Indian J. Genet., 68(2): 149-156 (2008)

Validation of chickpea-STMS markers and DNA fingerprinting in lentil (*Lens culinaris* subsp. *culinaris*) cultivars of India

P. K. Agrawal[1] and A. K. Katiyar

Indian Institute of Pulses Research, Kanpur 208 024

(Received: February 2007; Revised: January 2008; Accepted: April 2008)

Abstract

A set of 31 lentil genotypes having indigenous and exotic origin were screened using 42 STMS markers derived from chickpea genome. Out of the 42 STMS primers tested, all of them gave amplified products in lentil. Among them 36 (85.7%) primers were found to be polymorphic among the lentil genotypes. At genetic distance of less than 0.29, all the 31 genotypes could be grouped into five clusters where cluster IV contained 19 genotypes, including the exotic genotype Precoz. Many primers gave genotype-specific amplified products which in combination(s) could be used for DNA fingerprinting of lentil genotypes. A set of 16 STMS markers have been identified which could differentiate all the lentil cultivars of India and, along with the morphological data can be used for identification and confirmation of the purity of the lentil cultivars.

Key words: Lentil, fingerprinting, diversity, SSR, STMS

Introduction

Lentil is the second most important winter pulse crop of India with an acreage of 1.4 million hectors [1]. India has released more than 26 lentil cultivars which includes two varietal types *viz.:* small-seeded (*microsperma*) and large-seeded (*macrosperma*). Diversity analysis among these twenty six genotypes of lentil will help the breeders to choose the recipient and donor parents in their breeding programme. It will also help in bringing together the desirable alleles within one background, besides protecting them for intellectual property right. DNA markers based diversity analysis and fingerprinting is highly reliable over the conventional approaches like morphological and biochemical basis since it is free from the environment and genotype x environment interactions [2, 3]. There are various PCR and non-PCR based molecular markers available for these studies. Among these SSRs (Microsatellites, simple sequence repeats) are the markers of choice. The SSRs are generally spread over the whole genome and the flanking sequences at each of these loci are unique, therefore a sequenced SSR loci can be used as primers in a PCR reaction that can amplify only a single specific region containing the part of genome of interest, which is then referred to as a sequence-tagged microsatellite (STMS, sequence tagged SSR) [4]. STMSs are the DNA markers of choice for diversity, evolutionary and mapping purposes [5]. In the present investigation, STMS markers were used to study the diversity and DNA fingerprinting among the lentil cultivars of India.

The molecular profiles can also be utilized to demonstrate the uniqueness of the plants. Since the agro-economically important plants *per se* forms a major biological resource and considering the increasing demand and importance, it is imperative that the available Indian plant genetic resources especially agro economically plant should not only be conserved but also should be protected in terms of IPR. In many crops like rice, wheat and maize DNA markers has been used extensively for identification and classification of accessions, and predicting quantitative variation within germplasm. In lentil, since most of the studies have used markers like RAPD, ISSR and AFLP [6-11], and information using SSRs/STMSs is limited, the present investigation aims to study if the STMSs available in related genome like chickpea can be used among the lentil cultivars for the diversity analysis and DNA fingerprinting.

Materials and Methods

Genetic materials

Thirty-one genotypes of lentil including two exotic lines (Manson, Precoz) were taken for DNA fingerprinting and diversity analysis (Table 1).

