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Genetic Diversity Analysis in Grasspea (*Lathyrus sativus* L.) Using SSR Molecular Markers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study of genetic diversity among *Lathyrus sativus* L. may give fundamental insights into extent of genetic variation and provide options to meet the climate change challenge. 20 SSR loci were employed to assess the genetic diversity of 32 grasspea genotypes. Eleven markers proved to be polymorphic across examined genotypes in aggregation to allow detection of a total of 21 alleles with an average of 1.91 alleles per locus. The polymorphism information content (PIC) calculated as a relative measure of informativeness for each of the SSR markers, ranged from 0.11 to 0.34. The dendrogram from the neighbour-joining UPGMA cluster analysis of the pair-wise simple matching dissimilarity coefficients matrix grouped genotypes into three main clusters. Therefore the characters contributing maximum to genetic diversity may be given importance during grasspea hybridization program and these genotypes may be utilized in further grasspea breeding program aimed to enhance grain yield.

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Keywords: Genetic diversity; Lathyrus sativus; polymorphism information content; SSR marker.

1. INTRODUCTION

Grasspea (*Lathyrus sativus* L.) 2n=14, belongs to the genus Lathyrus of the family Fabaceae. It is the only species widely cultivated as a food crop in the genus *Lathyrus*. Grasspea has great economic potential as a food grain and forage legume on account of its ability to survive under extreme abiotic stress conditions such as drought, flood and salinity. Grasspea is a highly nutritive crop thus seeds are used as complimentary or sole source of calories; 351 cal/100 g of seed and endowed with 58% carbohydrates, 28-32% protein, 0.6% fat and 3 g minerals per 100 g of seed [1]. Amino acid profile of khesari grains indicates that, its protein contains tryptophan (0.4 - 0.5%). It also contain calcium (110 mg), Iron (5.6 mg), phosphorus (500 mg) vitamin B_1 (0.45 mg), B_2 (0.41 mg) and niacin (1.8 mg) per 100 g of seed (Sharma and Padmanaban, 1969). Lathyrus is safe for human consumption if variety is having ODAP content lower than 0.2% [2].

Genetic diversity can be estimated by using molecular markers. Molecular markers are useful and complement to morphological characterization of accession because they are highly efficient, independent of plant tissue or environmental effects and allow cultivar identification very early in plant development [3]. A few earlier works have highlighted the importance of Genetic Diversity analysis in grasspea. PCR based molecular markers utilized so far in *L. sativus* include Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP) and Amplified Fragment Length Polymorphism (AFLP) [4]. Simple sequence repeat (SSR) are common and informative molecular markers used for genetic diversity studies and up to now, there was little study of genetic diversity in *Lathyrus sativus* using SSR markers. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics. They make up the deficiency of the field morphological identification and biochemical marker identification methods with its more accurate, more convenient and faster approach. In this study, we used 20 SSR primers to study the genetic diversity among 32 accessions of *L. sativus.*

2. MATERIALS AND METHOD

A total of 32 grasspea genotypes (Table 1) procured from different districts of Bihar and adjoining districts of Jharkhand were used for conducting the present experiment. These genotypes along with three checks (Ratan and Prateek) were sown at Pulse Research Farm, Bhitti, Bihar Agricultural University, Sabour (Bhagalpur), Bihar, India.

Table 1. Details of grass root genotype

SL. No.	Genotypes	SL. No.	Genotypes
1	Nalanda L-1	17	Lakhisarai L-8
2	Nalanda L-2	18	Lakhisarai L-9
3	Chapra L-1	19	Lakhisarai L-10
4	Lakhisarai L-1	20	Lakhisarai L-11
5	Lakhisarai L-2	21	Lakhisarai L-12
6	Lakhisarai L-3	22	Lakhisarai L-13
7	Shahebjang Local	23	Lakhisarai L-14
8	Shahebjang Local Pahadi	24	Lakhisarai L-15
9	Shahebjang Local Diyara	25	Lakhisarai L-16
10	Lakhisarai L-4	26	Lakhisarai L-17
11	Lakhisarai L-5	27	Lakhisarai L-18
12	Lakhisarai L-6	28	Lakhisarai L-19
13	Lakhisarai L-7	29	Lakhisarai L-20
14	Samastipur Local	30	Lakhisarai L-21
15	Sabour Hatia L-1	31	Ratan
16	Sabour Local Diyara	32	Prateek

2.1 DNA Extraction

Genomic DNA was extracted from 4 week old seedlings of 32 genotypes using CTAB method [5]. Quantification of DNA was done to know the quantity of DNA present in 1 µl of extracted crude sample.After quantification, the DNA was diluted with DNAase, RNAase and protease free water such that the final concentration of DNA would beoame 40 ηg/µl for PCR amplification.

2.2 Primers

20 SSR primers related to different traits were used in the study. List of primers along with size of primer (bp) and annealing temperature is presented in Table 2.

