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56 Abstract

Genetic diversity was studied among 21 accessions of lentil using SSR markers and morphological traits in order to assess the diversification of Indian gene-pool of lentil through introgression of exotic genes and introduction of germplasm. Among these , 16 genotypes either had ‘Precoz’ gene, an Argentine line in their pedigree or genes from introduced lines from ICARDA. Sixty five SSR markers and eight phenotypic traits were used to analyse the level of genetic diversity in these genotypes. Forty three SSR markers (66 %) were polymorphic and generated a total of 177 alleles with an average of 4.1 alleles per SSR marker. Alleles per marker ranged from 2 to 6. The polymorphic information content ranged 0.33 to 0.80 with an average of 0.57, suggesting that SSR markers are highly polymorphic among the studied genotypes. Genetic dissimilarity based a dendrogram grouped these accessions into two main clusters (cluster I and cluster II) and it ranged 33 % to

		71 %, suggesting high level of genetic diversity among the genotypes. First three components of PCA based morphological traits explained higher variance (95.6 %) compared to PCA components based on SSR markers (42.7 %) of total genetic variance. Thus, more diversity was observed for morphological traits and genotypes in each cluster and sub-cluster showed a range of variability for size, earliness, pods/plant and plant height. Molecular and phenotypic diversity analysis thus suggested that use of germplasm of exotic lines have diversified the genetic base of lentil germplasm in India. This diversified gene-pool will be very useful in the development of improved varieties of lentil in order to address the effect of climate change, to adapt in new cropping systems niches such as mixed cropping, relay cropping, etc. and to meet consumers' preference.
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RESEARCH ARTICLE

Diversification of indigenous gene- pool by using exotic germplasm in lentil (*Lens culinaris* Medikus subsp. *culinaris*)

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Abstract Genetic diversity was studied among 21 accessions of lentil using SSR markers and morphological traits in order to assess the diversification of Indian gene-pool of lentil through introgression of exotic genes and introduction of germplasm. Among these , 16 genotypes either had 'Precoz' gene, an Argentine line in their pedigree or genes from introduced lines from ICARDA. Sixty five SSR markers and eight phenotypic traits were used to analyse the level of genetic diversity in these genotypes. Forty three SSR markers (66 %) were polymorphic and generated a total of 177 alleles with an average of 4.1 alleles per SSR marker. Alleles per marker ranged from 2 to 6. The polymorphic information content ranged 0.33 to 0.80 with an average of 0.57, suggesting that SSR markers are highly polymorphic among the studied genotypes. Genetic dissimilarity based a dendrogram grouped these accessions into two main clusters (cluster I and cluster II) and it ranged 33 % to 71 %, suggesting high level of genetic diversity among the genotypes. First three components of PCA based morphological traits explained higher variance (95.6 %) compared to PCA components based on SSR markers (42.7 %) of total genetic variance. Thus, more diversity was observed for morphological traits and genotypes in each cluster and sub-cluster showed a range of variability for size, earliness, pods/plant and plant height. Molecular and phenotypic diversity analysis thus suggested that use of germplasm of exotic lines have diversified the genetic base of lentil germplasm in India. This diversified gene-pool will be very useful in the development of improved varieties of lentil in order to address the effect of climate change, to adapt in new cropping systems niches such as mixed cropping, relay cropping, etc. and to meet consumers' preference.

Keywords Alien gene · Introgression · SSR marker · Morphological traits · Diversification · Lentil

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Introduction

Lentil is an important cool-season, which is grown mainly on marginal area under rainfed conditions. It is cultivated over 52 countries on 3.64 million ha area with annual production of 3.6 million tons as the rich source of protein (FAOSTAT, 2011). India is the major lentil producer in the world for both small- seeded (*microsperma*) and bold seeded (*macrosperma*) types of lentil and grown on an area of 1.56 m ha with a production of 1.06 m tons (AICRP on MULLaRP 2012-13). Considerable improvement in lentil was made in the past years using conventional breeding. However, in recent years, productivity of lentil crop has been shown stagnant and further improvement in yield potential of cultivars does not seem encouraging. One of the reasons for this yield stagnation is narrow genetic base of Indigenous *microsperma* germplasm (i.e. *pilosae* type), which led to repeated use of same genotypes in breeding programs (Ferguson et al. 1998; Kumar et al. 2004). Molecular diversity analysis has also suggested high genomic similarity among the Indian germplasm (Datta et al. 2011). Therefore, it has been suggested to introgress the alien genes from the exotic materials (*macrosperma* germplasm) for broadening the genetic base of lentil in South Asia