[1]Present address: Vivekanand Parvatiya Krishi Anusandhan Sansthan, Almora 263 601

Table 1. Genotypes of lentil used for the DNA fingerprinting and diversity analysis

Sl. No.	Genotype	Parentage
1	DPL 15 (Priya)	PL -406 x L4076
2	K 75 (Malika)	Local selection from Bundelkhand
3	LH 84-8 (Sapna)	L9-12x JLS-2
4	LH 82-6	Pusa 2 x No 4
5	DPL 62 (Sheri)	JLS-lxLG-171
6	T-36	Selection at Kanpur
7	LL147	PI 284-67 xNP21
8	L-4147 (Pusa Vaibhav)	(L 3875 x P4) x PKVL 1
9	L-4076	PL 234-67 x PL 639
10	L-9-12	Selection from local from Punjab
11	PL-406	Selection from P-495
12	PL-639	L9-12xT-8
13	PL-4	UPL-175x(PL184xP288)
14	PL-5	L4163xLG171
15	PL-234	Selection from P-230
16	L-4603	Precoz xL3 991
17	IPL-525	PL 639 x Precoz
18	Sehore 74-3	Local Selection from Sehore
19	PL-77-2	Mutant of B -25
20	Mason	Exotic collection from USA
21	DPL -58	PL 639 x Precoz
22	Precoz	Exotic collection from Argentina
23	B -77 (Asha)	Selection from Jorhat local
24	IPL-81 (Noori)	K -75 x PL 639
25	Ranjan	Mutant of B -77
26	WBL-58 (Subrata)	JLS 2 x T -36
27	VL-1	Selection from local germplasm of Almora
28	JL-1	Selection from local germplasm from M.P.
29	VL-103	Selection from local germplasm No. VHC 2771-1 collected from district Bageswar
30	VL-4	Local selection from Pithoragarh
31	NDL-1	Precoz x L 9- 12

DNA extraction

DNA was isolated using a modified method refined in our laboratory as follows, i) Collect 20-40 mg of young leaves, ii) These young leaves are added to 400 µl of extraction buffer (200mM Tris HCL ph 8.0, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) in a mortar and crushed so that the leaves are macerated and solution is turned deep green, iii) To this, another 400 µl of extraction buffer is added, mixed well with the pestle. 400 µl of the clear solution is taken carefully and transferred to an eppendorf tube. 30ug of RNAase was added to this, mixed well and incubated for 1 hour, iv) To this equal amount (400 µl) of solution of (Chloroform: Isoamyl alcoho::24:l) is added and gently mixed well, v) Centrifuge it at 13000 rpm for 5 minutes, vi) The supernatant (400 µl) is transferred to a new eppendorf tube and the DNA is pelleted by adding equal amount of absolute alcohol after centrifuging for 3 minutes at 13,000 rpm, vii) The pellet is washed with 70% alcohol, dried and solubilised with 100-200 ml of TE. The available DNA was used for PCR study.

DNA markers used in the study

The diversity analysis among the lentil genotypes was done using 42 STMS primers (Table 2).

General reaction conditions for STMSs

PCR amplification was performed in 200 µl thin-wall tubes using an MJ Research PTC-200 thermo cycler. Reaction solutions (25 µl) contained the following: 2.5 µl (2.5 x 25ng) of genomic DNA template, 1.0 unit of Taq DNA polymerase (Banglore Genei), 250 µM each of dATP, dCTP, dGTP and dTTP (Banglore Genei), 1.0 µM each of forward and reverse primer. The PCR buffer consisted of 10 mM Tris-HCl at pH 8.3, 50 mM KC1, 1.5 mM $MgCl_2$ (Banglore Genei). Cycle conditions were as follows: initial denaturation step at 94°C for 3 min followed by 40 cycles of 94°C for 1 min, 54°C for 1 min. and 72°C for 2 min with a final extension at 72°C for 7 min. The annealing temperatures for individual primers were standardized depending upon their *Tm* value.

Electrophoresis and statistical analysis

The amplified products were resolved by horizontal electrophoresis with 2% agarose + 1% metaphor gel. The gels were stained with Ethidium Bromide and were visualized under the UV light. The DNA fragment amplified by a primer for a genotype was scored '1' for the presence of the band and '0' for the absence of the band to create a binary matrix of the different genotypes.

