta K method was followed as suggested by Evanno *et al.* (2005).

Results and Discussion

To explore the pattern of genetic diversity among lentil accessions using microsatellite markers

The experimental material for the proposed study comprised 100 diverse lentil accessions and 12 microsatellite markers. Binary codes 0 and 1 were assigned based on the presence or absence of the amplified band, and the matrix was used for further analysis. Marker-wise values of genetic diversity (GD), polymorphic information content (PIC), and minor allele frequency (MAF) are presented in Table 1, and the complete sequence

Table 1. Minor allele frequency (MAF), Genetic diversity (GD) and Polymorphic Information Content (PIC) values of 12 microsatellite markers

S.No.	Marker	Seq	Sequence	Tm	MAF	GD	PIC
1	Le 1_1	F	5'-GGTTTGAGCCGTTTTTGTGT-3'	50.9	0.340	0.449	0.348
		R	5'-TTTGCCGTCAAGTGATTCAA-3'				
2	Le 1_2	F	5'-TGTTGAACTATCGAATCGTC-3'	50.9	0.070	0.130	0.122
		R	5'-CACCTATATTATTGGCTACG-3'				
3	Le 1_13	F	5'-GCGACAACTCCCACACACTA-3'	50.9	0.320	0.435	0.341
		R	5'-AAGGGCCTCAAAAGCACTTA-3'				
4	Le 1_17	F	5'-CTCTTTCTTTTTCGGTGTGT-3'	55.6	0.490	0.500	0.375
		R	5'-AAAGTAAAAATCGCTGCCCA-3'				
5	Le 1_26	F	5'-ATGTCGGTGACGGATTGATT-3'	50.3	0.460	0.497	0.373
		R	5'-GCATGCTTATGATGAACTTGACC-3'				
6	Le 1_31	F	5'-AGATTGCTATTGGTTTGACCC-3'	50	0.340	0.449	0.348
		R	5'-TCATATCAATCCCACAACGG-3'				
7	Le 1_34	F	5'-GAATTGCGGCGAGAGTAGAT-3'	50.9	0.310	0.428	0.336
		R	5'-TTCCTCTCTGTGTCGTTCCA-3'				
8	Le 1_45	F	5'-TCGATCACGTCATTCGTAAA-3'	53.1	0.110	0.196	0.177
		R	5'-TGTGTGTTTGCCTGATCCAT-3'				
9	Le 2_2	F	5'-GCCCATGGTCAATAATCCTC-3'	50.9	0.060	0.113	0.106
		R	5'-TCCTAATCTAACTCGTGCTCAA-3'				
10	Le 2_4	F	5'-AAGTGGCAAAGTGCTTGGTT-3'	50.9	0.070	0.130	0.122
		R	5'-GGCCATTGTTGACAAGAAAA-3'				
11	Le 2_22	F	5'-TGGTTCAAGTTACCAAGTCCA-3'	50.9	0.230	0.354	0.292
		R	5'-CAACCAAACTCAATATGATGGC-3'				
12	Le 3_1	F	5'-CGCGTTAACAGAGGAAGAGG-3'	50.9	0.400	0.480	0.365
		R	5'-GCGGTGAACTTTTACGAGG-3'				
				Mean	0.267	0.347	0.275

*F: Forward; R: Reverse

of microsatellite marker-based genetic diversity and population structure of lentil is summarised in Figure 1.

The genetic diversity analysis pattern is depicted in Figure 4. The estimation of the number of subpopulations using the Delta K value for K ranging from 2 to 10 is shown in Figure 4. The peak at K=3 is depicted in Figure 2, the population structure in Figure 3, and a circular dendrogram in Figure 4.

The need to address stress-related agricultural issues has led to an increase in research studies on lentil using molecular markers. Several studies have shown the importance of understanding genetic diversity for developing lentil varieties that can withstand stress (Singh *et al.,* 2020). Because of their high polymorphism, microsatellite markers are an effective tool for evaluating genetic diversity (Jin *et al.,* 2008; Mekonnen *et al.,* 2016; Reddy *et al.,* 2010 Yadav *et al.,* 2016). Previous research highlights how molecular markers might help to decipher complex genetic patterns and identify accessions that are stress-tolerant (Afzal *et al.,* 2023; Atta *et al.,* 2023).

