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Short Communication

Genetic Analysis of Indian Lentil (*Lens culinaris* Medikus) Cultivars and Landraces Using RAPD and STMS Markers

M K Rana*, Ratna Kumari, Sonika Singh and K V Bhat
National Research Center on DNA Fingerprinting, National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110 012, India

Molecular analysis of 29 lentil (*Lens culinaris*) cultivars and landraces of Indian origin was carried out using twenty RAPD and ten cross-species STMS primers. A total of 97 markers (72 RAPD and 25 STMS) were amplified of which 42.3% were polymorphic. Genetic similarity among the cultivars and landraces was 89.7%. The observed results indicated low level of genetic diversity in the studied material. UPGMA cluster analysis for the combined data of RAPD and STMS revealed two broad clusters – Cluster I with three landraces and Cluster II containing all remaining landraces and cultivars except Precoz. Germplasm line Precoz was found to be the most distinct in individual as well as combined analyses. All cultivars and landraces except K-75 and L4076 could be discriminated from one another using combined data for the two techniques. Germplasm lines Precoz, L830 and cultivars L4147 and JL3 were quite distinct and could be potential germplasm resource.

***Key words*:** genetic diversity, *Lens culinaris*, lentil, RAPD, STMS.

Lentil (*Lens culinaris* Medikus*)*, a self pollinating diploid (2n = 2x = 14) (1), is one of the valuable sources of vegetable proteins in the diet of Indian population. It is grown in an area of 1.45 million hectares producing 1.10 million tons with an average yield of 759 Kg ha^{-1} (2). Most of the lentils grown throughout the world are local landraces and only a few released cultivars are available to the farmers (3). Genetic gain in any crop plant depends on the use of genetically diverse parents in the pedigree while breeding. Conventionally, the identification of genetically distinct genotypes is accomplished using morpho-agronomical characteristics. With the availability of large number of DNA marker techniques in many crops, the morphological characteristics are supplemented with the molecular markers. These DNA markers not only have better genomic coverage which is essentially required for diversity analyses, but also provide more reliable genetic estimates. In the present study, diversity was studied in twenty-nine lentil cultivars and landraces using for the first time RAPD and STMS markers.

Twenty-nine lentil genotypes including fourteen released cultivars and thirteen landraces from thirteen different lentil growing states of India were included in the study (Table 1). Additionally, two germplasm lines IC 240921 and IC 241430 were also included in the analysis. Total genomic DNA from three-week-old seedlings was extracted by following the method of Saghai-Maroof *et al* (4) with slight modifications. Subsequently the isolated DNA was purified and fluorometrically quantified. A part of the DNA sample was diluted to yield a working concentration of 10 ng μl^{-1}.

Thirty RAPD primers from kits A and B (M/S Operon Technologies Inc) were initially screened on four samples, and ten primers were finally selected on the basis of reproducibility and scorability of the bands (Table 2). PCR reaction mixture (25μl) consisted of 1x PCR buffer; 1 Unit of *Taq* DNA polymerase; 0.2mM each of dATP, dCTP, dGTP and dTTP; 3mM of $MgCl_2$ (all these reagents from M/S Banglore Genei Pvt Ltd); 0.25 μM primer (M/S Operon Technologies Inc) and 50 ng of genomic DNA. PCR amplification was carried out using PTC-200 thermal cycler (M/S MJ Research) and the cycling conditions were as follows: initial DNA denaturation at 94 °C for 2 min, followed by 39 cycles of denaturation (94 °C), annealing (35 °C) and extension (72 °C) for one, one and two minutes, respectively. There was an additional extension (72 °C) step for four minutes. The RAPD amplification products were electrophoresed on 1.6% agarose gel at 80 volts, stained with ethidium bromide and photographed using Polaroid film 667. STMS anlalysis was carried out as described earlier (5). Ten primer pairs already identified based on cross-species/ genera amplifications were used.