Table 2. List of the STMSs used for the diversity analysis and fingerprinting

S.No.	Sequence (5' to 3')	Tm value (°C)	% gel for electrophortesis	P/NP	Band size (bp)
1	TGA AAT ATG GAA TGA TTA CTG AGT GAC	60.05	2 % agarose	P	70,95
	TAT TGA AAT AGO TCA GGC TTA TAA AAA	57.07			
2	AAA ATA ATC TCC ACT TCA CAA ATT TTC	57.01	2% agarose	P	80, 100
	ATA AGT GCG TTA TTA GTT TGG TCT TGT	60.05			
3	ATA TAT CGT AAC TCA TTA ATC ATC CGC	60.05	Metaphor	P	80, 150
	AAA TTG TTG TCA TCA AAT GGA AAA TA	55.13			
4	TCC TCT TCT TCG ATA TCA TCA	56.71	2% agarose	P	30,75
	CCA TTC TAT CTT TGG TGC TT	56.30			
5	ACA CTA TAG GTA TAG GCA TTT AGO CAA	61.57	2% agarose	P	70,150
	TTC TTT ATA AAT ATC AGA CCG CCG GAA AGA	58.53			
6	ATT TGG CTT AAA CCC TCT TC	56.30	2% agarose	P	70,90
	TTT ATG CTT CCT CTT CTT CG	56.30			
7	CGT ATG ATT TTG CCG TCT AT	56.30	2% agarose	NP	90
	ACC TCA AGT TCT CCG AAA GT	58.35			
8	ACC TGC TTG TTT AGC ACA AT	56.30	Metaphor	P	70, 160
	CCG CAT AGA AAT TTA TCT TC	56.30			
9	GTT GAG CAA CAA AGA CAC AA	58.35	2% agarose	P	30,50
	TTC TTG TCT GGT TGT GTG AGC	60.61			
10	CTT GTG AAT TCA TAT TTA CTT ATA GAT	55.49	2% agarose	P	80, 110
	ATC CGT AAT TTA AGG TAG GTT AAA ATA	57.01			
11	CTC TTC CTC CTC GAG ATC	59.90	2% agarose	P	75,80
	ATA GAT ACA ATA CTC TGT GAG TTG G	59.66			
12	GAT GAA GAT AAA AGC ATA ATT AAG G	56.38	2% agarose	P	90, 100
	TTT CTT CTT CTA TGA TAG ACA CAC T	58.02			
13	CTT GTG AAT TCA TAT TTA CTT ATA GAT	55.49	Metaphor	NP	60,75
	ATC CGT AAT TTA AGG TAG GTT AAA ATA	57.01			
14	CCC TTC TAG TGA TAT TTT G	53.69	2% agarose	NP	50
	AAA TGT GTT TTA TGG AAT AAG TCA T	54.74			
15	TGC TGA CTG CTT GCT ATT CGT	60.61	2% agarose	P	60,145
	GTA CTT CAT CTT GAG CGA CGC AA	62.77			
16	ATC TAA AGA GAA ATC AAA ATT GTC GAA	57.01	2% agarose	P	30,50
	GCA AAT GTG AAG CAT GTA TAG ATA AAG	60.05			
17	TGT TTT GGA GAA GAG TGA TTC	56.71	2% agarose	P	60, 100
	TGT GCA TGC AAA TTC TTA CT	54.25			
18	TTT TTG GCT TAT TAG ACT GAC TT	55.64	Metaphor	P	40,85
	TTG CCA TAA AAT ACA AAA TCC	52.8			
19	CTC TAT ATT TGT TTG TTT TTC GTT TTG	57.01	2% agarose	NP	60
	TAA AAT GTG TAG GGT GCA GAA TAA ATA	58.53			
20	AGT TTA ATT GGC TGG TTC TAA GAT AAC	60.05	2% agarose	NP	55
	AGG ATG ATC TTT AAT AAA TCA GAA TGA	57.01			