Genetic diversity (GD)

Minor allele frequency (MAF) ranged 0.070 to 0.490 with mean value of 0.267. The minimum MAF was observed as 0.060 (Le 2_2) followed by 0.070 (Le 1_2), 0.070 (Le 2_4), 0.110 (Le 1_45) whereas maximum as 0.490 (Le 1_17), 0.460 (Le 1_26), 0.400 (Le 3_1). Genetic diversity (GD) for SSRs ranged from 0.113 to 0.500. The maximum GD was observed as 0.500 (Le 1_17) followed by 0.497 (Le 1_26), 0.480 (Le 3_1) whereas minimum as 0.113 (Le 2_2) followed by 0.130 (Le 1_2), 0.130 (Le 2_4). The polymorphic information content (PIC) which ranges from 0 to 1 is the measure of the degree of polymorphism, ranged 0.123 to 0.375 in present study. The maximum PIC was

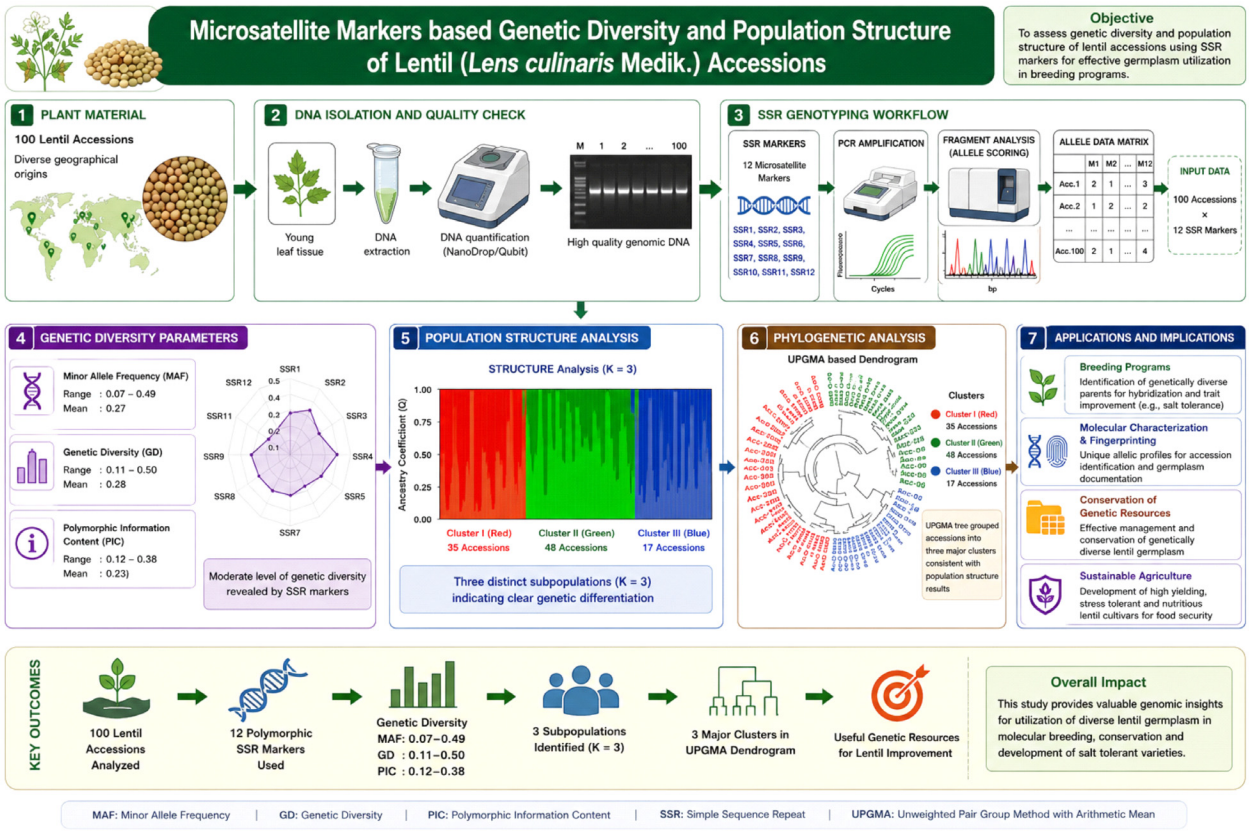

Fig. 1 Microsatellite marker-based genetic diversity and population structure of lentil

