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Short Communication

Genetic relatedness among indigenous and exotic cultivars of lentil based on RAPD, SSRs and morphological traits

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Lentil (*Lens culinaris*) is the second most important cool season legume crop of India after chickpea. In India mostly *pilosae* types of lentil are grown and released cultivars have narrow genetic base [1]. The development of various PCR based molecular markers has enabled to characterize genotypes and measure their genetic relationship [2-4]. RAPD (randomly amplified polymorphic DNA) markers have been used in identification of cultivars and for assessing genetic diversity in several legume crops like mungbean [5], pea [6], chickpea [7] etc. Microsatellites marker are co-dominant markers, usually single locus, which is often multi-allelic because of high mutation rate [8]. Inder *et al.* [9] constructed a DNA fingerprinting database for 10 Lens genotypes using STMS markers. In addition to molecular markers, information from morphological traits can also be integrated to provide comprehensive knowledge of relatedness as studied by Dikshit *et al.* [10] in Vigna *spp.* The present study was undertaken to estimate genetic relationship among ten lentil genotypes (Table 1) of diverse nature in respect to seed size, origin and wilt reaction using RAPD and SSR markers along with morphological traits.

For morphological characterization, lentil genotypes were grown during *Rabi* season of years 2007-08 and 2008-09 in randomized block design with three replications. All ten genotypes were characterized using 14 morphological descriptors (Table 2) as per national guidelines of DUS test. The qualitative data were recorded in binary data matrix. For molecular characterization, genomic DNA was isolated from 15 day old seedlings using cetyltrimethyl ammonium bromide (CTAB) method [11]. Thirty SSR primers reported by Hamwieh *et al.* [12] and 50 RAPD primers (Table 3) were used to screen ten lentil genotypes. PCR amplifications were carried out in a total volume of 20 µL containing 25-30 ng of genomic DNA. A 100 bp ladder was used for approximate sizing of the products while separation during electrophoresis. DNA samples were electrophoresed for 1 h at a constant voltage of 100 V in 1X TBE buffer and photographed with a CCD camera attached to a Gel Documentation System (GBox-EF, Syngene). Fragments amplified by primer sets were scored for presence or absence which coded as 1 or 0,

Table 1. Materials used in the present study

Genotypes	Pedigree
Precoz	Argentine variety
JL-3	Selection from local germplasm
Sehore-74-3	Local selection from Sehore
DPL-62	JLS 1 x LG171
PL- 406	Selection From P 495
L -4076	PL 234 x PL 639
DPL- 58	PL639 x Precoz
L -4147	(L 3875 x P 4) x PKL 1
PL- 05	L 4606 x LG 171
PL- 02	-

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Table 2. Morphological descriptors used in the present study

Sl.No.	Morphological character	Description
1.	Stem pigmentation	1. Absent 2. Present
2.	Leaf size	1. Small 2. Medium 3. Large
3.	Leaf color	1. Yellowish green 2. Ash green 3. Green 4. Dark green
4.	Leaf pubescence	1. Absent 2. Present
5.	Time of flowering	1. Extra early 2. Early 3. Medium 4. Medium late 5. Late
6.	Flower colour	1. White 2. White with blue veins 3. Blue 4. Violet 5. Pink
7.	Tendril length	1. Rudimentary 2. Prominent
8.	Pod pigmentation	1. Absent 2. Present
9.	Cotyledon colour	1. Yellow 2. Orange 3. Olive green
10.	Seed size	1. Small <2g 2. Medium2-2.5g 3. Large2.6-3.0g 4. Very large>3.0g
11.	Plant growth habit	1.Erect 2. Semi erect 3. Horizontal
12.	Seed coat colour	1. Green, 2. Grey, 3. Pink, 4. Brown, 5. Black
13.	Seed testa mottling	1. Absent, 2. Present
14.	Plant height	1. Low (<40cm), 2. Medium(40-60cm) and 3. High(>60 cm)

respectively. Both SSR and RAPD data sets were combined to obtain the more informative and precise result depicting genetic relationship.