(Ladizinski et al. 1984; Erskine 1997; Erskine et al. 1998; Rahman et al. 2009). Initially, cross incompatibility of *macrosperma* type germplasm due to the long duration with Indian germplasm has restricted their use in Indian lentil breeding program. However, identification of an early-flowering exotic germplasm of *macrosperma* type, 'Precoz' led to its introduction in India and involved in Indian lentil breeding program for improving the earliness, seed size and rust resistance (Asghar et al. 2010; Erskine et al. 1998; Kumar et al. 2004; Singh et al. 2006). As a result, breeding lines having Precoz genes in its derivatives have been developed. However, there is a view among Indian breeders that direct introduction and use of Mediterranean germplasm had negative impact on Indian gene-pool because it increased crop duration and reduced the biomass. Moreover, recently, it has observed on the basis of molecular markers that Indian germplasm have higher genomic similarity among themselves (Datta et al. 2011). However, use of molecular markers along with agro-morphological traits can be a better way to explain the genetic base of Indian germplasm.

Earlier molecular markers have been preferred for genetic diversity analysis in lentil (Udupa et al. 1999; Abe et al. 2003; Hamwieh et al. 2009; Reddy et al. 2009). Among the various molecular markers, microsatellites or simple sequence repeats (SSR) have shown to be very useful, because these markers showed high polymorphism, reproducible and easy to handle (Varshney et al. 2005, 2009; Datta et al. 2011). Though in lentil, a number of SSR markers have been developed (Hamwieh et al. 2005; Kaur et al. 2011; Datta et al. 2011), availability of polymorphic SSR markers and their use in analysis of the genetic diversity is still limited in lentil compared to other pulses such as chickpea (Hamwieh et al. 2005, 2009; Kaur et al. 2011; Datta et al. 2011). Therefore, present investigation was aimed to assess diversification of Indian gene-pool on the basis of SSR markers and morphological traits among 21 lentil genotypes involving the exotic lines.

Materials and methods

Plant materials

The present study included 21 lentil genotypes comprising local and exotic germplasm, elite breeding lines and improved cultivars released in India and frequently used donors in hybridization programs (Table 1). Breeding lines developed at the Indian Institute of Pulses Research (IIPR) were derived from crosses involving parents adapted to short-season, drought prone environments. These genotypes represent diversity with regard to morpho-phenological traits and are adapted to mild winter environments.

SSR markers

Sixty-five (65) SSR markers developed in lentil at ICRADA by Dr. A. Hamwieh (personal communication) were used in present study. Description of primers sequence and expected product size are shown in Table 2. The primers were custom synthesized from Eurofins Genomics India, India.

DNA extraction and SSR marker analysis

Genomic DNA was extracted from young leaves of each genotype using CTAB extraction protocol described by (Doyle and Doyle 1987; Abdelnoor et al. 1995) with certain modifications. These modifications were made in grinding of the fresh tissue in liquid nitrogen and mixing the grounded powder unpreheated extraction buffer, while centrifuges were made on 10,000 rpm for 10 min. Thus extracted highly purified DNA of each genotype was used for SSR marker analysis through PCR amplification.

PCR reaction contains a total volume of 20 µl volume consisting of 50–100 ng genomic DNA, 1X PCR buffer with 1.5 mM $MgCl_2$, 200 µM each of dNTPs (Bangalore Genei, Bengaluru), 0.5 U Taq DNA Polymerase (Bangalore Genei, Bengaluru) and 40 pmoles each of forward and reverse primers. The PCR amplification was performed in a G-STORM PCR System with a programme for an initial denaturation of 94 °C for 4 min followed by 39 cycles of 94 °C for 1 min, annealing 50–55 °C for 1 min, elongation 72 °C for 1 min and final extension at 72 °C for 15 min . The amplified product were run on 10 % polyacrylamide gels along with 1 kb DNA ladder (Babglore Genei, Bengaluru) and visualized by silver staining.