21	TAA TTG ATC ATA CTC TCA CTA TCT GCC	61.57	Metaphor	P	80, 110
	TGG GAA TGA ATA TAT TTT TGA AGT AAA	55.49			
22	TCA TTA AAA TTC TAT TGT CCT GTC CTT	58.53	2% agarose	P	60, 170
	ATC GTT TTT CTA AAC TAA ATT GTG CAT	57.01			
23	ACT TAG ATG AAT TAT CTT TCT TGG TCC	60.05	2% agarose	P	60, 125
	CGT ATT CAA ATA ATC TTT CAT CAG TCA	58.53			
24	TCC TCT TCT TCG ATA TCA TCA	56.71	2% agarose	P	80, 100
	CCA TTC TAT CTT TGG TGC TT	56.3			
25	CGG TAA ATA AGT TTC CCT CC	58.35	2% agarose	P	70, 180
	CAT CGT GAA TAT TGA AGG GT	56.3			
26	CGA ATT TTT ACA TCC GTA ATG	54.76	2% agarose	P	90, 150
	AAT CAA TCC ATT TTG CAT TC	52.2			
27	ACA AGT CAC ATG TGT TCT CAA TA	57.42	2% agarose	P	180,200
	GGA AAG GTT AAG AAA TTT TAG AAT AC	56.71			
28	TTT AGA GAC TAT TTA GGA TTG TCG T	58.02	2% agarose	P	50,75
	GTT CCA TTT TTC TTT CTT TCT TTA T	54.74			
29	AAT GCT ACA AAT TAT TAA AAA CAG TC	55.13	2% agarose	P	60, 100
	AAC TTT TAG TGT GCT GAC GAG T	58.94			
30	TGG TTG GAA ATT GAT GTT TT	52.2	2% agarose	P	30,60
	GTG GTG TGA GCA TAA TTC AA	56.3			
31	TCT GAT TTA ATT TCC TAT CAT TAG TTG C	58.76	2% agarose	P	40,70
	ATT TTT GTC GGG GAG TAC ATA ATA	57.73			
32	TTG CCT GAT GTT CGA ATT TTT CAA CTA A	60.22	2% agarose	P	60,75
	GGG ATC ATC ATT GCA TCG ATT TTT ATG A	61.69			
33	AAC ATT CAT GAA CCT ACC TCA ACT TA	59.86	Metaphor	P	60,80
	CCA TAT ATG ACT ACA CTA CCT CTC GG	64.59			
34	AAG TTT GGT CAT AAC ACA CAT TCA ATA	58.53	2% agarose	P	70,80
	TAA ATT CAC AAA CTC AAT TTA TTG GC	56.71			
35	ATT CAA CAC TCA GTA CTA CCA TTT T	58.02	2% agarose	P	200, 250
	GAT TGT TAA AAG CTT ATA TCC CTA A	56.38			
36	AAA AAT CAG AGC CAA CCA AAA A	55.22	2% agarose	P	330, 430
	AAG TAG GAG GCT AAA TTA TGG AAA AGT	60.05			
37	CAT TGC TTA GAA CCA AAA TGG	56.71	2% agarose	NP	75
	CAA TTT TAC ATC GAC GTG TGC	58.66			
38	TAT AGA GTG AGA AGA AGC AAA GAG GAG	63.08	2% agarose	P	75, 100
	TAT TTG CAT CAA TGT TCT GTA GTG TTT	58.53			
39	TGA ACA AAG AAA AAC CCG TTC	56.71	2% agarose	P	70, 120
	TGG CAA TTT GTC TGA GAT GC	58.35			
40	TCT TCA ACA CCT CCA TCT AAC CTA	61.15	2% agarose	P	30,65
	GAC ATG AAA CCA AAG CAT CAC A	58.94			
41	ATC CAT CAC AAC CCT CAA CTC A	60.81	2% agarose	P	55, 165
	CTC CGT CAA CCT TTC CGC AA	62.45			
42	CCA CAA AGO ACG ACA ACA ACG A	62.67	2% agarose	P	30,60
	CCC AAC ACG AAC CAC ACG A	62.32			