*Corresponding author. E-mail: mkrana@nbpgr.ernet.in

Table1. List of lentil (*Lens culinaris* Medikus) varieties and landraces used in the study

Acc Number	Cultivar name	Pedigree	Breeding centre/ Source	Year of release
IC-393703	L4147 (Cultivar)	[(L3875 x P4) x PKVL1]	IARI	1995
IC-73120	B256 (Cultivar)	Mutant of B77	Berhampore, WB	1984
IC-118932	K-75 (Cultivar)	Local selection from Bundelkhand landrace	CSAUAT, Kanpur	1986
IC-118931	L4076 (Cultivar)	PL234 x PL639	IARI	1993
IC-118930	PL406 (Cultivar)	Selection from P495	GBPUAT, Pantnagar	1980
IC-266109	JL-3(Cultivar)	Selection from local landrace	JNKVV, Sehore	1999
IC-296107	Pant L-5 (Cultivar)	L41426 x LG171	GBPUAT, Pantnagar	2000
IC-118929	PL-639 (Cultivar)	L-9-12 x T-8	GBPUAT, Pantnagar	1982
IC-73122	B-77 (Cultivar)	Local selection from Jorhat	Berhampore, WB	1982
IC-73562	VL Masoor1 (Cultivar)	Selection from hills	VPKAS, Almora	1984
IC-73524	LL 56 (Cultivar)	L-9-12 x L32-1	PAU, Ludhiana	1985
IC-73525	L9-12 (Cultivar)	Selection from local variety	PAU, Ludhiana	1975
IC-296108	IPL-81 (Cultivar)	K-75 x PL639	IIPR, Kanpur	2000
IC-73121	B177 (Cultivar)	Mutant of B77	Berhampore, WB	1984
IC-240921	Precoz (Germplasm line)	Germplasm line	-	-
IC-241430	L-830 (Germplasm line)	Germplasm line	-	-
IC-127578	Landrace	-	Uttaranchal	-
IC-139821	Landrace	-	Uttar Pradesh	-
IC-34679	Landrace	-	West Bengal	-
IC-127568	Landrace	-	Bihar	-
IC-63453	Landrace	-	Andhra Pradesh	-
IC-60968	Landrace	-	Jammu & Kashmir	-
IC-53237	Landrace	-	Rajasthan	-
IC-32367	Landrace	-	Assam	-
IC-27659	Landrace	-	Orissa	-
IC-16458	Landrace	-	Maharashtra	-
IC-201561	Landrace	-	Punjab	-
IC-98394	Landrace	-	Himachal Pradesh	-
IC-98362	Landrace	-	Madhya Pradesh	-

'-' – Not known

Table 2. RAPD primers used and their characteristics for diversity analysis in lentil (*Lens culinaris* Medikus)

Primer	Sequence (5′-3′)	Size range	Total number of bands	Number of polymorphic bands	Per cent polymorphism
OPA-05	AGGGGTCTTG	500-2000	10	1	10.0
OPA-16	AGCCAGCGAA	500-1500	8	6	75.0
OPB-08	GTCCACACGG	500-1000	7	4	57.1
OPA-20	GTTGCGATCC	350-1020	10	4	40.0
OPB-11	GTAGACCCGT	550-1250	7	3	42.8
OPA-17	GACCGCTTGT	250-700	2	0	0.0
OPA-12	TCGGCGATAG	450-950	8	2	25.0
OPB-05	TGCGCCCTTC	400-950	4	1	25.0
OPA-10	GTGATCGCAG	400-900	7	4	57.1
OPA-11	CAATCGCCGT	240-1015	9	3	33.3
		Total	72	28	38.9

Each band was scored for presence (1) or absence (0) for all samples. Jaccard's similarity coefficient (6) was used to calculate similarity between pairs of varieties, which was as follows: $J = n_{xy} / n_t - n_z$, where, n_{xy} is the number of bands common to varieties x and y; n_t is the total number of bands present in all samples; and n_z the number of bands absent in x and y but, found in all other samples. The 29 x 29 similarity matrix was subjected to UPGMA (unweighted pair group method for arithmetic mean) analysis and a dendrogram was construced. These data were analyzed using the program NTSYS-pc Ver. 2.1 software (7). STMS and RAPD data were pooled together for combined analysis. Analysis of molecular variance (AMOVA) (8) on the pooled data was applied on the Euclidean distance matrix between individuals to partition the total genetic variation 'between' and 'among' populations (varieties and landraces as the two major groups) using computer software Arlequin (9).

