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Research article

Molecular diversity analysis in lucerne (*Medicago sativa* L.) genotypes using SSR markers

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Abstract

The present study examined the genetic diversity in 40 lucerne (*Medicago sativa* L.) germplasm using 30 SSR markers. Lucerne is an autotetraploid, allogamous, and heterozygous species. Out of 30 primers investigated, 24 showed polymorphism and amplified 78 alleles. The number of alleles ranged from 2 to 7. The PIC values ranged from 0.25 to 0.99. The primers AW101 (0.99) and AW332 (0.99) were found to be highly informative for molecular diversity studies. The genetic dissimilarity values varied from 0.27 to 0.89. A high dissimilarity index of 0.89 was found between CO 1 and GETL 21 (0.89) and CO 2 and AWL 6 (0.89). Genotypes RBB 07-01 and LLC 9 (0.27) had the least dissimilarity index. Cluster analysis based on molecular diversity revealed two clusters. Among the two clusters, cluster II comprised a maximum of 26 germplasm, followed by cluster I with 14 germplasm. The study indicated that the diverse lucerne genotypes such as CO 1, CO 2, GETL 21 and AWL 6 could be utilized as one of the parents for developing promising diverse lucerne genotypes through a polycross breeding approach.

Keywords: Lucerne, Molecular diversity, Polymorphism, SSR marker

Introduction

Lucerne, or alfalfa (*Medicago sativa* L.) (2n = 4x = 32), is one of the most important legume fodder crops belonging to the family Fabaceae, that is widely cultivated in arid and semi-arid regions of the world. This crop is often described as 'Queen of forage crops' (Barnes *et al*., 1988). Lucerne is perennial in growth habit, has relatively high water demand and is exposed to periodic harvesting (Singh *et al*., 2007). It is rich in protein and grown worldwide due to its high nutritive value, yield potential, quality, and survival in contrasting environments (Takawale *et al*., 2019). As it is a legume fodder, it fixes atmospheric nitrogen and can be easily converted into hay and silage for preservation. The protein content of lucerne is higher than that of all other fodder crops (Alla *et al*., 2013). Apart from this, lucerne green fodder contains significant amounts of minerals like phosphorus, magnesium, calcium, and vitamins like A and D (Antony *et al*., 2024). Lucerne is a highly cross-pollinated crop that expresses high inbreeding depression on hybridization followed by selfing and it is also highly self-incompatible (Vinodkumar *et al*., 2024). Intermating among the selected germplasm or lines through polycross breeding design followed by selection on selfing is the common breeding method followed in the lucerne crop improvement program. To select diverse parents for crop improvement, knowledge of the genetic diversity available in the existing gene pool (Poonia *et al*., 2020; Kaur *et al*., 2024) is a prerequisite.

Molecular marker analysis serves as an excellent tool for assessing genetic variation, nullifying the effect of the environment on different trait expressions. Researchers used various DNA markers to estimate the heritable difference in lucerne. Among them, simple sequence repeat (SSR) markers are extremely polymorphic and co-dominant (Raveendar *et al*., 2016). Falahati-Anbaran *et al*. (2007) found that SSR markers are highly useful in assessing the genetic diversity in lucerne, both in inter and intra-population. They emphasized that the SSR markers are very informative and appropriate for characterizing the populations of lucerne. The current study was designed to use SSR markers to analyze the molecular diversity among lucerne genotypes.

Materials and Methods

Plant materials and methods: A total of 40 germplasms in the domestic gene pool were utilized in this study (Table 1). The study was carried out at the Department of Forage Crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, from October 2022 to December 2022. The plant samples for DNA extraction were collected during the early vegetative phase and molecular analysis was carried out using 30 SSR markers (Table 2).

DNA extraction: Genomic DNA was extracted from young leaves of each plant per germplasm according to the modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). DNA concentration was determined spectrophotometrically. The OD_{260}/OD_{280} ratio of 1.8 and 2.0 was used to detect the contaminants. A value lower than 1.8 notifies the contamination by proteins, whereas a value greater than 2 implies contamination by salts. The DNA concentration (ng/μL) and quality were measured at 260 and 280 nm using Tecan's infinite 200 NanoQuant 200. The DNA samples were diluted by adding sterile water based on the concentration measured.