Recording of data

Amplification products were recorded on the basis of presence or absence of marker alleles across studied genotypes. The length of DNA fragment was calculated by DNA Fragment Size Calculator available freely on-line (http://www.basic.northwestern.edu/biotools/sizecalc.html). The polymorphism information content (PIC) values were calculated following Botstein et al. (1980) as follows:

$$(\mathrm{PIC}) = 1 - \sum_{j=1}^{n} P_{ij}^{2}$$

where, P_{ij} is the frequency of the j^{th} allele for the i^{th} marker, and summed over n alleles (Table 1).

The binary data on 21 genotypes for 43 polymorphic markers were subjected to similarity co-efficient analysis (Jaccard 1908) based on which dendogram was constructed using unweighted pair group method with arithmetic average (UPGMA) using NTSYS pc- 2.11x (Rohlf 1998) software. Similarity/dissimilarity matrix formed from above analysis

t1.1 **Table 1** Description of pedigree, type of material and source of lentil genotypes used in present study

t1.2 Genotype	Pedigree	Type of material	Source/origin	Country
t1.3 Exotic line/breeding line having exotic line in their pedigree				
t1.4 ILL 7663	Cross between two locals	Exotic line	ICARDA	Syria
t1.5 ILL 6002	ILL 4349×Precoz	Exotic line	ICARDA	Syria
t1.6 Precoz	Argentina cultivar	Exotic line	ICARDA	Syria
t1.7 ILL 7723	Land race of Pakistan	Exotic line	ICARDA	Syria
t1.8 ILL 10011	Unknown	Exotic line	ICARDA	Syria
t1.9 ILL 9941	Unknown	Exotic line	ICARDA	Syria
t1.10 IPLS 9-35	ILL 7938×ILL 6037	Breeding line	IIPR, Kanpur	India
t1.11 IPLS 9-23	ILL 8072×ILL 6037	Breeding line	IIPR, Kanpur	India
t1.12 IPLS 9-17	Masan×DPL 62	Breeding line	IIPR, Kanpur	India
t1.13 L 4603	Precoz×L3991	Breeding line	PAU, Ludhiana	India
t1.14 IPL 99/209	PL639×Precoz	Breeding line	IIPR, Kanpur	India
t1.15 DPL-58	PL639×Precoz	Breeding line	IIPR, Kanpur	India
t1.16 IPL 98/193	[Sehore 74-3×DPL 44]×DPL 35	Breeding line	IIPR, Kanpur	India
t1.17 IPL 533	IPL 98/155×DPL 62	Breeding line	IIPR, Kanpur	India
t1.18 IPLS118	Selection from ILWL118	Wild (*L. orientalis*)	IIPR, Kanpur	India
t1.19 PL02	PL 4×DPL 55	Cultivar	GBPUA&T	India
t1.20 Breeding line/cultivar/land races originated from Indian germplasm				
t1.21 EC 208362	Unknown	Land race from India	NBPGR, New Delhi	India
t1.22 DPL 15	PL406×L4076	Cultivar	IIPR, Kanpur	India
t1.23 JL 1	Local selection from Madhya Pradesh	Cultivar	JNKVP, Jabalpur	India
t1.24 LL 864	LL 498×LH 84-8	Breeding line	PAU, Ludhiana	India
t1.25 DPL 53	Sehore 74-3×LG 171	Breeding line	IIPR, Kanpur	India

was used to analyse the principal component analysis (PCA) for both morphological traits and SSR markers using same software (Rohlf 1998).

Results

A total 65 SSR markers developed in lentil were used to test their polymorphism among the 21 lentil genotypes. Out of these SSR markers, 43 markers (66 %) were observed polymorphic. These polymorphic markers generated a total of 177 alleles with an average of 4.1 alleles per SSR marker. Alleles per marker ranged from 2 to 6. The polymorphic information content ranged from 0.31 to 0.80 with an average of 0.57 suggesting that SSR markers are highly polymorphic among the studied genotypes (Table 2).