P: Polymorphic, NP: Non-polymorphic bands, Metaphor: 2% agarose + 1% metaphor

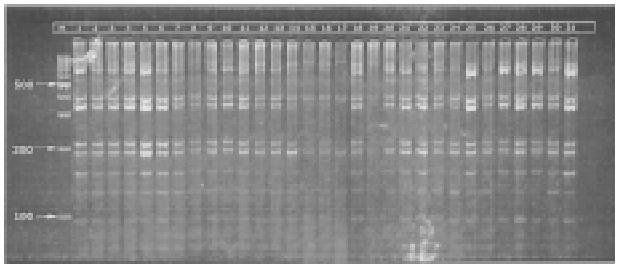

Fig. 1. Polymorphic banding pattern of 31 lentil genotypes (1-31) using SSR primer SSR-54, M-50 bp ladder

A cluster analysis based on the similarity matrix was performed using the unweighted pair group method with arithmetical averages (UPGMA). NTSYS-ps version-2.0 software was used for cluster analysis. The relationship among the 31 lentil accessions was presented graphically in the form of a Dendrogram (Fig. 2).

DNA fingerprinting

The banding pattern of the lentil cultivars were observed for any unique band(s) associated with any particular cultivar. A set of primers were selected which in combination gives a distinguishable characteristic to individual cultivars.

Results and discussion

Transferability of markers and diversity among the lentil cultivars

Among all the 42 STMSs tried during the experiment, 13.9% (6/42) primers were non-polymorphic whereas all the other STMSs (36/42, 85.7%) gave polymorphism, which were used for diversity analysis and DNA fingerprinting. The results were reproducible. All those makers derived from chickpea could be transferred and amplified among the lentil genotypes. In a similar study while working on four major pulses *viz.,* field pea, chickpea, lentil and vetch, Pandian *et al.* [12] observed that a high level of sequence conservation among those species exists. The cross transferability between chickpea and lentil observed during the investigation is similar to their study. The estimates of genetic similarity based on Jaccards' coefficients observed during the investigation ranged from 0.65 to 0.94, which indicates the existence of high degree of variability among the lentil cultivars of India. During the study, exotic lines like Precoz do not form separate clusters. The reason being, Precoz (ILL 4605) was the first early flowering, macrosperma used in lentil crossing programme of India in 1990 and since then, it has been frequently used in Indian lentil breeding programme. The existence of