SSR analysis: PCR amplification was carried out in a 10 μL reaction volume containing 2 μL (25 ng) of genomic DNA, 1.0 μL primer (10 μM), 5.0 μL master mix (2X), and 2.0 μl sterile double distilled water. The PCR reaction was carried out as follows: initial denaturation temperature (at 94°C for 4 minutes), denaturation temperature (at 94°C for 1 min), then annealing temperature (35 cycles of 1-minute at 55°C), extension temperature (at 72°C for 2 minutes), and a final extension temperature (at 72°C for 6 minutes). The PCR products were fractionated with 3% agarose gel in 10X TBE at 120 V for 2 hours. The ethidium bromide-stained gels were documented using a gel documentation unit (Bio-Rad, Gel Doc^{TM}XR^{+}, USA).

Statistical analysis: The distinguishable polymorphic bands were scored visually for their presence or absence. The resultant data was used to estimate Jaccard dissimilarity coefficients (Jaccard, 1908). The clustering of genotypes was carried out using the unweighted pair group method with arithmetic means (UPGMA) through DARwin version 6 software (Perrier *et al*., 2003). The polymorphism information content (PIC) was manually calculated for each SSR marker to measure the allelic diversity using the formula $PIC = 1- \Sigma P_i^2 - \Sigma\Sigma\ 2\ P_i^2\ P_j^2$.

Results and Discussion

Molecular diversity analysis assists crop breeders in ascertaining the genetic variability prevailing in crop species. The current investigation showed that out of 30 SSR primers employed, 24 showed polymorphism and amplified 78 alleles. Ferchichi *et al*. (2021) reported similar findings and recorded 54 alleles. Among the polymorphic primers, the number of alleles ranged from two to seven. The average number of polymorphic alleles per primer was 3.25. Similar findings were documented by Nourredine *et al*. (2014).

Polymorphic information content (PIC): Polymorphic information content is a quantitative measure used to assess the informativeness of genetic markers by quantifying the diversity of alleles at a specific locus. The PIC value of the SSR primers ranged from 0.25 to 0.99 (Table 3), which indicated that most of the loci might be intermediate or highly diverse (López-Román *et al*., 2024). The primers *viz*., AW172, AW379, AW347, AW776153, BI40 and BG150 showed no polymorphism among the germplasm. The PIC value was highest for primers AW101 (0.99) and AW332 (0.99). It was followed by AW11 (0.95), BE92 (0.95), MTR58 (0.95), AW300 (0.94), AFca1 (0.90), MTIC153 (0.88), AFca11 (0.87), AW127 (0.86), AW289 (0.85), BI86 (0.82), AL46-1 (0.80) and AW261 (0.80). The primer AFca16 (0.25) had the lowest PIC value. Riasat

Table 1. List of lucerne germplasm employed in the study

S. No.	Genotype	S. No.	Genotype	S. No.	Genotype	S. No.	Genotype
1	GETL 1	11.	GETL 11	21.	GETL 21	31.	ANAND 2
2	GETL 2	12.	GETL 12	22.	GETL 22	32.	RL 88
3	GETL 3	13.	GETL 13	23.	GETL 23	33.	LLC 5
4	GETL 4	14.	GETL 14	24.	GETL 24	34.	KRISHNA
5	GETL 5	15.	GETL 15	25.	AWL 6	35.	AL 3
6	GETL 6	16.	GETL 16	26.	LLC 9	36.	AL 4
7	GETL 7	17.	GETL 17	27.	AL 115	37.	CO 1
8	GETL 8	18.	GETL 18	28.	AL 104	38.	CO 2
9	GETL 9	19.	GETL 19	29.	RBB 07-01	39.	CO 3
10	GETL 10	20.	GETL 20	30.	TNLC 12	40.	CO 4