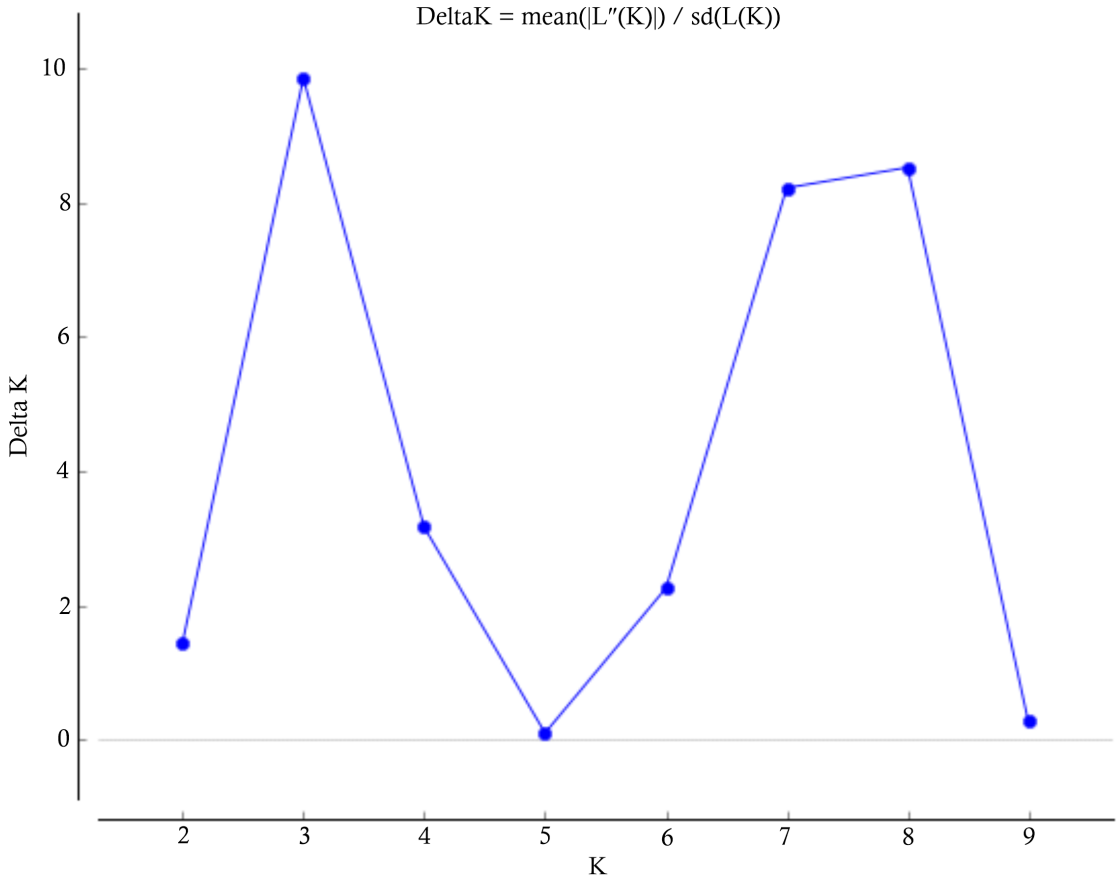

Fig. 2 Estimation of number of subpopulations using Delta K value for K ranging from 2 to 10, the peak maxima at the K=3