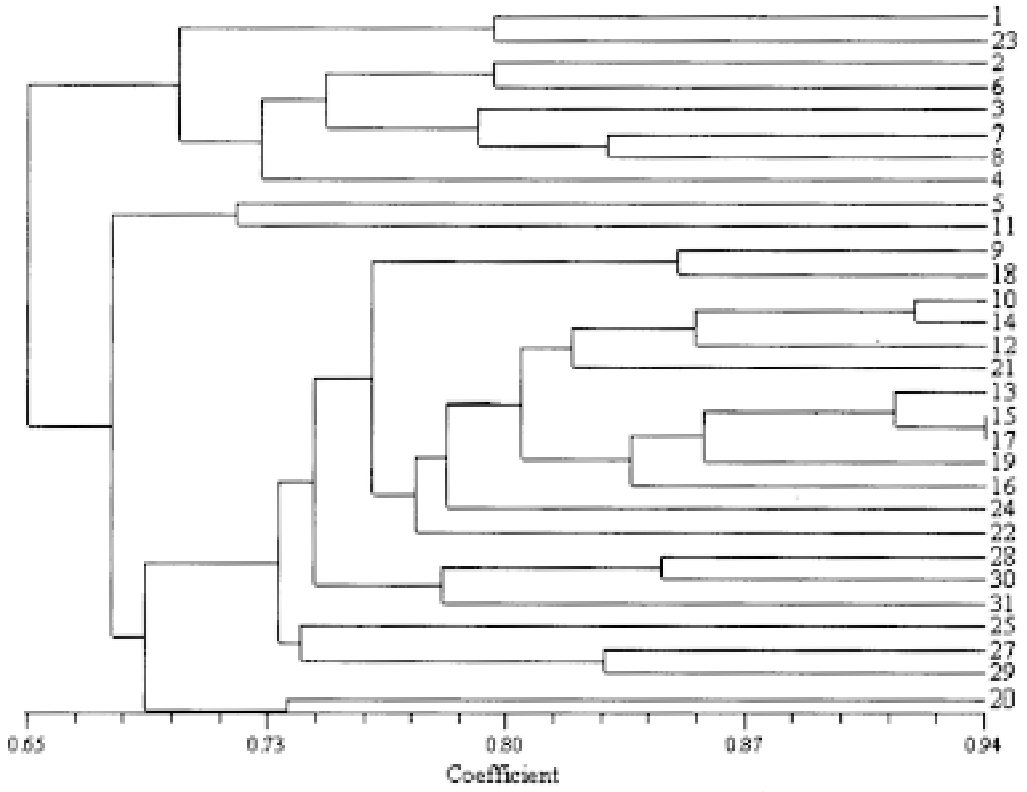

Fig. 2. Dendrogram depicting the genetic relatedness among the lentil cultivars using STMS data

diversity among the lentil varieties found to be quite high, and introgression of this genome (Precoz) into different backgrounds took place during the development process of the varieties. This finding was similar to Ford *et al,* [7] working on an Australian lentil-breeding programme where they observed a maximum dissimilarity value of 0.36. Similar results were also observed by other workers [9, 10, 11]. Duran, *et al.* [9] studied the genetic variation among a collection of varieties of *Lens* including cultivated and wild relatives by employing RAPD and ISSR markers, while Sharma *et al.* [9] used AFLP and RAPD markers. DNA polymorphism also been detected by analysis of variable number of tandem repeats (VNTR), inter-simple sequence repeats (ISSR), and amplified fragment length polymorphism (AFLP) to distinguish between commercial lentil cultivars (*Lens culinaris*) that are closely related or identical judging from pedigree and agronomical and morphological traits [10, 11]. However results obtained from use of STMS markers are considered to be more reliable than other PCR based methods since they are locus specific and spread well within the entire genome.

Major clusters

All the 31 genotypes under study were grouped into five clusters at 0.71 similarity index value (Table 3, Fig. 2). Among them, group IV had highest number of genotypes (19) while group V had only one genotypes i.e. Mason (from USA). Among the population taken for investigation, two known genotypes with exotic origin were Mason and Precoz.

Precoz has been frequently used in the Indian breeding programme whereas the use of Mason is limited and none of the varieties has been released using Mason as one of the parents. That is why Precoz finds a place within group IV where as Mason makes a separate group. This result is similar to other workers [6, 7] working on cultivated and wild lentil.

Table 3. Clustering of lentil cultivars using STMS data

Group	Genotypes
I	DPL 15, B 77(Asha)
II	K 75 (Malika), T 36, LH 84-8 (Sapna), LL 147, L 4147 (Pusa Vaibhav), LH 82-6
III	DPL 62 (Sheri), PL 406
IV	L-4076, Sehore 74-3, L-9-12, PL-5, PL 639, DPL 58, PL 4, PL 234, IPL 525, PL 77-2, L 4603, IPL 81 (Noori), Precoz, JL 1, VL 4, NDL 1, Ranjan, IPL 525, VL 103
V	Mason

Detection of genotype specific markers

During the investigation, not a single STMS marker could be identified which was specific to any cultivar/genotype. However, combination of two or more primers could differentiate each cultivar to other. The fingerprint pattern of the 31 genotypes with selected STMS markers are given in Table 4. This can be helpful in identifying a lentil cultivar without any error, supported by morphological and related characters. A set of 16 STMS primers (Table 4) are good enough to differentiate all the lentil cultivars of India and also further be used to differentiate other genotypes. Similar work has been reported in rice extensively for a variety of purpose which include identification and classification of accessions and hybrids [13]. Since SSR have been shown to provide a powerful means for discrimination between closely related genotypes in many plant species, the results observed during the present investigation will be useful for other accessions of lentil.