Table 2. Details of SSR primers used in this study

S. No.	Marker Name	Forward sequence 5′ - 3′	Reverse sequence 5′ - 3′
1	AFca1	CGTATCAATATCGGGCAG	TGTTATCAGAGAGAGAAAGCG
2	AFca11	CTTGAGGGAACTATTGTTGAGT	AACGTTTCCCAAAACATACTT
3	AFca16	GGTCGAACCAAGCATGT	TAAAAAACATTACATGACCTCAAA
4	AL22	TGCATTGAAGCAAATTAACGA	ACGGGAAGGAGTTAGGTTCC
5	AL89	CAAAGGCACTTCATCAGCAA	TGAAGATTGAGAGGCGGTCT
6	AL46-1	TTTTTCCCAAGGTGGTATCAA	TTCCAATTCCAACAACAAACA
7	AW11	ATTCGCAGTGAGCTGATCCT	GACATTTGCAGACCACCATT
8	AW365	CACCACTATCTCTTCCCTCACC	TGTTGGTAATGTTCAAGCTCCA
9	AW127	GCAACCAACAACAACAATGG	TAGGATTTGAATAAGGCGAGGA
10	AW300	CCACGTTGTGTCATTGTCTACTC	GTCGAAGAAAGAGGTGGTTGTT
11	AW172	CATCAGGCAGGTTCCTTCTC	CAACAGCTAGGAAGACCCTTG
12	AW101	GCAACCAACAACAACAATGG	TTTCTGGTGAAAACCCACAA
13	AW213	ACCCTTGTGGGTTCTTCTTCTT	CATGTACGGGGATTGTTGTTTT
14	AW379	GTCTCTCTCTATTCTCTTCCCTTTTC	TTCTCGAAATCTTCTGCTCTCG
15	AW261	ACCCCGATTTGATTTCTTTCTC	CTTGTGGGAGATTTTGGATTGT
16	AW282	CGACCAAATCACTCTTCTTCAA	AATCCAAGACCATTCACCTGAG
17	AW347	CCATGTCTCTCAATCTTCGTCA	GAACGGGTTTGCGATCTT
18	AW776153	TGGGTGGAGGAAATTACGAC	CCACATATGTTGCTGTTTCCA
19	AW289	ACGAGGCACACACTCTCTCTCT	GGTGCTTTCATTACATCCCATA
20	AW332	TGAGAGATTGATGGGCAATACA	AAGTTGAAGGAAGGTGGTGGT
21	AW312	CTGTGGGGAACAAGAAGAAGAG	CCAGTAACAACAGTCCCATTTG
22	BE92	AGTTCAAACCCTTACCCTTCA	GATGAGGATGATGATGAATTGG
23	BG283	AGCAAACTACGCCTCTTCAGAT	GTTGGTGAATTTGGGATTTAGG
24	BI40	CCAACAAAAATCCCATCACC	GTGTCGATCAAGGAGGCAAT
25	BI86	GAAAAGAAATCACCCCGAAGAT	CGTCGAAGTCAAAATCAATCTC
26	BG150	GGACGCCTTCTTTGTATTCTGT	GATTGGGATTGAGATTGTGGTT
27	MTIC153	TCACAACTATGCAACAAAAGTGG	TGGGTCGGTGAATTTTCTGT
28	MTIC338	TCCCCTTAAGCTTCACTCTTTTC	CATTGGTGGACGAGGTCTCT
29	MTIC343	TCCGATCTTGCGTCCTAACT	CCATTGCGGTGGCTACTCT
30	MTR58	GAAGTGGAAATGGGAAACC	GAGTGAGTGAGTGTAAGAGTGC

et al. (2021) also reported high polymorphic information content. The high PIC value indicated that the SSR marker was more informative and practically useful in detecting the genetic diversity of crop species. The PIC value provided a clear picture for diversity assessment as it took into account the relative frequencies of each available band (Taran *et al.,* 2005). The obtained results coincided with the findings of earlier workers for high

Table 3. PIC value of different polymorphic SSR primers in lucerne germplasm

S. No.	Marker name	Polymorphic alleles (numbers)	PIC value
1.	AFca1	4	0.90
2.	AFca11	3	0.87
3.	AFca16	3	0.25
4.	AL22	3	0.55
5.	AL89	4	0.48
6.	AL46-1	3	0.80
7.	AW11	2	0.95
8.	AW365	3	0.77
9.	AW127	4	0.86
10.	AW300	2	0.94
11.	AW101	3	0.99
12.	AW213	4	0.58
13.	AW261	5	0.80
14.	AW282	3	0.76
15.	AW289	2	0.85
16.	AW332	7	0.99
17.	AW312	3	0.69
18.	BE92	5	0.95
19.	BG283	3	0.63
20.	BI86	2	0.82
21.	MTIC153	4	0.88
22.	MTIC338	2	0.76
23.	MTIC343	2	0.75
24.	MTR58	2	0.95

polymorphic information content (Falahati-Anbaran *et al*., 2007; Riasat *et al*., 2021). The primers AW101 (0.99) and AW332 (0.99) were considered to be highly informative and could be employed for molecular diversity studies of different lucerne gene pools.