Genetic diversity analysis

The molecular diversity was studied among the 21 genotypes of lentil. Jaccard similarity coefficient between the genotypes ranged from 0.33 to 0.71. Minimum disimilarity (33 %) was observed between IPL 533 and IPLS 09-17, while it was maximum between EC-208362 and ILL7663 and followed by other pairs of genotypes. The UPGMA analysis grouped these genotypes into two major clusters (Fig. 1). Cluster I comprised of nine genotypes (Precoz, IPL98/193, ILL-6002, LL864, EC 208362, Pl-02, JL-1, DPL-58, IPLWS-118). Broadly, in this cluster, genotypes belonging to cultivated species had 71–86 days for 50 % flowering and variable seed size ranging from 2.2 to 3.7 g per 100 seeds. This cluster further had two sub clusters (Ia and Ib) and each one had variable plant height. The sub cluster Ia had four genotypes (PL-02, JL-1, DPL-58, IPLWS-118) with a plant height ranging from 28.0 cm to 42.4 cm. The sub cluster Ib had five genotypes with a plant height varying from 28.8 cm to 43.8 cm. The cluster II had 12 genotypes (ILL7663, IPL-99/209, DPL-15, L4603, ILL10011, IPLS 09-17, DPL 53, IPL 533, IPLS 9-23, IPLS 9-35, ILL 9941 and ILL 7723) and this cluster was further divided into two sub-clusters (IIa and IIb). All genotypes of cluster IIa had early flowering except DPL-15 (63–66 days of 50 % flowering) and seeds size less than <3.0 g/100 seeds. However, genotypes of sub cluster IIb had early to late flowering (63–91 days, small to large seed size (2.4–5.4 g/100 seeds) and medium to tall plant height (28.2–45.1 cm) and low to high biological and grain yield (4.57–17.54 and 1.39–5.9 g/plant, respectively). In each cluster and sub cluster, further variability for pods/plant, harvest index and other traits were also observed (see Table 3).

PCA analysis based on SSR marker data and morphological traits data resulted in clustering of 21 genotypes

t2.1 **Table 2** Sequence of forward and reverse primer, allele size, number of alleles and PIC value for SSR marker used in present study