To analyze morphological and molecular data, binary matrices were then transformed to genetic similarity (GS) matrices using Jaccard's coefficient [13]. To measure the association between similarity matrices of morphological and molecular data, Mantel's test was performed using MXCOMP option in NTSYS-PC2.02. A dendrogram based on similarity coefficients was prepared by using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) for both kinds of data. Cluster analysis was performed using software package NTSYS-PC2.02 [14]. Strength of the clusters was supported and evaluated by bootstrap analysis using Win Boot software [15]. One thousand samples were generated by re-sampling with replacement of characters within the combined 1/0 data matrix. Principal coordinate analysis (PCoA) based on Jaccard distances and UPGMA method using NTSYS-pc 2.02 was used to estimate the actual number of groups that may be obtained by cluster analysis for molecular data.

In the present study, fifteen out of 50 SSR primers and thirty-five RAPD primers yielded polymorphic amplicons (Table 3). SSR primers amplified 54 scorable bands of which 47 were polymorphic. Percentage of polymorphism ranged from 25 (LC11) to 100%. The number of bands amplified by individual primer ranged from 2 to 9. Polymorphism information content (PIC) ranged from 0.10 to 0.88. Thirty-five RAPD markers produced 186 bands, out of these 147 were polymorphic. Polymorphism information content (PIC) ranged from 0.10 to 0.90. Percentage of polymorphism ranged from 0 to 100%. The number of bands amplified by individual primer ranged from 2 (OPV17) to 9 (OPB15). The PIC value ranged from 0.50 (OPV19) to 0.94 (OPB04).Unique alleles serve the purpose for identification and distinguishing the cultivar with specific identity with regard to particular band. The marker LC2 produced specific amplicon in Precoz and PL02 (Fig. 1). In this case, genotype, Sehore 74-3 has got the

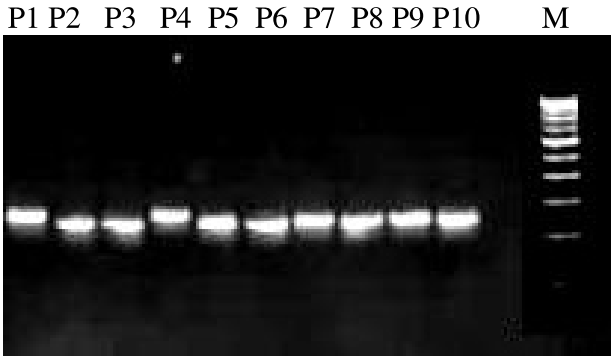

Fig. 1. SSR profile of lentil genotypes by LC2 primer (P1: Precoz, P2: Sehore 74-3, P3: L 4076, P4: PL 02, P5: JL3, P6: PL 05, P7: DPL 62, P8: DPL 58, P9: L4147, P10: PL406)

Table 3. Polymorphic information content (PIC), number of scorable bands and polymorphic bands produced by 15 SSR and 35 RAPD markers in 10 lentil genotypes