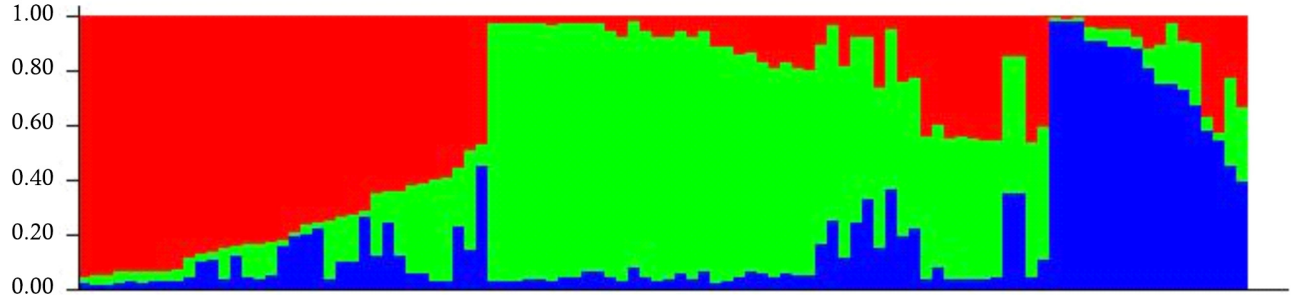

Fig. 3 Population structure of diversity set depicting three subpopulations

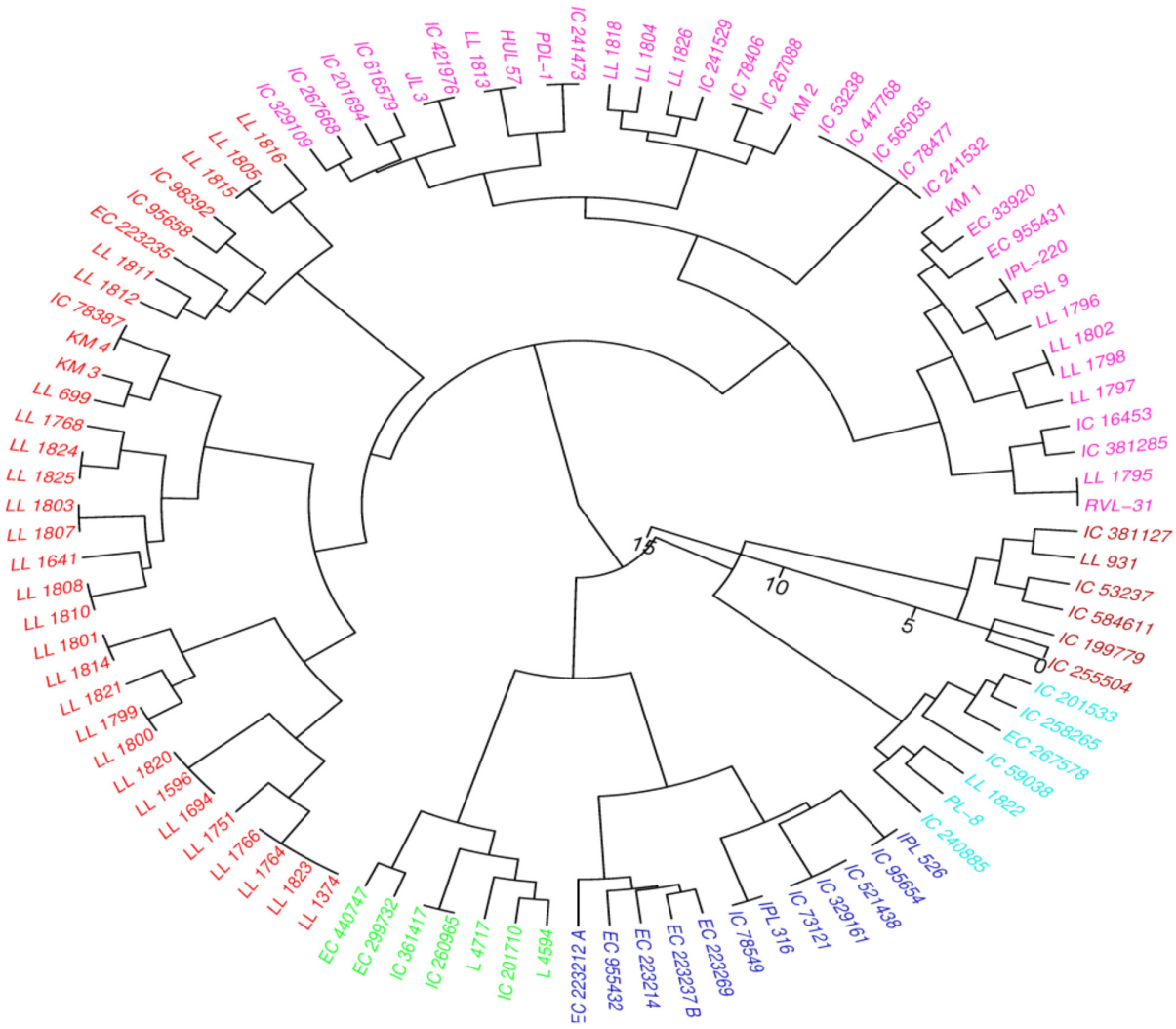

Fig. 4 Phylogenetic tree construction of lentil genotype using UPGMA based clustering method

observed as 0.375 (Le 1_17) followed by 0.373 (Le 1_26), 0.365 (Le 3_1), 0.348 (Le 1_1) where as minimum value was observed as 0.106 (Le 2_2), 0.122 (Le 1_2), 0.122 (Le 2_4), 0.177 (Le 1_45) and 0.292 (Le 2_22).

The knowledge gained from these studies might help to create resilient lentil varieties, which are essential for long term agricultural sustainability in areas overwhelmed by stress. Examining genetic diversity and understanding the evolutionary relationship among accessions are important components of breeding and enhancement efforts. Despite global breeding efforts, genetic enhancement initiatives and the

use of diverse genotypes of lentils display a limited genetic background, primarily due to the frequent utilization of the common parents (Ibrahim *et al*., 2023; Safhi *et al*., 2022). Assessing genetic diversity is essential for introducing novel genetic material and advancements in molecular marker methods have proven effective in this regard. In this study, genetic diversity was assessed using microsatellite markers, revealing polymorphisms across all samples, indicating genetic variation.

Population structure analysis

The dataset comprising information from 12 SSR markers was used for STRUCTURE analysis to investigate potential sub-groups. A membership probability cutoff of 60% was applied to assign accessions to clusters using the admixture model. The results revealed the existence of three primary sub-groups, identified based on ΔK values across K values ranging from 2 to 10. These sub-groups, denoted as the red, green and blue clusters, encompassed of 35, 48 and 17 lentil accessions, respectively. Notably, some accessions did not meet the membership probability threshold for any specific cluster and were classified as admixtures.