t2.2	Marker	Forward primer	Reverse primer	Expected size	Size range	No. of allele	PIC value
t2.3	ALD 3	GAACAGATGTCTTGAGC	GAACATTTTCTCTCGTGTG	211	199–230	5	0.59
t2.4	ALD 6	GCCTGATAGTGGACTTTCATC	CTGTTGATTAGTGCTGCTC	228	220–261	4	0.65
t2.5	ALD 13	CAGCTGTCCTATTGGTTTG	GATGAATGTCCCTTACGATG	300	268–324	3	0.57
t2.6	ALD 14	CTATAGCTTCTGCCTGTAG	CAACAACACATCACATACG	260	249–313	6	0.52
t2.7	ALD 15	CAAGCATGACGCCTATGAAG	CTTCACTCACTCAACTCTC	289	261–321	5	0.52
t2.8	ALD 16	GACTCTCCAAGGATTCACTC	GCACAGGTCGTCATTATTAC	262	233–321	4	0.68
t2.9	ALD 18	GATTCATGAGCTAGGGGATG	GATGGGCGTGGGGAATTTTC	203	167–236	5	0.73
t2.10	ALD 19	GCCTCGTTTCATCAAAGACG	GAGTGAGTGTGTGTGTAGATG	180	182–209	3	0.39
t2.11	ALD 20	CATGGTGGAATAGTGATGGC	CTCCATACACCACTCATTCAC	165	120–170	5	0.69
t2.12	ALD 21	CTGCACGCTAGGCTTGTC	GTAAAGTGCGCCAGCTCG	130	100–160	2	0.33
t2.13	ALD 22	CATCTGAGGAGTTGCTTGC	GTTCACACGGCTGTAAGTC	309	250–378	2	0.54
t2.14	ALD 28	GGTAGTGGTGAGGAATGAC	GCATCACTGCAACAGACC	253	191–294	5	0.78
t2.15	ALD 29	CATAGGTACCAAGGAGATG	GCGAAGTCTCTGACAACAC	433	367–433	3	0.43
t2.16	ALD 30	CAAACAGTACAAGGAAAGGAG	CTGACTGAGCTGCTTGAAC	253	183–284	5	0.64
t2.17	ALD 31	GGTCTATTTGCGTGCC	GCAAGTCCTTATACCAAG	188	173–206	3	0.31
t2.18	ALD 33	CCGTGTACACCCCTAC	CGTCTTAAAGAGAGTGACAC	181	151–224	3	0.39
t2.19	ALD 34	CGTGGGAAAATGTGTTG	GTGTGTCGATAGGTCG	200	145–237	6	0.58
t2.20	ALD 35	CGCTGCAACAACACTG	GCGGCATAGAGTGCTAT	135	110–216	5	0.58
t2.21	ALD 37	GTTATCTTCCAGCGTC	GATATACAATCAGAGATG	210	211–297	3	0.62
t2.22	ALD 38	GACTCTTAATGTAACAC	GACAGAACCTACACTTCAG	273	241–293	5	0.48
t2.23	ALD 39	GGGAATTTGTGGGAGGAAG	CCTCAGAATGTCCCTGTC	161	163–201	3	0.53
t2.24	ALD 40	GCGGCGAGCAAATAAAT	GGAGAATAAAGAGTGAAATG	168	154–201	6	0.60
t2.25	ALD 41	CTTCTCACTTCTCTCCC	CTTGGTGTATTCTTGGTTTC	172	168–221	6	0.58
t2.26	ALD 42	CCGTAAGAATTAGGTGTC	GGAAAATAGGTGGAAAG	211	211–265	3	0.53
t2.27	ALD 44	GTATGGGTTATTAACATTGAAAAG	CACCACCATTTTCACACAC	185	186–213	4	0.75
t2.28	ALD 45	GAACTCAGTTTCTCATTG	GAACATATCCAATTATCATC	266	272–322	6	0.60
t2.29	ALD 46	GCCTCTCTCGGTTTGTTTC	GCACATGCGTGTGTGC	130	119–153	3	0.41
t2.30	ALD 47	GTATGTGACTGTATGCTTC	GCATTGCATTTCACAAACC	174	174–224	5	0.54
t2.31	ALD 48	CACACCTTCCCATCTCC	GAAAGGAGATTAACAGTGGG	157	140–188	3	0.55
t2.32	ALD 49	CCACGTATGTGACTGTATG	GAAAGAGAGGCTGAAACTTG	196	164–200	3	0.44
t2.33	ALD P2	CGGCGGATGAAACTAAAG	CATTTCCTTCACAAACCAAC	185	153–201	4	0.44
t2.34	ALD P4	GGTAGTGGTGAGGAATGAC	GCATCACTGCAACAGACC	–	223–280	5	0.66
t2.35	ALD P5	CAAACAGTACAAGGAAAGGAG	CTGACTGAGCTGCTTGAAC	–	139–278	5	0.68
t2.36	ALD P6	CCGTGTACACCCCTAC	CGTCTTAAAGAGAGTGACAC	–	146–156	5	0.73
t2.37	ALD P7	CCAGAACAAACGTAAACC	CTATCGCATATGAGTGAAC	397	145–385	4	0.63
t2.38	ALD P8	GCTCGCATTGGTGAAAC	CATATATAGCAGACCGTG	119	102–143	4	0.52
t2.39	ALD P9	GCAAATTTCTTGGTCTACAC	GGGCACAGATTCATAAG	238	184–257	4	0.70
t2.40	ALD P1	CACCAATCACCAACACAC	GAGCTGTGAAGTCTTATCTG	173	138–189	3	0.80
t2.41	ALD P1	CAACCTCACTTACCTTAC	GCTCTTTATCATCATTCTAC	220	181–250	4	0.59
t2.42	ALD P1	GAGAGATACGTCAGAGTAG	GATTGTGCTTCGGTGGTTC	227	237–277	2	0.32
t2.43	ALD P1	CCAACAACAATTCACCATAC	AACATTGTACTGAGAGGT	251	162–265	4	0.64
t2.44	CEDAATOO2	GAAAAAGTAAGGCTGAGGAAGG	CAAACCTCGTCATTCCACCATG	–	225–310	4	0.53
t2.45	CEDAAT001	GCATGAACTATGAACGTGTAG	GCTTTCTCTCGTATTAGTGG	–	226–303	5	0.62