Primers	Tm c	NSB	NPB	%PB	PIC	Primers	Tm°c	NSB	NPB	%PB	PIC
LC2	56	2	2	100	0.32	OPB15	38	9	9	100	0.90
LC3	56	3	3	100	0.34	OPB17	38	5	4	80	0.60
LC6	56	7	6	86	0.42	OPM04	38	9	9	100	0.88
LC9	51	3	3	100	0.56	OPM06	38	6	6	100	0.84
LC11	49	4	1	25	0.68	OPM17	38	5	4	80	0.71
LC13	55	2	2	100	0.18	OPN05	38	6	4	66	0.81
LC17	54	3	3	100	0.62	OPN13	38	6	6	100	0.81
LC19	51	3	2	66	0.12	OPN19	38	4	4	100	0.73
LC23	50	9	9	100	0.72	OPT01	38	4	1	25	0.73
LC24	52	2	1	50	0.50	OPU08	38	7	4	57	0.83
LC25	56	2	1	50	0.10	OPU11	38	3	1	33	0.72
LC26	50	4	4	100	0.25	OPU12	38	4	4	100	0.81
LC28	53	5	5	100	0.88	OPU13	38	4	2	50	0.79
LC29	51	3	3	100	0.66	OPU15	38	5	3	60	0.59
LC30	54	2	2	100	0.18	OPU19	38	4	3	75	0.74
OPA01	38	5	4	80	0.77	OPV01	38	4	4	100	0.55
OPA09	38	6	6	100	0.82	OPV03	38	3	3	100	0.79
OPB02	38	3	2	66	0.64	OPV06	38	5	5	100	0.80
OPB04	38	8	7	87	0.94	OPV08	38	6	4	66	0.65
OPB05	38	7	6	86	0.83	OPV12	38	7	6	85	0.83
OPB08	38	8	8	100	0.85	OPV17	38	2	2	100	0.60
OPB10	38	4	2	50	0.75	OPV18	38	5	3	60	0.72
OPB12	38	6	6	100	0.49	OPV19	38	3	0	0	0.50
OPB13	38	4	3	75	0.75	OPV20	38	7	3	43	0.77
OPB14	38	8	8	100	0.83	OPW09	38	4	1	25	0.81

N.B. Tm- Annealing temperature, NSB- number of scorable bands, NPB- number of polymorphic bands, %P-% polymorphism

unique allele by primers LC3, LC6, LC25, LC23, LC17 and OPN13, the exotic line Precoz amplified the unique band in case of primers LC2, LC17, LC19, LC30, OPM06, OPM17, OPN5 and OPN19. The small seeded variety, L4147 from IARI, New Delhi showed unique allele amplified by LC11, OPA09 and OPB14 and DPL62, the bold seeded variety from IIPR, Kanpur exhibited specific allele amplified by OPV01, LC23 and LC25.

The UPGMA based cluster of molecular data classified the genotypes into one major group consisting of 8 genotypes and two minor clusters with only one genotype each (Precoz and JL3 separately) (Fig. 2). The morphological data based dendrogram depicted relatively different relationship among the studied genotypes (Fig. 3). Precoz was separated in different cluster in this case also. The Mantel's test provided the degree of association between correlation matrices of molecular and morphological data. The low value (r=0.32) of matrix correlation supported the findings of non-conformity of molecular and morphological data. The reason behind this might be low number of morphological markers. Although morphological descriptors used are mainly qualitative in nature, some of them are being quantitative influenced by environment and epistatic interaction. Nevertheless the grouping based on these has some extent of conformity with pedigree information. To get the more clear picture of

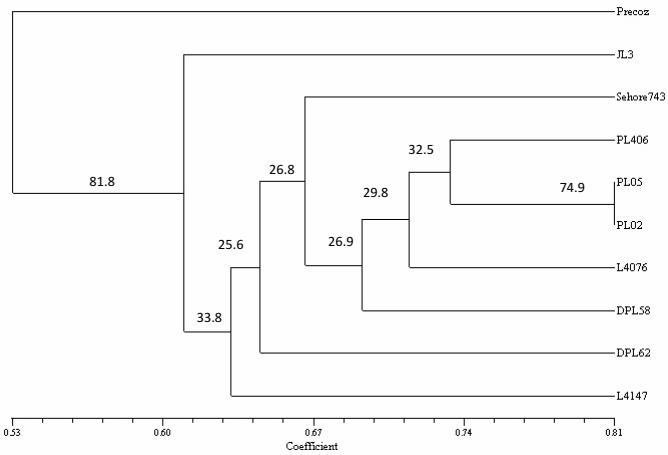

Fig. 2. Dendrogram depicting genetic relationship among ten lentil genotypes using SSR and RAPD markers, numbers at branch point indicate support for cultivars clustered to the right of the number, values are percent of bootstrap samples that exhibited the cluster