The polymorphic Information Content (PIC) value, as indicated by Pervaiz *et al*. (2009), serves as an indicator of diversity among the assessed varieties. The assessment of PIC values can be conducted on a locus-by-locus basis, with each SSR locus potentially having a distinct PIC value. The calculated average PIC value of approximately 0.28 indicates diversity among the lentil genotypes. The STRUCTURE analysis classified all one hundred genotypes into three distinct groups, aligning with the evolutionary tree clustering. Similarly, Mekonnen *et al*. (2016) and Singh *et al*. (2016) also reported comparable groupings based on SSR markers. These research studies also conducted comparisons of dissimilarity indices among lentil accessions using SSR markers, revealing nearly identical results. Additionally, the population structure analysis revealed additional admixtures and the expected GD values ranged 0.11 to 0.50.

To further elucidate the genetic relationships among diverse lentil genotypes by additional analyze, UPGMA-based phylogenetic tree was constructed using DARwin 6. This analysis aimed to evaluate both the genetic resemblance within subpopulations and across the entire population, providing insights into the intricate genetic connections among the lentil accessions. Finally, the circular dendrogram was created via multivariate hierarchical cluster analysis using group mean method and Jaccard's coefficient of similarity, also congregated all genotypes into three main clusters. These supplementary analyses provided consistent support for the identified population structure.