into three groups and distinct position of each genotype was observed within each group (Fig. 2a, b). First three most informative components in PCA analysis of SSR markers individually accounted 20.2 %, 13.2 % and 9.3 % of total variation, respectively, and collectively these three components explained 42.7 % of total variability. However, first three components of morphological traits individually accounted 77.6 %, 11.5 % and 6.6 % of total

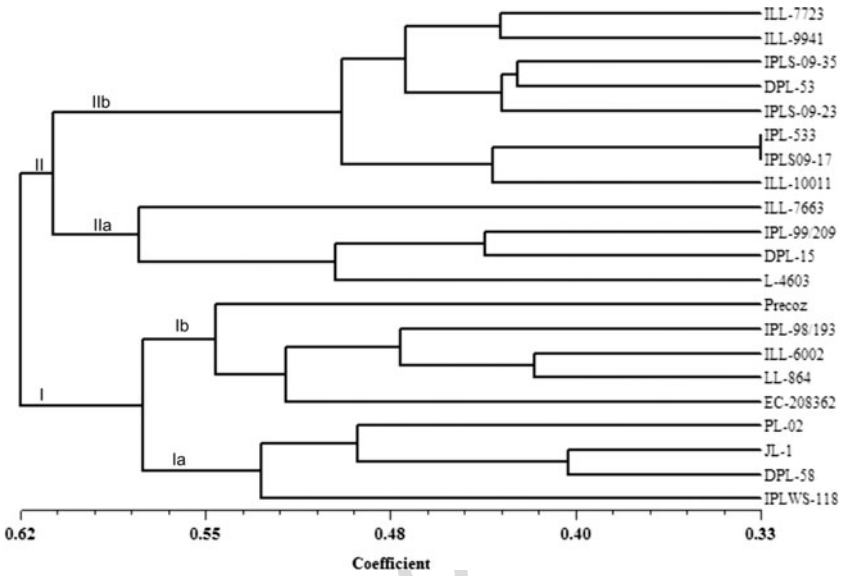

Fig. 1 Dendrogram showing genetic relationships among lentil genotypes based on molecular data of SSR markers (Scale indicates Jaccard's coefficient of dissimilarity)

variability, which is commutatively equal to 95.6 % of total variability.

Discussion

The molecular markers have been developed and used widely in crop improvement. Simple sequence repeat (SSR) markers are accepted as breeder friendly markers for utilization in marker aided breeding programs (Gupta et al. 2013). However, use of these markers in lentil is restricted due to their unavailability. Thus, availability of more number of polymorphic SSR markers in lentil will help to enrich the genomic resources in lentil and also to cover whole genome analysis. In present study, 66 % (43 out of 65) SSR markers were polymorphic when studied on 21 genotypes of lentil. It

Table 3 Mean performance of 21 genotypes over the 2 years for five important traits

Genotype	Days to 50 % flowering (no.)	Pods/Plant (no.)	Days to maturity (no.)	Plant height (cm)	100-seed weight (g)	Biological yield/ plant (g)	Grain yield/ plant (g)	Harvest index (%)
ILL 7723	84	120	121	36.42	2.8	16.68	4.49	27.65
ILL 9941	91	121	128	34.08	2.9	11.76	3.42	29.67
IPLS 09-35	66	67	128	38.70	5.2	8.30	3.15	37.51
IPLS 09-23	68	65	120	32.58	5.4	5.71	2.46	46.19
DPL 53	83	97	121	45.12	2.9	14.63	5.51	38.07
IPL 533	81	67	121	43.83	2.9	8.33	1.39	16.92
IPLS 09-17	68	55	114	28.92	3.1	4.57	2.04	44.33
ILL 10011	88	150	126	41.20	2.4	17.54	5.90	35.08
ILL 7663	63	70	126	35.90	2.5	9.04	2.75	31.73
IPL 99/209	63	88	116	31.80	2.7	8.88	6.00	42.75
DPL 15	84	101	123	45.07	2.9	10.09	5.16	51.17
L 4603	66	94	117	38.57	2.4	13.69	4.44	30.08
Precoz	74	52	117	28.80	3.7	8.89	2.74	30.59
IPL 98/193	86	113	121	36.77	2.6	11.98	3.81	31.74
ILL 6002	79	74	127	36.07	3.3	10.50	2.53	24.31
LL 864	85	148	120	43.83	2.6	21.13	8.17	38.43
EC 208362	78	102	129	36.60	2.5	15.93	5.68	34.40
PL 02	71	81	111	38.60	3.0	9.21	2.59	28.37
JL 1	72	99	116	42.40	2.2	14.44	5.33	34.37
DPL 58	83	63	123	40.37	3.4	14.53	3.26	22.41
IPLWS 118	55	66	77	28.00	2.7	8.89	2.50	28.12