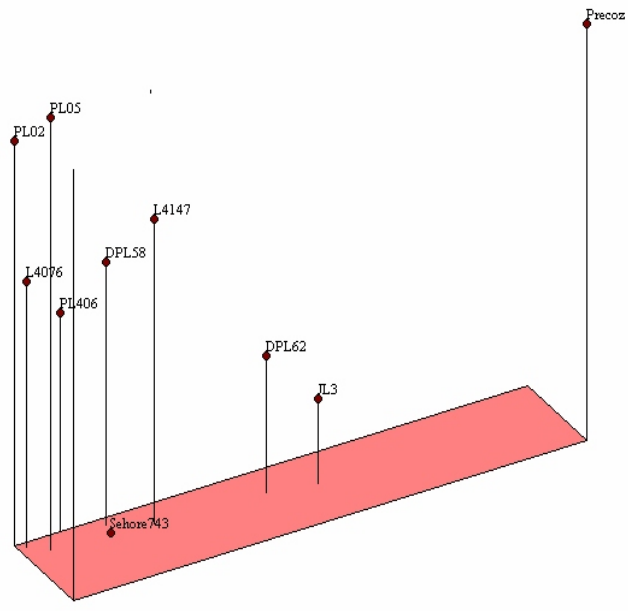

Fig. 3. Three dimensional view of genetic diversity among lentil genotypes based on principle coordinate analysis

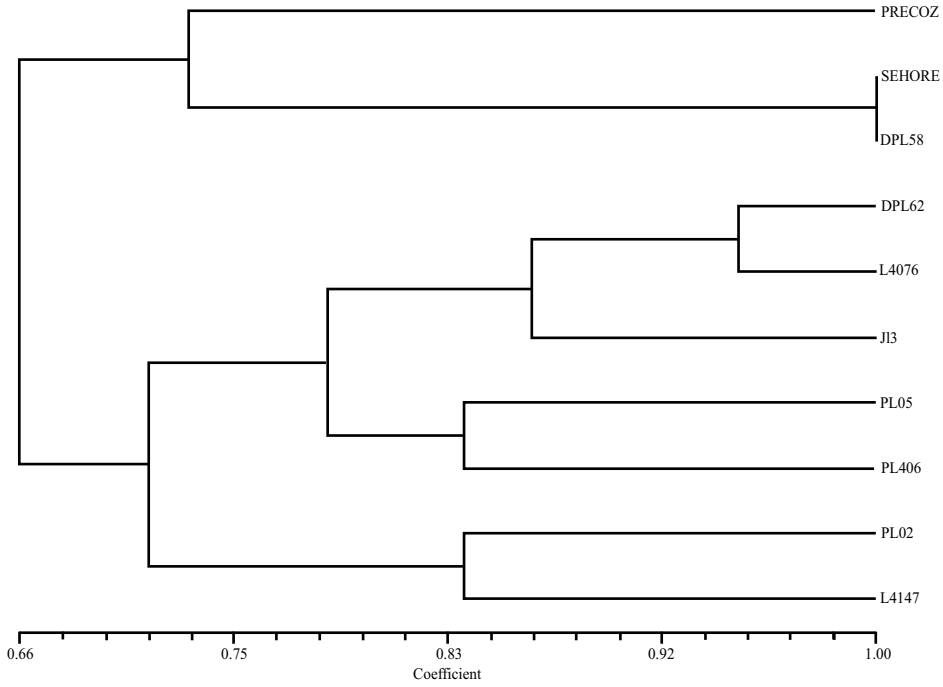

Fig. 4. Cluster analysis of ten lentil genotypes based on morphological attributes

diversity among the genotypes, Principal coordinate analysis was performed by NTSYS 2.02 package with options of ordination and eigen, which resulted into wide separation of two genotypes Precoz and Sehore 74-3 (Fig. 4). PL02 and PL05 are grouped together in both kinds of analyses, with bootstrap value also very high 74.9 indicating strength or high precision of clustering. The clustering pattern of precoz into distinct class was also supported by high bootstrap value (81.8%).

On the basis of present study it could be suggested that locus specific alleles can be useful for DNA fingerprinting and identification of elite lentil genotypes. The information generated will assist lentil breeders to maintain varietal purity during seed production and enable the accurate identification of seed in the commercial production.

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