These findings underscore the effectiveness of microsatellite markers-based genotyping in understanding the genetic diversity and structure of accessions. The findings regarding genetic diversity and PIC values align with the conclusions drawn by Saidi *et al*. (2022), who utilized SSR markers to assess the genetic diversity among various lentil accessions. Hamwieh *et al*. (2009) developed 14 microsatellite markers by creating an additional set of 14 markers to assess genetic diversity and relationships within a core collection of lentil germplasm maintained at ICARDA. The microsatellite markers developed in their research exhibited high polymorphism, locus specificity, and mostly operated as co-dominant markers, proving invaluable analytical tools for evaluating genetic diversity in numerous other studies. Liu *et al*. (2008) analysed 440 lentil accessions from the National Gene Bank of China using 14 SSR markers, while Babayeva *et al.* (2009) investigated the genetic relationships among 39 lentil accessions with five SSR markers. Both studies confirmed the effectiveness of these microsatellites in exploring cultivar diversity in lentils.

Similarly, Bacchi *et al*. (2010) utilised six SSR markers to study the genetic relationship among 25 lentil accessions, suggesting that a subset of SSR markers could provide valuable information about genetic connections. Zaccardelli *et al*. (2012) employed 16 SSR markers to categorise Italian lentil landraces into clusters based on geographical origin. Furthermore, SSR markers developed by others, as demonstrated by Verma *et al*. (2014), also confirmed their efficiency in analyzing genetic relationships between cultivated and wild *Lens species*. In a study conducted by Pandey *et al*.

(2018), genetic divergence among ten lentil genotypes was assessed using SSR markers, identifying 26 discernible and reproducible bands, of which 25 were polymorphic and one was monomorphic resulted in the identification of two distinct clusters among the genotypes, with polymorphic information content values ranging from 0.16 (SSR 204) to 0.65 (SSR 124), averaging 0.42 across all loci. The genetic distance or coefficient of similarity among lentil accessions ranged 0.14 to 0.58. This research provides insights into the genetic diversity among the examined lentil accessions, revealing specific markers with high polymorphic potential.

Conclusions

The result findings suggest that SSR markers are important tools for assessing genetic diversity among lentil accessions. The process of selecting accessions as potential donors in a crop breeding strategy aimed at enhancing specific attributes in lentil necessitates the evaluation of genetic diversity among individuals. The presence of different clusters in both phylogenetic as well as population structure analysis indicates a high level of genetic variation in the investigated lentil accessions that can be used as a criterion for the selection of diverse parents in the improvement programme to get better transgressive segregants.

Author Contributions: VS – conceptualization, planning, data acquisition, data analysis; PS **-** data acquisition, data analysis; JS – Editing and reviewed the manuscript; VS – Editing and reviewed the manuscript; AK - Data curation; ZS - data analysis.

Data Availability Statement: The data supporting the outcomes of this research are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this manuscript.

References

Afzal M, Hindawi SE, Alghamdi SS, Migdadi HH, Khan MA, Hasnain MU, Arslan M, Habib UR, Rahman M and Sohaib M (2023) Potential breeding strategies for improving salt tolerance in crop plants. *Journal of Plant Growth Regulation* **42** (6): 3365-3387.

Atta K, Mondal S, Gorai S, Singh AP, Kumari A, Ghosh T, Roy A, Hembram S, Gaikwad, DJ, Mondal S and Bhattacharya S (2023) Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science* **14**: 1241736

Babayeva S, Akparov Z, Abbasov M and Mammadov A (2009) Diversity analysis of Central Asia and Caucasian lentil (*Lens culinaris* Medik.) germplasm using SSR fingerprinting. *Genetic Resources and Crop Evolution* **56**: 293-298.

Bacchi M, Leone M, Mercati F, Preiti G, Sunseri F and Monti M (2010) Agronomic evaluation and genetic characterization of different accessions in lentil (*Lens culinaris* Medik.). *Italian Journal of Agronomy* **4**: 303-314.

Chowdhury MM, Haque MA, Malek MA, Rasel M, Molla MR and Ahamed KU (2020) Morphological and SSR marker based diversity analysis of lentil (*Lens esculenta*) genotypes using yield and yield contributing characters. *Indian Journal of Agricultural Research* **54** (4): 429-436.