A total of 72 RAPD bands across all the cultivars and landraces were amplified using ten primers of which 28 (38.9%) were found to be polymorphic (Table 2). A representative profile of the cultivars and landraces using primer OPA-16 is depicted in Fig.1. The observed bands were found in the size range of 240 (OPA-11) to 2000 base pairs (OPA-05) and their number per primer varied from two (OPA-17) to ten (OPA-05 & OPA-20). Per cent polymorphism for the primers varied from 0.0% (OPA-17) to 75% (OPA-16).

Data of these 72 RAPD and 25 STMS markers from an earlier study (5) were pooled and pair-wise genetic similarities among lentil cultivars and landraces were calculated. Average genetic similarity among the cultivars and landraces was found to be 89.7% using 97 markers. These observed genetic similarity estimates along with per cent polymorphism indicate low level of genetic diversity in all the accessions. The fourteen varieties included in the study were found to have very high genetic similarity (91.8%). Although these have been bred and developed at eight different centers and released over a span of 25 years, yet the diversity in them at the analyzed molecular marker loci is low. The high genetic relatedness among these cultivars can be attributed to common parents in their pedigrees. For example, the variety PL 639 is in the pedigrees of varieties L4076 and IPL 81 and the variety L-9-12 in the pedigrees of PL 639 and LL56. Varieties B177 and B256 have been derived from the same source variety B77 through induced mutagenesis. Six of the fourteen varieties have been derived through selection from local landraces. The selected 13 landraces which were representative of 13 different lentil growing states of India possess very low genetic diversity (7.3%). It is evident from the pedigree column of Table 1 that six of the fourteen varieties have been developed through selection from the local landraces which have narrow genetic base.

Low level of genetic variability has been reported in *Lens culinaris* earlier as well. Dixit and Katiyar (10) carried out pedigree analysis of 35 lentil varieties released in India and observed that 22 ancestors were involved in the development of 22 varieties. Four of the ancestors alone contributed 37% of the genetic base. Sharma *et al* (11) using RAPD markers observed similar extent of genetic similarity of 89.7% as we did in this study, in most of the Indian cultivars belonging to *macrosperma* and *microsperma* groups. However, slightly higher diversity (19.7%) has been reported by Abo-elwafa *et al* (12) in the cultivars which might be due to the diverse nature of the genetic material used in their study. Ford *et al* (13) have also reported more genetic diversity (36%) than what has been observed in the Indian cultivars and landraces. Higher levels of genetic diversity in their study again might be due to very diverse genetic material obtained from nine countries. The overall consensus from these studies can

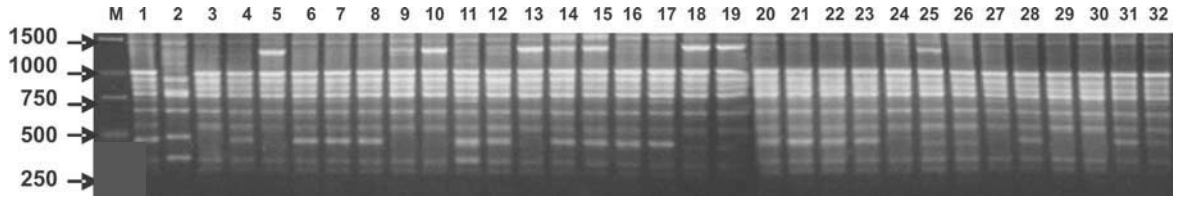

Fig. 1. RAPD profile of lentil cultivars and landraces using primer OPA-16. Lane M = 1 kb molecular weight size standard (M/S MBI Fermentas). Lanes 1 to 32 are :1 = L4076; 2 = Precoz; 3 = L830; 4 = L4147; 5 = B256; 6 = K-75; 7 = L 4076; 8 = PL406; 9 = JL-3; 10 = B256; 11 = Pant L5; 12 = PL639; 13 = B77; 14 = VL Masoor1; 15 = LL56; 16 = L9-12; 17 = B77; 18 = IPL81; 19 = B177; 20 = IC 139819; 21 = IC 127578; 22 = IC 34679; 23 = IC 127568; 24 = IC 63453; 25 = IC 60968; 26 = IC 53237; 27 = IC 32367; 28 = IC 27659; 29 = IC 16458; 30 = IC 201561; 31 = IC 98394; and 32 = IC 98362.

be derived that diversity in *Lens culinaris* is very limited and extensive use of few ancestors might have led the genetic erosion and narrowing down of genetic base. Analysis of molecular variance conducted on the landraces and the varieties as two separate populations also revealed no separation of lentil material into these groupings as significant fraction of the variance was explained by "within population" variance (81%). Narrow genetic base of cultivated lentil genome is indicative of its vulnerability to various insect-pests and diseases and warrants immediate efforts to broaden the genetic base for further genetic gains.