Dissimilarity index: To evaluate the genetic relationship between genotypes, Jaccard's dissimilarity index was used. The dissimilarity index values were obtained for each pairwise comparison among the 40 lucerne germplasm (Table 4). The dissimilarity index varied from 0.27 to 0.89, as reported earlier by Haliloglu *et al*. (2022). Among the 40 germplasm, the highest dissimilarity index was found between CO 1 and GETL 21 (0.89) and CO 2 and AWL 6 (0.89). These were followed by GETL 22 and GETL 2 (0.88), GETL 19 and GETL 2 (0.87), and RBB 07-01 and GETL 2 (0.87). The lowest dissimilarity index was recorded between RBB 07-01 and LLC 9 (0.27). These results were in line with the previous report by Ertus and Sensoy (2021). The germplasm with a high dissimilarity index revealed the presence of high genetic diversity that could be harnessed to improve crop performance. The germplasm with a low dissimilarity index is limited in its capacity to contribute to genetic diversity because it indicates that these genotypes possess less genetic diversity compared to the germplasm with a higher dissimilarity index. Indeed, less genetic divergence among the existing genetic resources of lucerne is a major limiting factor for its improvement. The genotypes identified in the present study with higher genetic dissimilarity have opened up avenues for enhancing the genetic gain in improved lucerne genotypes developed through poly cross derivatives or synthetics.

Genetic distance between accessions: The present study utilized Jaccard's dissimilarity coefficient and grouped genotypes following the unweighted pair-group method of arithmetic average (UPGMA). The clustering of accessions using UPGMA analysis was based on genetic distance expressed more detailed relationships among the accessions (Li *et al*., 2022). The lucerne germplasm was grouped into two major clusters based on UPGMA (Fig 1). Among the two clusters, cluster II, comprised of a maximum of 26 germplasm, was further subdivided into two sub-clusters. Cluster I, with 14 germplasm, was further subdivided into two sub-clusters. This was in agreement with the earlier findings in lucerne (Riasat *et al*., 2021) and feral alfalfa (Ferchichi *et al*., 2021; Mabry *et al*., 2023). According to the results, it is advisable to consider germplasm with a higher dissimilarity index when aiming to develop plants with increased biomass production. Knowledge of the extent of genetic diversity present in the available germplasm will pave the way for the selection of parents and the selection of breeding