2.3 PCR Amplification Programme through SSR Marker

Template DNA was initially denatured at 94ºC for 5 minutes followed by 35 cycles (30 sec denaturation at 94ºC, 59 sec annealing at 55ºC, 45 sec of primer extension at 72ºC) of PCR amplification, and final extension of 72ºC for 5 min followed by hold at 4ºC. On completion of reaction 2 µl 6X gel loading buffer was added. The amplified fragments were separated through 2.0% Agarose gel containing ethidium bromide and run for 2 hours in 1X TAE buffer and image was taken under gel documentation system.

2.4 Allelic Diversity Analysis

The frequency of SSR polymorphism calculated on the basis of presence or absence of common bands where presence was denoted as 1 and absence was denoted as 0. Polymorphism Information Content (PIC) values were calculated by using the given formula.

$$PIC = \frac{1}{n}\sum 2F(1-F)$$

Where,

F = Proportion of a particular allele among the genotypes,

n = No. of alleles generated.

3. RESULTS

The genotypes included the study were subjected to allelic diversity analysis using 20 SSR markers. For understanding genetic diversity, the average PIC values were computed over all loci [6]. For the relationships among grasspea genotypes analysed, allelic data were used to develop dendrograms by using neighbour joining unweighted pair-group method with arithmetic averages (UPGMA) clustering of simple matching dissimilarity indices with the help of the DARwin-6.0 program [7]. The dendrogram was showed in Fig. 1.

3.1 Microsatellite Allelic Diversity of All the Twenty SSR Markers

Eleven markers proved to be polymorphic across the 32 grasspea genotypes examined; as such, in aggregate they allow detection of a total of 21 alleles. Among the eleven SSR markers, the number of alleles detected per locus ranged from one for the markers (AY839517 and DY396423) to 3 for the single marker (DY396387) with an average of 1.91 alleles per locus. The PIC, calculated as a relative measure of informativeness for each of the SSR markers, ranged from 0.11 for marker DY396412 to 0.34 for marker AY370647, AY839517and DY396369, with an average value of 0.24. Almost all the SSR markers were found to be polymorphic, though to less extent and informative. These results demonstrated polymorphic SSR markers which we used were good enough for further genetic diversity analysis.

3.2 Genetic Diversity and Relationships among Grasspea Genotypes

The average genetic dissimilarity index among the thirty-two grasspea genotypes computed as the weighted mean for all of the pair-wise comparisons of the simple matching dissimilarity indices based on all of the SSR markers ranged from 0.0 between genotypes LKH L-3 and Samastipur local, Samastipur local and LKH L-15, LKH L-8 and LKH L-10, LKH L-20 and Ratan to 0.71 between LKH L-12 and LKH L-16. High average genetic distance values were noted in between LKH L-9 and LKH L-30, LKH L-12 and LKH L-21genotype(0.67), LKH L-4 and LKH L-21 (0.65), Sabour local diyara and LKH L-16, LKH L-7 and LKH L-19 (0.64) indicating that these genotypes shared the lowest number of alleles with the rest of the test grasspea genotypes. The dendrogram from the neighbour-joining UPGMA (DARwin Program 6.0) cluster analysis of the pair-wise simple matching dissimilarity

coefficients matrix taking into account all of the SSR loci profiles resulting from the twenty SSR markers resolved all thirty-two grasspea genotypes examined into three clusters.

Assessment of genetic variation and understanding genetic relationships in germplasm collections are indispensable for effective management and use of genetic resources in crop breeding, as well as providing insurance against unforeseen threats (e.g. climate change) to agricultural production. In our study, most of the SSR markers used showed polymorphism and detected a total of 21 alleles with an average of 1.91 alleles per locus and average PIC value of 0.24.

Table 2. Annealing temperature of SSR markers used under study

SN	Primer detail	Species	Annealing temp. (ºC)	Nature
1.	AY370647	*L. sativus*	55.7	polymorphic
2.	AY839517	*L. sativus*	57	polymorphic
3.	DY396279	*L. sativus*		Not amplify
4.	DY396317	*L. sativus*	48	polymorphic
5.	DY396353	*L. sativus*	50	polymorphic
6.	DY396360	*L. sativus*	48	polymorphic
7.	DY396369	*L. sativus*	50	polymorphic
8.	DY396380	*L. sativus*	48	Monomorphic
9.	DY396387	*L. sativus*	50	polymorphic
10.	DY396412	*L. sativus*	51	polymorphic
11.	DY396423	*L. sativus*	48.5	polymorphic
12.	DQ201798	*L. japonicus*	55.7	Monomorphic
13.	DQ201799	*L. japonicus*		Not amplify
14.	DQ201800	*L. japonicus*		Not amplify
15.	DQ201801	*L. japonicus*	49.5	Monomorphic
16.	DQ201802	*L. japonicus*	55.7	Monomorphic
17.	DQ201803	*L. japonicus*		Not amplify
18.	DQ208039	*L. japonicus*	49	Monomorphic
19.	DQ208040	*L. japonicus*	57	polymorphic
20.	DQ208041	*L. japonicus*	46.5	polymorphic