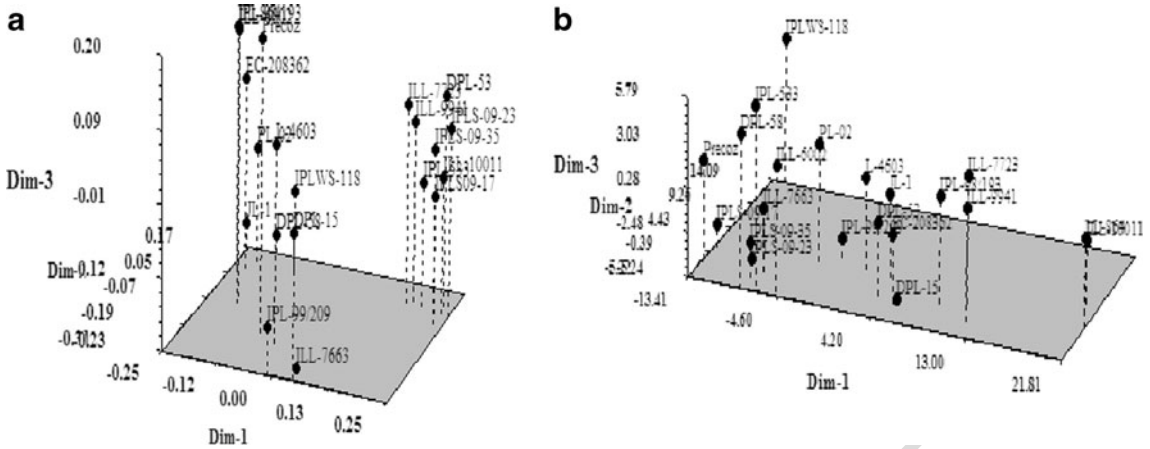

Fig. 2 PCA analysis of 21 genotypes of lentil based on **a** SSR markers and **b** morphological data used

was comparatively similar to earlier study where 47.5 % SSR markers developed from lentil genome were polymorphic (Kaur et al. 2011). In another study, transferrable SSR markers were also highly polymorphic in lentil (Datta et al. 2011). However, in contrast to this, Gupta et al. (2012) identified only 4.43 % SSR markers polymorphic. It was because they screened SSR markers only in two genotypes and used agrose gel for separation of PCR amplified product. The high PIC value for most of the SSR markers (average 0.57) indicates their usefulness in differentiating closely related accessions. Similarly, on an average, 5.1 alleles per locus and PIC ranging from 0.06 to 0.89 with an average of 0.58 was observed for newly developed SSR markers in lentil (Andeden et al. 2013). The most polymorphic SSR markers obtained in the study could effectively be used in DNA fingerprinting of lentil genotypes (Agrawal and Katiyar 2008).