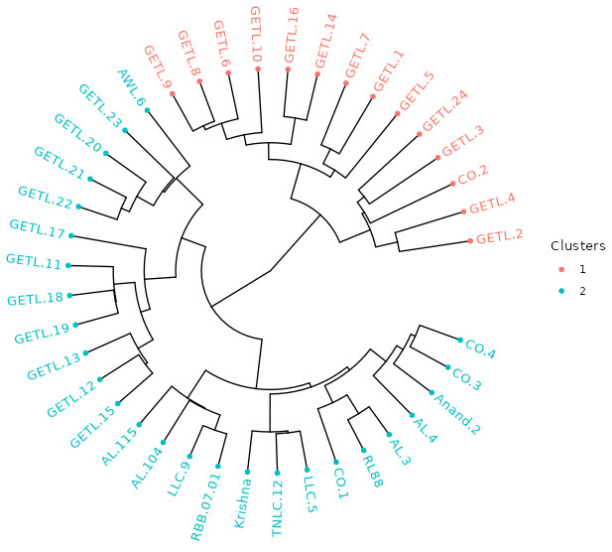

Fig 1. Cluster tree diagram showing molecular diversity in lucerne germplasm

Table 4. Dissimilarity index for molecular diversity in lucerne germplasm

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0.00																			
2	0.65	0.00																		
3	0.74	0.73	0.00																	
4	0.55	0.58	0.62	0.00																
5	0.61	0.81	0.69	0.64	0.00															
6	0.52	0.74	0.66	0.48	0.56	0.00														
7	0.54	0.70	0.78	0.63	0.67	0.46	0.00													
8	0.46	0.82	0.70	0.65	0.54	0.34	0.59	0.00												
9	0.50	0.74	0.67	0.61	0.54	0.39	0.55	0.34	0.00											
10	0.62	0.68	0.73	0.61	0.77	0.44	0.52	0.48	0.48	0.00										
11	0.67	0.84	0.81	0.79	0.79	0.64	0.81	0.53	0.56	0.66	0.00									
12	0.64	0.85	0.77	0.73	0.74	0.60	0.73	0.51	0.58	0.65	0.37	0.00								
13	0.60	0.86	0.75	0.80	0.71	0.64	0.75	0.52	0.60	0.76	0.37	0.40	0.00							
14	0.62	0.78	0.67	0.65	0.64	0.44	0.68	0.43	0.43	0.58	0.53	0.58	0.49	0.00						
15	0.70	0.82	0.71	0.76	0.74	0.69	0.76	0.58	0.61	0.72	0.40	0.37	0.37	0.55	0.00					
16	0.50	0.76	0.74	0.70	0.71	0.48	0.65	0.43	0.47	0.59	0.50	0.44	0.49	0.38	0.48	0.00				
17	0.68	0.84	0.81	0.75	0.80	0.70	0.74	0.65	0.62	0.67	0.45	0.61	0.53	0.65	0.55	0.69	0.00			
18	0.66	0.84	0.73	0.74	0.65	0.63	0.77	0.49	0.58	0.73	0.36	0.43	0.36	0.52	0.43	0.58	0.53	0.00		
19	0.64	0.87	0.78	0.78	0.72	0.63	0.73	0.54	0.56	0.73	0.36	0.51	0.42	0.54	0.56	0.54	0.54	0.36	0.00	
20	0.64	0.85	0.74	0.73	0.69	0.53	0.66	0.48	0.55	0.65	0.50	0.57	0.57	0.55	0.51	0.51	0.64	0.57	0.45	0.00
21	0.63	0.84	0.75	0.77	0.68	0.63	0.71	0.52	0.58	0.70	0.42	0.57	0.50	0.49	0.55	0.52	0.61	0.50	0.33	0.36
22	0.68	0.88	0.72	0.78	0.65	0.62	0.73	0.52	0.60	0.78	0.45	0.52	0.37	0.49	0.46	0.55	0.63	0.45	0.39	0.40
23	0.73	0.86	0.77	0.80	0.69	0.69	0.69	0.67	0.67	0.79	0.69	0.65	0.64	0.60	0.57	0.61	0.69	0.65	0.58	0.50
24	0.65	0.79	0.67	0.60	0.68	0.60	0.76	0.62	0.62	0.67	0.73	0.72	0.70	0.65	0.70	0.66	0.77	0.76	0.66	0.60
25	0.67	0.85	0.77	0.84	0.79	0.72	0.76	0.61	0.64	0.72	0.56	0.65	0.54	0.58	0.60	0.64	0.61	0.57	0.48	0.48
26	0.66	0.84	0.80	0.80	0.77	0.65	0.74	0.58	0.63	0.73	0.45	0.57	0.50	0.55	0.60	0.58	0.66	0.47	0.33	0.52
27	0.61	0.82	0.71	0.70	0.66	0.57	0.72	0.50	0.53	0.71	0.52	0.58	0.48	0.53	0.47	0.56	0.67	0.56	0.45	0.47
28	0.60	0.78	0.71	0.73	0.69	0.63	0.66	0.58	0.64	0.75	0.56	0.65	0.54	0.61	0.54	0.64	0.67	0.46	0.48	0.48
29	0.68	0.87	0.77	0.79	0.75	0.65	0.67	0.60	0.60	0.76	0.50	0.59	0.52	0.54	0.54	0.60	0.63	0.46	0.38	0.48
30	0.59	0.81	0.67	0.65	0.67	0.58	0.71	0.49	0.52	0.67	0.54	0.61	0.58	0.52	0.58	0.56	0.71	0.53	0.46	0.49

