Genetic Diversity Analysis of Lentil (Lens culinaris Medik) Cultivars Using Inter Simple Sequence Repeats Markers

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Abstract Molecular markers have emerged as useful tools to assess the genetic diversity across crops. In lentil, molecular markers are limited. The objective of the study was to explore genetic diversity and relatedness Indian and exotic lentil accessions. Genetic diversity was studied in 25 lentil cultivars using 100 ISSR markers. Out of 100 markers, 24 amplified PCR products and total of 156 alleles were identified with a mean of 6.5 alleles per marker. Genetic similarity among the genotypes ranged from 37 to 84%. UPGMA cluster analysis revealed two main clusters, and the second cluster comprised majority of genotypes with the exception of germplasm line Precoz, which did not fall in either cluster. Eleven genotype specific unique bands were also obtained which showed amplification only in particular genotypes. These unique bands can serve as potential diagnostic markers and therefore, may be of immense importance. The polymorphic markers will enhance marker repertoire to study genetic diversity in lentil and also improve understanding about the genetic base lentil cultivars.

Keywords Lens culinaris, Dendrogram, Genetic diversity, ISSR

Introduction

Lentil (Lens culinaris Medikus), an autogamous diploid (2n = 2x = 14) species with haploid genome size of 4 063 Mbp, is an important cool-season food legume crop of South Asia, North America, West Asia and North Africa and Australia (Hamwieh et al., 2009). Globally, it is cultivated for its protein-rich grains in as many as 52 countries on 3.64 million ha area with annual production of 3.60 million ton (FAOSTAT, 2011). However, about 95% of the global production comes from just ten countries, namely Canada, India, Turkey, Nepal, Australia, China, Iran, USA, Syria, and Ethiopia. India accounts for 39% (1.47 million ha) of the global acreage with 0.90 million ton production.

Assessment of genetic diversity in germplasm is prerequisite for any breeding program so that genetic gain is not limited because of narrow genetic base of parental lines (Kumar et al., 2004). Though morphological data and pedigree information have been used in the past to assess genetic diversity among the lentil varieties, these studies could not make much contribution to our knowledge due to limited phenotyping, high genotype X environment interaction, and paucity of accurate record of ancestry.

In earlier studies, molecular markers such as Simple Sequence Repeat (SSR), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD) have been preferred for genetic diversity analysis in lentil (Havey and Muehlbauer, 1989; Abo-Elwafa et al., 1995; Sharma et al., 1995, 1996; Ahmad et al., 1996; Ford et al., 1997; Udupa et al., 1999; Abe et al., 2003; Hamwieh et al., 2005, 2009; Reddy et al., 2010) and gene mapping (Eujayl et al., 1998; Tullu et al., 2003; Duran et al., 2004; Kahraman et al., 2004; Hamwieh et al., 2005). SNPs were identified across Palestinian lentil accessions for development of cost-effective and robust genotyping assays (Basheer-Salimia et al., 2015).

Kaur et al. (2014) identified SSR and SNP markers from transcriptome and EST for construction of gene-based
genetic linkage map in lentil. Several reports from our lab have established transferability of SSR markers from one legume genera/species to other (Datta et al., 2010a, 2010b; 2011; 2012; 2013a, 2013b; 2015), and observed high level of sequence conservation of microsatellite markers in legumes.

ISSR (Inter Simple Sequence Repeat) markers are inexpensive and readily adaptable technique for routine germplasm fingerprinting and evaluation of genetic relationship between accessions or genotypes (Sardana et al., 1998; Dixit et al., 2004, Edossa et al., 2007) and construction of genetic linkage maps (Abo-Elwafa et al., 1995). This technique has been used to assess genetic diversity in germplasm collection (Gilbert et al., 1999; Salimath et al., 1995), to identify cultivars (Prevost et al., 1999). The present investigation was undertaken to assess the diversity and genetic relatedness in Indian and exotic lentil genotypes with the objectives, to study the polymorphism and genetic relationship among and to identify genotype specific markers.

1 Results and Analysis
A total of 100 ISSR markers were used to test their ability in detection of DNA polymorphism. Only 24 markers produced reproducible amplification in all the selected genotype and therefore only these were considered for polymorphism assay (Table 1). The ISSR profiles generated with markers UBC-888, UBC-891, UBC-886, UBC-815, UBC-889 and UBC-858 respectively showed (Figure 1).

Table 1 Characteristics and details of 24 ISSR markers amplified in 25 lentil genotypes