Dissanayake R, Cogan NO, Smith KF and Kaur S (2021) Application of genomics to understand salt tolerance in Lentil. *Genes* **12** (3): 332.

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611- 2620.

Gupta PK and Varshney R (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica* **113** (3): 163-85.

Hamwieh A, Udupa SM., Sarker A and Jung C and Baum M (2009) Development of new microsatellite markers and their application in the analysis of genetic diversity in lentils. *Breeding Science* **59**: 77-86.

Hoshino AA, Bravo JP, Nobile PM and Morelli KA (2012) Microsatellites as tools for genetic diversity analysis. Genetic diversity in microorganisms: InTechOpen, p 26.

Ibrahim AA, Salem KFM, Abdein MA and Ramadan SM (2023) Advances in summer squash (*Cucurbita pepo* L.) molecular breeding strategies. In: Singh S, Sharma D, Sharma SK, Singh R (eds) *Smart Plant Breeding for Vegetable Crops in Post-genomics Era*, Springer, Singapore.

Idrissi O, Udupa MS, De Keyser E, Van Damme P and De Riek J (2016) Functional genetic diversity analysis and identification of associated simple sequence repeats and

amplified fragment length polymorphism markers to drought tolerance in lentil (*Lens culinaris ssp. culinaris* Medicus) Landraces. *Plant Molecular Biology Reporter* **34**: 659-680.

Jin LIU, Jian-Ping GUAN, Dong-Xu XU, Zhang XY, Jing GU and Xu-Xiao ZONG (2008) Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. *Acta Agronomica Sinica* **34** (11): 1901-1909.

Johnson N, Boatwright JL, Bridges W, Thavarajah P, Kumar S, Shipe E and Thavarajah D (2021) Genome wide association mapping of lentil (*Lens culinaris* Medikus) prebiotic carbohydrates toward improved human health and crop stress tolerance. *Scientific Reports* **11** (1): 13926.

Kushwaha UKS, Ghimire SK, Yadav NK, Ojha BR and Niroula RK (2015) Genetic characterization of lentil (*Lens culinaris* L.) germplasm by using SSR markers. *American Journal of Agricultural and Biological Sciences* **1** (2): 16-26.

Liu J, Guan JP, Xu DX, Zhang, XY, Gu J and Zong XX (2008) Genetic diversity and population structure in lentil (*Lens culinaris* Medik.) germplasm detected by SSR markers. *Acta Agronomica Sinica* **34**: 1901-1909.

Liu K and Muse SV (2005) Power Marker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**: 2128-2129.

Mekonnen F, Mekbib F, Kumar S, Ahmed S and Sharma TR (2016) Molecular diversity and population structure of the Ethiopian lentil (*Lens Culinaris* Medikus) genotype assessment using SSR markers. *Journal of Crop Science and Biotechnology* **19**: 1-11.

Mondini L, Noorani A, Pagnotta MA (2009) Assessing plant genetic diversity by molecular tools. *Diversity* **1** (1): 19–35.

Pandey AK, Sengar RS, Kumar A, Chand P and Yadav R (2018) Molecular characterization of Lentil (*Lens culinaris* Medikus) genotypes through simple sequence repeats (SSR) markers. Biotech Today: *An International Journal of Biological Sciences* **8** (1): 65-72.

Pervaiz ZH, Rabbani MA, Pearce SR and Malik SA (2009) Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. *African Journal of Biotechnology* **8**: 21.

Pritchard JK, Stephens M and Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959.

Reddy MRK, Rathour R, Kumar N, Katoch P and Sharma TR (2010) Cross genera legume SSR markers for analysis of genetic diversity in *Lens species*. *Plant Breeding* 129 (5): 514-518.

Safhi FA, Alshamrani SM, Jalal AS, El-Moneim DA, Alyamani AA and Ibrahim AA (2022) Genetic characterization of some Saudi Arabia's accessions from *Commiphora gileadensis* using physio-biochemical parameters, molecular markers, DNA barcoding analysis and relative gene expression. *Genes* **13**: 2099.