Genome wide distribution of the markers

The DNA markers selected during the study were distributed in all the linkage groups of chickpea. Since chickpea and lentil are very close to each other during their evolution, we expected that the markers are well distributed in the whole genome of lentil. This makes the purpose of DNA fingerprinting more reliable. STMSs are the DNA markers of choice for diversity, evolutionary and mapping purpose [5] sine they are specific to locus and highly reliable. During the present investigation, the markers reported were found to be robust and repeatable. Similar to earlier reports [12, 13], we observed cross transferability of the STMS markers from chickpea to lentil. Hence it can be reliably used to distinguish the lentil genotypes.

Supplementation of phenotyping data to the marker data for practical utility

Phenotypic data *per se* may not be sufficient to distinguish all the released lentil cultivars of India. However those data can be supplemented with the DNA fingerprint data to give robustness to the test and help in reducing the number of primers required for the test, to distinguish and identify the cultivars.

Table 4. DNA profile of individual cultivars for selected STMSs

Genotypes	SSR2 A:102 B:204	SSR4 A:103 B:273	SSR22 A:95 B:300	SSR23 A:80 B:135 C:195	SSR29 A:105 B:218	SSR32 A:90 B:330	SSR34 A: 109 B:325	SSR39 A:70 B:216	SSR41 A:92 B:166	SSR53 A:113 B:102	SSR54 A:73 B:215	SSR55 A:100 B:300	SSR56 A:86 B:207	SSR60 A:80 B:98	SSR61 A:82 B:95	SSR71 A:85 B:220
DPL 15 (Priya)	A	AB	A	B	AB	B	A	AB	A	A	A	AB	B	A	B	AB
K 75 (Malika)	B	AB	A	A	AB	B	A	AB	A	B	AB	A	AB	A	A	A
LH 84-8 (Sapna)	B	AB	B	B	AB	AB	A	AB	B	B	AB	B	B	B	B	AB
LH 82-6	B	AB	B	C	A	AB	A	A	B	B	AB	A	B	A	B	A
DPL 62 (Sheri)	A,B	A	B	C	A	A	A	B	B	B	A	A	A	A	A	AB
T-36	B	A	B	A	AB	A	A	AB	A	B	A	AB	B	A	A	AB
LL147	B	AB	AB	AB	AB	B	A	AB	B	B	A	AB	B	B	A	AB
Pusa Vaibhav	B	AB	AB	B	AB	B	A	B	B	A	A	AB	B	A	A	AB
L-4076	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	AB
L-9-12	B	A	A	AC	A	A	A	AB	A	B	B	A	A	A	A	AB
PL-406	A, B	A	A	AC	A	B	AB	AB	B	A	A	A	AB	A	A	B
PL-639	B	A	A	AC	A	A	A	AB	A	B	AB	A	A	A	A	B
PL-4	B	A	A	A	AB	AB	AB	A	B	AB	A	B	A	A	AB	
PL-5	B	A	A	C	A	A	A	A	A	B	B	A	A	A	A	AB
PL-234	B	A	A	AC	A	B	A	AB	A	B	AB	A	B	A	A	A
L-4603	A	A	A	A	AB	B	A	AB	A	B	AB	A	A	A	A	A
IPL-525	B	A	A	A	A	B	A	AB	A	B	AB	A	B	A	A	AB
Sehore 74-3	B	A	A	A	A	A	A	B	B	B	A	AB	A	B	AB	
PL-77-2	B	A	A	A	A	AB	A	AB	A	B	A	A	AB	A	A	A
Mason	A	A	A	C	A	B	A	AB	A	B	A	A	A	B	A	B
DPL -58	A	A	A	C	A	A	A	A	B	B	A	AB	A	A	AB	
Precoz	A	A	A	C	A	A	A	AB	A	B	AB	A	B	A	A	B
B -77 (Asha)	A	AB	A	AB	AB	B	A	A	A	A	A	A	AB	A	A	B
IPL-81 (Noori)	A	A	A	AC	A	A	A	A	A	B	AB	B	AB	A	A	AB
Ranjan	A	A	AB	A	A	A	A	A	A	B	AB	A	AB	A	A	B
WBL -58	A	A	A	AC	A	B	A	A	A	B	A	A	A	B	A	AB
VL-1	B	A	AB	ABC	AB	A	AB	A	A	A	B	A	A	A	A	B
JL-1	A	A	A	C	A	B	AB	A	A	B	AB	A	AB	A	A	AB
VL-103	B	A	AB	A	A	A	AB	A	A	B	B	A	A	A	A	AB
VL-4	B	A	A	AC	AB	B	AB	A	A	B	AB	A	B	A	A	AB
NDL-1	B	A	A	ABC	A	B	AB	A	B	B	AB	A	AB	B	A	AB