Assessment of diversification of Indian gene pool

On the basis of the 177 alleles amplified by 43 SSR markers, the genetic dissimilarity among 21 lentil genotypes was assessed and a dendrogram grouped these accessions into two main clusters at a boot strap value of 100 % (Fig. 1). The genetic dissimilarity among genotypes was ranged from 33 % to 71 % suggesting high amount of genetic diversity among the present genotypes. First three components of PCA explained 42.7 % and 95.6 % of total variance for SSR markers and morphological traits, respectively. These results suggested that most of the genotypes are diverse for maximum morphological traits compared SSR markers and hence first three components could be able to explain the most of variance available among the genotypes. However, existence of genetic diversity among the present genotypes found to be quite high compared to previous studies that showed high genetic similarity among the accessions originating from South Asia (Agrawal and Katiyar 2008; Hamwieh et al. 2009; Datta et al. 2011). The limited genetic variability observed in the Indian lentils was probably due to the founder effect, restriction brought to genetic variability in the indigenous gene pool, which is further narrowing the gene pool due to the adaptation in specialized environments in south Asia (Erskine et al. 1989, 1998; Ferguson et al. 1998; Kumar et al. 2004).

In order to identify the reason of high level of genetic diversity among the present genotypes, we have gone through the pedigree of these genotypes. Among the present genotypes, 16 genotypes either had Precoz in their pedigree (i.e. breeding lines generated by involving Precoz or its derivative lines as one of parents) or were introduced from ICARDA (i.e. breeding material of Mediterranean origin). The breeding line IPL 98/193 has DPL 44 as one of parents, which has Precoz in its ancestry while IPL 533 has IPL 98/155 in its pedigree which is derived from cross involving DPL 44 as one of the parents. Argentinean landrace, Precoz, is short duration and yellow cotyledon line, which has extensively been used as donor for rust resistance and earliness in the Indian breeding programs. However, clustering of these genotypes on the basis of UPGMA has not separated these 16 genotypes together in one cluster. Moreover, Precoz has also not formed a separate cluster (Fig. 1). Instead, these genotypes were clustered with genotypes bred/collected in/from India. For example, in cluster I and II, JL 1 and DPL15, respectively, were grouped with lines having Precoz in their pedigree. This is because of high selection intensity in the Indian lentil program for microsperma red lentil type (Datta et al. 2011) and frequent use of Precoz in lentil crossing programme of India in 1990 and after that (Agrawal and Katiyar 2008). Analysis of variability for morphological traits has also suggested that crossing of Precoz with indigenous lines has generated a lot of genetic variability for earliness, seed size, plant height and seeds/pod, biological and grain yield/plant leading to widen the genetic base of cultivated species. Broadly genotypes of cluster I and II are differed for seed size and days to 50 % flowering. Moreover, for plant height, genotypes of sub

cluster Ia (40–46 cm) differed from of sub-cluster Ib (31.8–43.8 cm) and cluster II (31.8–45.1 cm). Similarly variation for pods/plant was observed in sub-clusters [Ia (63–200) and Ib (70–148)] and cluster II (70–101). Also breeding line DPL 58 involved alien gene introgression from Precoz showed comparatively at par in biomass (i.e. biological yield) over the Indian cultivar JL1 developed though local selection from Indian land race (Tables 1 and 3). Breeding lines having exotic lines in their pedigree (i.e. IPLS 09-23 and IPLS 09-35) have been shown early (66–68 days to flowering) and large seeded (5.2–5.4 g/100 seeds) over the Indian cultivars but they are poor in biomass. Therefore, these genotypes can be important donors for developing early maturing and extra large seeded cultivars. This diversification of lentil gene-pool is probably due to inherent genetic differences at DNA level generated through introgression of genes from exotic lines and inclusion of exotic lines in present study. These results clearly demonstrate that introgression of alien genes into indigenous material have not only widen the genetic base of lentil at molecular level but also diversified the breeding material for agronomically important traits. The recent and past studies also showed that introgression of alien genes from exotic lines (i.e. wild species including *L. culinaris* ssp. *orientalis*, *odemensis*, *lamottei* and *ervoides*) has substantially exhibited higher variations for seed yield and its attributing traits in segregating F_2 generation indicating transgressive segregation (Gupta and Sharma 2007; Singh et al. 2013).

In conclusion, this study clearly demonstrated that involving exotic genetic materials of diverse origin in the crossing with indigenous genotypes widens the genetic base of lentil germplasm and diversified genotypes can be developed through introgression of alien genes which can help the breeders to choose the trait specific recipient and donor parents to use in their breeding programs.

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