Saidi A, Sarvmeili J and Pouresmael M (2022) Genetic diversity study in lentil (*Lens culinaris* Medik.) germplasm: a comparison of CAAT box derived polymorphism (CBDP) and simple sequence repeat (SSR) markers. *Biologia* **77** (10): 2793-2803.

Singh A, Sharma V, Meena J, Gupta S, Dikshit HK, Aski M, Mishra GP and Sarker A (2017) Genetic variability for grain iron and zinc concentration in lentil. *Chemical Science Review and Letters* **6**: 1327-1332.

Singh D, Singh CK, Kumari S, Tomar RSS, Karwa S, Singh R, Singh RB, Sarkar SK and Pal M (2017) Discerning morpho-anatomical, physiological and molecular multiformity in cultivated and wild genotypes of lentil with reconciliation to salinity stress. *Plos One* **12** (12): e0190462.

Singh D, Singh, CK, Tomar RSS, Chaturvedi AK, Shah D, Kumar A and Pal M (2016) Exploring genetic diversity for heat tolerance among lentil (*Lens culinaris* Medik.) genotypes of variant habitats by simple sequence repeat markers. *Plant Breeding* **132** (2): 215-223.

Singh D, Singh CK, Tomar RSS, Sharma S, Karwa S, Pal M, Singh V, Sanwal SK and Sharma PC (2020) Genetics and molecular mapping for salinity stress tolerance at seedling stage in lentil (*Lens culinaris* Medik). *Crop Science* **60** (3): 1254-1266.

Skliros D, Kalloniati C, Karalias G, Skaracis GN, Rennenberg H and Flemetakis E (2018) Global metabolomics analysis reveals distinctive tolerance mechanisms in different plant organs of lentil (*Lens culinaris*) upon salinity stress. *Plant and Soil* **429**: 451-468.

Tomar S, Sharma S, Tripathi N, Thakur S, Pathak N, Sharma R and Tiwari P (2023) SSR marker-based molecular characterization of lentil (*Lens culinaris* Medik.) genotypes. *Legume Research* **46** (7): 837-842.

Tsanakas GF, Mylona PV, Koura K, Gleridou A and Polidoros AN (2018) Genetic diversity analysis of the Greek lentil (*Lens culinaris*) landrace 'Eglouvis' using morphological and molecular markers. *Plant Genetic Resources* **16** (5): 469-477.

Urbano G, Porres JM, Frias J, Vidal-Valverde C (2007) nutritional value. In: Lentil: An Ancient Crop for Modern Times. (Edited by Yadav SS, McNeil D and Stevenson PC) Berlin: Springer, Dordrecht **3**: 47- 93.

Verma P, Sharma TR, Srivastava PS, Abdin MZ and Bhatia S (2014) Exploring genetic variability within lentil (*Lens culinaris* Medik.) and across related legumes using a newly developed set of microsatellite markers. *Molecular Biology Reports* **41**: 5607- 5625.

Wong MM, Gujaria-Verma N, Ramsay L, Yuan HY, Caron C, Diapari M, Vandenberg A and Bett KE (2015) Classification and characterization of species within the genus *Lens* using genotyping by sequencing (GBS). *PLoS One* **10** (3): e0122025.

Yadav NK, Ghimire SK, Sah BP, Sarker A, Shrestha SM and Sah SK (2016) Genotype x environment interaction and stability analysis in lentil (*Lens culinaris* Medik.). *International Journal of Environment, Agriculture and Biotechnology* **1** (3): 238539.

Yadav NK, Ghimire SK, Shakya SM, Sah SK, Sah BP, Sarker A and Kushwaha UKS (2016) Genetic diversity analysis of lentil (*Lens culinaris* L.) germplasm using DNA based SSR markers. *American Journal of Food Science and Health* **2** (3): 18-24.

Zaccardelli M, Lupo F, Piergiovanni AR, Laghetti G, Sonnante G, Daminati MG, Sparvoli F and Lioi L (2012) Characterization of Italian lentil (*Lens culinaris* Medik.) germplasm by agronomic traits, biochemical and molecular markers. *Genetic Resources and Crop Evolution* **59**: 727-738.

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