The UPGMA clustering pattern of the cultivars and landraces using RAPD and STMS markers is depicted in Fig. 2. Precoz emerged out to be the most distinct accession whereas the rest of the material could be grouped in two broad clusters – Cluster I and Cluster II. Cluster I contained three accessions only (two varieties and one germplasm line) whereas all the landraces were grouped in Cluster II. Cluster II could be further divided in two sub-clusters i.e. sub-cluster IIa and sub-cluster IIb. Sub-cluster IIa had 16 accessions whereas IIb had nine accessions. Seventy five per cent of the accessions of the sub-cluster IIa were landraces. All the landraces included in this study grouped in sub-cluster IIa except for the landrace from Assam which grouped in sub-cluster IIb. Cultivar B77 and its derivative cultivar B256 obtained through induced mutations clustered together in this sub-cluster. However, another mutant derivative B177 from the same B77 source was placed distantly with the cultivars of sub-cluster IIb. This could be due to the reason that drastic changes would have occurred in the genome owing to mutagenic treatment which might have affected the primer binding sites. Sub-cluster IIb contained 89% of the cultivars of the present study. In this sub-cluster, cultivars K-5 and L4076 although from different sources and selections from the local landraces, could not be differentiated from each other. It is likely that these two might have been selected from the same original source material or these might be duplicates as their identity numbers are also very close to each other, or else there could be some labeling error. Cluster analysis revealed that germplasm lines Precoz, L830 and cultivars L4147 and JL3 were found to be quite distinct. These accessions can be used as germplasm resource for broadening the genetic base of cultivated lentils in India. Besides, these can be used in generating intra-specific mapping populations by utilizing their specific traits like seed boldness and yield particularly of Precoz and L4147 for tagging purposes. For broadening genetic base of lentil genome, these alone, however, would not be sufficient as we additionally need to screen large number of germplasms and identify the putative diverse lentil genetic resources. Pre-breeding employing diverse germplasms of exotic origin in the background of well adapted Indian cultivars, inter-specific hybridizations and introgressions are other viable options to diversify the genetic base of lentil.

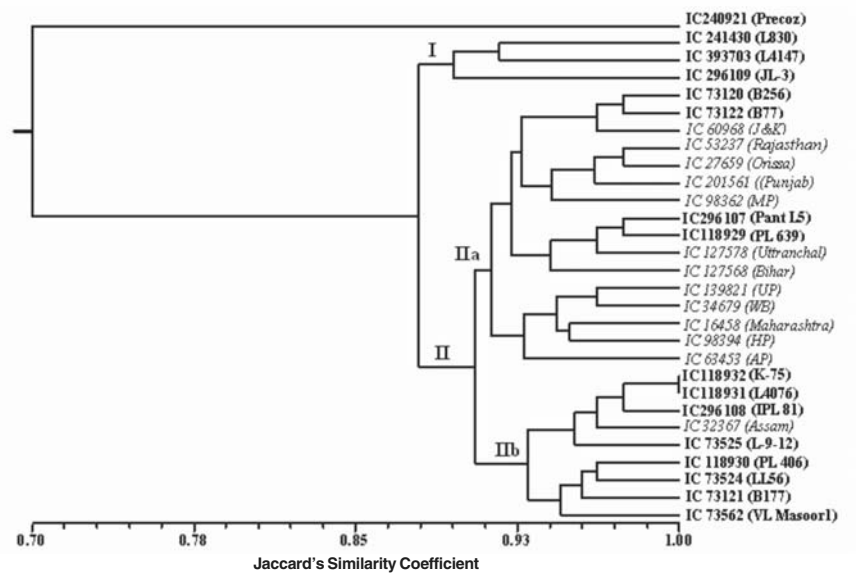

Fig. 2. UPGMA-clustering-based dendrogram generated using combined RAPD and STMS markers for lentil cultivars (bold) and landraces (italics).

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