n.b.: Size of the band is approximate value (base pair); A, B and C stands for the different size bands for a given primer

References

1. **FAO (Crop Statistics).** 2006. (web site, http:// faostat.fao.org)
2. **Smith J. S. C. and Smith O.** S. 1992. Fingerprinting crop varieties. Rev. Agric. Acad. Press, USA, p. 85-140.
3. **Westman A. L. and Kresovich S.** 1997. Use of molecular marker techniques for description of plant genetic variation. *In*: Callow J. L., Ford-Lloyd B. V. and Newburry H. J. (eds.), Biotechnology and Plant Genetic Resources. CAB Int., p. 9-45.
4. **Morgante M. and Olivieri A. M.** 1993. PCR-amplified microsatelilites as markers in plant genetics. Plant J., **3**: 175-182.
5. **Gupta P. K. and Varshney R. K.** 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica, **113**: 163-185.
6. **Sharma S. K., Dawson I. K. and Waugh R.** 1995. Relationships among cultivated and wild lentils revealed by RAPD analysis. Theor. Appl. Genet., **91**: 647-654.
7. **Ford R., Pang E. C. K. and Taylor P. W. J.** 1997. Diversity analysis and species identification in Lens using PCR generated markers. Euphytica., **96**: 247-255.
8. **Ahmad M., McNeil D. L., Fautrie A. G., Armstrong K. F. and Paterson A. M.** 1996. Genetic relationships in Lens species and parentage determination of their interspecific hybrids using RAPD markers. Theor. Appl. Genet., **92**: 1091-1098.
9. **Duran Y. and Perez.de.la.Vega M.** 2004. Assessment of genetic variation and species relationships in a collection of Lens using RAPD and ISSR. Spanish J. Agricultural Research, **2**: 538-544.
10. **Sharma S. K., Knox M. R. and EHis T. H. N.** 1996. AFLP analysis of diversity and phylogeny of Lens and its comparison with RAPD analysis. Theor. Appl. Genet., **93**: 751-758.
11. **Zavodna M., Kraic J., Paglia G., Gregova E. and Morgante M.** 2000. Differentiation between closely related lentil (*Lens culinaris* Medik.) cultivars using DNA markers. Seed Sci. Tech., **28**: 217-219.
12. **Pandian A., Ford R. and Taylor P. W. J.** 2000. Transferability od sequence tagged microsatellite site (STMS) primers across four major pulses. Plant Molecular Biology Reporter, **18**: 395a-395h.
13. **Fukuoka S., Hosaka K. and Kamijima O.** 1992. Use of random amplified polymorphic DNAs (RAPDs) for identification of rice accessions. The Japanese Journal of Genetics, **67**: 243-252.