Table 3. Details of SSR markers used under study

Marker name	Major allele frequency	No. of allele	Gene diversity	PIC value
AY370647	0.69	2.00	0.43	0.34
AY839517	0.69	1.00	0.43	0.34
DY396317	0.88	2.00	0.22	0.19
DY396353	0.84	2.00	0.26	0.23
DY396360	0.97	2.00	0.06	0.06
DY396369	0.68	2.00	0.44	0.34
DY396387	0.72	2.00	0.40	0.32
DY396412	0.94	3.00	0.12	0.11
DY396423	0.88	1.00	0.22	0.19
DQ208040	0.77	2.00	0.36	0.29
DQ208041	0.84	2.00	0.25	0.21
Mean	**0.81**	**1.91**	**0.29**	**0.24**

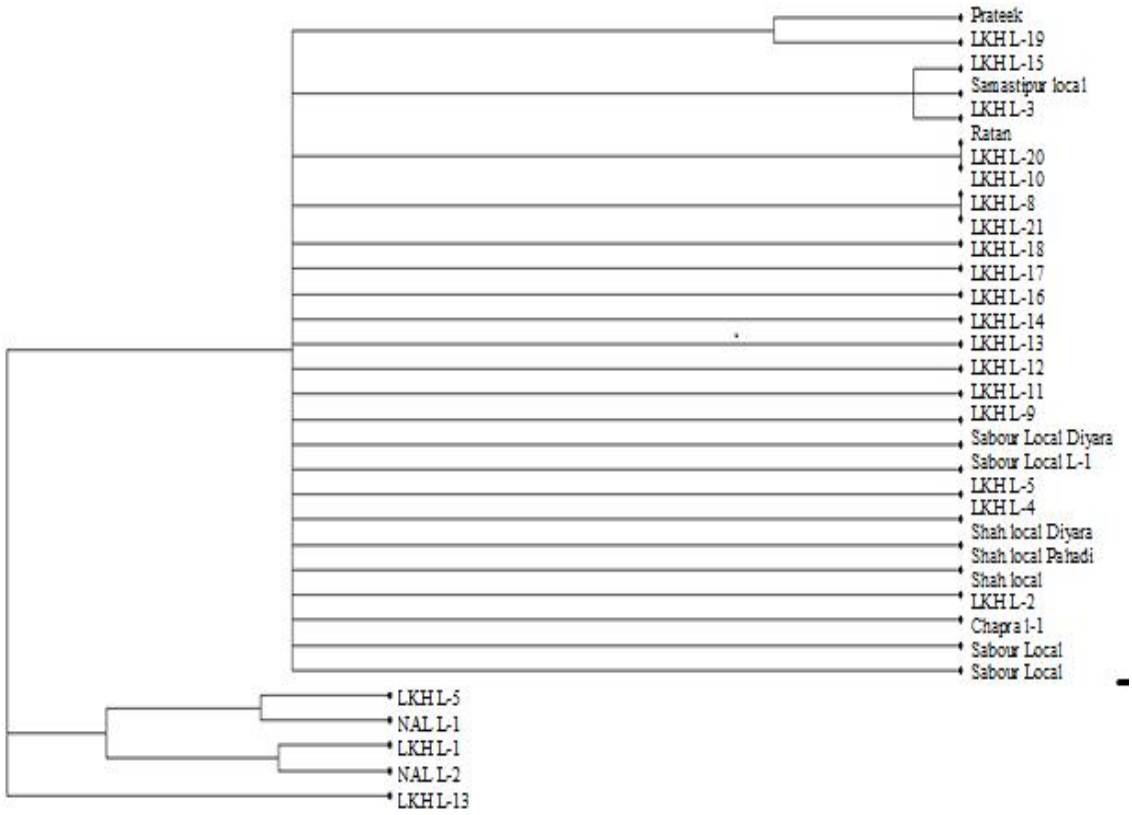

Fig. 1. Clustering of Grasspea genotypes by UPGMA using molecular data

4. DISCUSSION

Grasspea, as an underutilized legume is very popular among the resource poor farmers in marginal lands due to its stress tolerance nature. Assessment of genetic variation and understanding genetic relationships in germplasm collections are indispensable for effective management and use of genetic resources in crop breeding, as well as providing insurance against climate change challenges in agricultural production. Knowledge of genetic diversity will assist germplasm utilization in *Lathyrus* breeding, and more climate-resilient varieties would be bred in the near future. In our study, most of the SSR markers used showed polymorphism and detected a total of 21 alleles with an average of 1.19 alleles per locus and average PIC value of 0.24.

5. SUMMARY AND CONCLUSION

Molecular genetic diversity studies using twenty SSR markers (Table 3) was performed of all the twenty SSR markers and eleven markers proved to be polymorphic across 32 grasspea cultivars examined; as such, in aggregate they allow detection of a total of 21 alleles with an average of 1.19 alleles per locus. The PIC, calculated as a relative measure of informativeness for each of the SSR markers, ranged from 0.11 to 0.34 with an average value of 0.24. They revealed a genetic diversity of 29%. This underlies the utility of these marker loci to generate specific genetic fingerprints for each genotype, useful for variety identification and protection, genetic purity analysis, and other studies. In other words, these results suggest the reliability of SSR markers for DNA fingerprinting and genetic diversity analysis of grasspea cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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