31	0.53	0.79	0.71	0.70	0.72	0.51	0.69	0.42	0.54	0.61	0.49	0.50	0.51	0.50	0.47	0.46	0.67	0.57	0.58	0.47
32	0.63	0.84	0.75	0.74	0.70	0.63	0.71	0.55	0.55	0.73	0.51	0.57	0.47	0.49	0.46	0.55	0.61	0.47	0.44	0.43
33	0.58	0.82	0.74	0.71	0.67	0.57	0.67	0.49	0.59	0.69	0.59	0.63	0.60	0.59	0.63	0.52	0.70	0.63	0.46	0.39
34	0.62	0.85	0.73	0.72	0.64	0.53	0.59	0.51	0.62	0.71	0.65	0.67	0.57	0.51	0.64	0.59	0.71	0.58	0.54	0.51
35	0.64	0.86	0.81	0.78	0.74	0.63	0.75	0.61	0.67	0.71	0.48	0.60	0.57	0.55	0.60	0.59	0.64	0.55	0.47	0.49
36	0.64	0.85	0.72	0.71	0.69	0.57	0.70	0.56	0.62	0.69	0.54	0.55	0.55	0.56	0.58	0.62	0.67	0.44	0.49	0.52
37	0.60	0.89	0.77	0.79	0.65	0.62	0.67	0.54	0.54	0.70	0.53	0.62	0.54	0.54	0.59	0.63	0.55	0.49	0.40	0.48
38	0.71	0.81	0.69	0.61	0.77	0.66	0.65	0.78	0.81	0.80	0.83	0.75	0.79	0.74	0.81	0.68	0.83	0.81	0.82	0.75
39	0.63	0.83	0.70	0.72	0.65	0.56	0.68	0.51	0.63	0.71	0.50	0.57	0.49	0.48	0.51	0.51	0.66	0.52	0.51	0.41
40	0.59	0.81	0.73	0.75	0.64	0.61	0.64	0.52	0.62	0.74	0.60	0.61	0.50	0.56	0.61	0.56	0.75	0.58	0.59	0.55

Genotype	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**22**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
21	0.00																			
22	0.33	0.00																		
23	0.48	0.51	0.00																	
24	0.66	0.63	0.73	0.00																
25	0.49	0.55	0.57	0.78	0.00															
26	0.44	0.47	0.67	0.74	0.55	0.00														
27	0.43	0.43	0.55	0.55	0.56	0.40	0.00													
28	0.46	0.49	0.63	0.67	0.54	0.40	0.41	0.00												
29	0.43	0.38	0.56	0.71	0.54	0.27	0.35	0.35	0.00											
30	0.44	0.44	0.67	0.51	0.64	0.44	0.42	0.45	0.46	0.00										
31	0.54	0.51	0.65	0.63	0.65	0.48	0.39	0.43	0.43	0.40	0.00									
32	0.44	0.33	0.62	0.63	0.55	0.38	0.40	0.40	0.31	0.37	0.38	0.00								
33	0.41	0.41	0.57	0.55	0.61	0.47	0.39	0.49	0.43	0.32	0.37	0.38	0.00							
34	0.49	0.46	0.64	0.65	0.69	0.49	0.50	0.48	0.44	0.32	0.42	0.46	0.33	0.00						
35	0.44	0.42	0.70	0.72	0.65	0.41	0.54	0.49	0.43	0.50	0.44	0.28	0.40	0.42	0.00					
36	0.56	0.50	0.63	0.70	0.69	0.44	0.56	0.42	0.43	0.50	0.40	0.44	0.53	0.49	0.44	0.00				
37	0.49	0.49	0.64	0.73	0.59	0.52	0.53	0.57	0.45	0.55	0.53	0.40	0.55	0.48	0.43	0.46	0.00			
38	0.81	0.75	0.85	0.75	0.89	0.81	0.79	0.78	0.78	0.70	0.76	0.73	0.73	0.67	0.74	0.76	0.73	0.00		
39	0.52	0.40	0.59	0.69	0.60	0.52	0.50	0.54	0.45	0.55	0.40	0.43	0.43	0.48	0.46	0.39	0.45	0.68	0.00	
40	0.56	0.44	0.67	0.71	0.67	0.53	0.54	0.49	0.46	0.47	0.36	0.44	0.43	0.45	0.53	0.47	0.52	0.67	0.35	0.00

methodology for the lucerne crop improvement program. The diverse germplasm accessions identified in the present study could be utilized in the development of high green fodder-yielding polycross varieties by intermating among the selected diverse genotypes in a polycross mating design followed by selection.

Conclusion

The current study provides evidence that the SSR markers were more informative and a suitable approach to studying the molecular polymorphism and phylogenetic relationships in lucerne germplasm. The study also characterized 40 lucerne genotypes using 30 SSR primers to understand their genetic nature. The microsatellites considered reliable and repeatable markers for this purpose, unraveled the high level of genetic polymorphism among the studied genotypes. From the study, it was concluded that the diverse lucerne germplasm lines, such as CO 1, CO 2, GETL 21 and AWL 6 could be used in future breeding programs. Besides this, the diverse genotypes identified in the present study could also be evaluated for novel traits such as high moisture tolerance, high-temperature tolerance, high crude protein content, etc., in order to utilize them as a source of genetic material for future crop improvement.

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