| Primers     | Sequence (5’-3’) | Tm(°C) | ES (bp) | NA | NP | % P | PIC (H_o) | MI | UA No | G(i) |
|-------------|-----------------|--------|---------|----|----|-----|----------|----|-------|------|
| UBC-808     | AGAGAGAGAGAGAGAG | 51.3   | 160-1000| 6  | 5  | 83.33 | 0.46   | 0.092 | 0.49 | 1    | DPL44(900) |
| UBC-815     | CTCTCTCTCTCTCTCTG | 51.3   | 280-3000| 14 | 14 | 100.00 | 0.79   | 0.056 | 0.36 | 2    | Sehore74-3(1120), IPL525 (400) |
| UBC-824     | TCTCTCTCTCTCTCTG | 51.3   | 1100-2000| 1 | 1  | 50.00  | 0.88   | 0.440 | 1.43 | 0    | - |
| UBC-826     | ACACACACACACACACC | 51.3   | 820-2500 | 8  | 8  | 100.00 | 0.84   | 0.105 | 0.68 | 0    | - |
| UBC-827     | ACACACACACACACACG | 51.3   | 400-200  | 10 | 10 | 100.00 | 0.79   | 0.079 | 0.51 | 0    | - |
| UBC-847     | CACACACACACACACAR | 52.1   | 520-1600 | 7  | 6  | 85.77  | 0.54   | 0.074 | 0.41 | 1    | IPL525 (500) |
| UBC-854     | TCTCTCTCTCTCTCRG | 52.1   | 900-3000 | 5  | 5  | 100.00 | 0.72   | 0.144 | 0.93 | 1    | ILL8114 (3000) |
| UBC-859     | TGTTGTTGTGTTGTTG | 52.1   | 300-1400 | 7  | 7  | 100.00 | 0.52   | 0.074 | 0.48 | 0    | - |
| UBC-882     | TCTCTCTCTCCTCTCA | 48.9   | 650-3000 | 8  | 8  | 100.00 | 0.71   | 0.088 | 0.57 | 1    | DPL15 (3000) |
| UBC-810     | GAGAGAGAGAGAGAGAT | 48.9   | 300-1600 | 8  | 8  | 100.00 | 0.46   | 0.057 | 0.37 | 1    | IPL525 (300) |
| UBC-812     | GAGAGAGAGAGAGAGAA | 48.9   | 370-1200 | 8  | 8  | 100.00 | 0.29   | 0.036 | 0.23 | 1    | DPL44 (1000) |
| UBC-814     | CTCTCTCTCTCCTCTA | 48.9   | 500-2000 | 7  | 7  | 100.00 | 0.66   | 0.094 | 0.61 | 1    | P2016 (500) |
| UBC-816     | CACACACACACACACAT | 48.9   | 600-1000 | 2  | 1  | 50.00  | 0.30   | 0.300 | 0.97 | 0    | - |
| UBC-817     | CACACACACACACACAA | 48.9   | 480-2200 | 6  | 6  | 100.00 | 0.63   | 0.105 | 0.68 | 0    | - |
| UBC-819     | GTGTGTGTGTGTGTGTGA | 48.9   | 480-2500 | 6  | 6  | 100.00 | 0.71   | 0.118 | 0.77 | 0    | - |
| UBC-834     | AGAGAGAGAGAGAGAGYT | 48.9   | 900-3000 | 5  | 3  | 60.00  | 0.17   | 0.056 | 0.22 | 1    | DPL44 (3000) |
| UBC-858     | TGTGTGTGTGTGTGTGR | 49.8   | 710-1160 | 3  | 2  | 66.66  | 0.98   | 0.490 | 2.12 | 0    | - |
| UBC-865     | VDVTCTCTCTCCTCT | 46.5   | 300-1450 | 9  | 9  | 100.00 | 0.75   | 0.083 | 0.53 | 0    | - |
| UBC-888     | DVVCACACACACACA | 46.5   | 310-2000 | 10 | 10 | 100.00 | 0.57   | 0.057 | 0.37 | 0    | - |
| UBC-889     | DVDCACACACACACA | 46.5   | 400-1600 | 6  | 6  | 100.00 | 0.59   | 0.098 | 0.63 | 0    | - |
| UBC-891     | HVHTGTGTGTGTGTGTG | 46.5   | 400-1200 | 5  | 5  | 100.00 | 0.43   | 0.086 | 0.55 | 0    | - |
| UBC-861     | ACACACACACACACAC | 58.9   | 860-1160 | 3  | 2  | 66.66  | 0.16   | 0.080 | 0.34 | 0    | - |
| UBC-881     | GGGTGGGTGGGGTTTTG | 57.7   | 850-3000 | 6  | 6  | 100.00 | 0.45   | 0.075 | 0.48 | 1    | VL103 (1570) |
| UBC-865     | CCGCGCGCGCGCGCGCG | 72.6   | 960-2000 | 5  | 5  | 100.00 | 0.50   | 0.100 | 0.65 | 0    | - |

Note: Tm: Temperature of melting; ES: Estimated size of amplicons in bp; NA: Total number of amplicons; NP: Number of polymorphic amplicons; % P: Percentage polymorphism; PIC: Polymorphism information content; (H_o): Average polymorphic heterozygosity; MI: Marker index; UA: Unique amplicons; G(i): Genotype and size of unique amplicon
Figure 1 Amplification profile of lentil genotypes with ISSR markers

Note: a: UBC-888; b: UBC-891; c: UBC-886; d: UBC-815; e: UBC-889; f: UBC-858; Lane M: 100 bp DNA ladder; Lane 1-25: lentil genotypes

Total 156 amplicons were generated by the 24 ISSR markers with an average of 6.5 bands per marker with the amplicons size ranging between 160-3000 bp. One hundred and forty eight fragments (94.87%) were found polymorphic. Among the 24 polymorphic markers, 17 (UBC-810, UBC-812, UBC-814, UBC-815, UBC-817, UBC-819, UBC-822, UBC-826, UBC-827, UBC-854, UBC-859, UBC-865, UBC-881, UBC-886, UBC-888, UBC-889, UBC-891) showed 100% polymorphic bands (Table 2), among these markers most contained dinucleotide repeats. The maximum numbers of amplicons were amplified by UBC-815 (14) followed by UBC-827 (10), while UBC-816 and UBC-816 amplified only two amplicons. UBC-858 and UBC-861 amplified three amplicons each. On the other hand, UBC-816 and UBC-824 exhibited minimum polymorphism (50%).

1.1 DNA polymorphism and genetic relationship

ISSR markers analysis of lentil genotypes using Jaccard’s (1908) coefficient of genetic similarity are given in Table 1. The similarity coefficients varied from 0.37 to 0.84, the average being 0.69. Genotypes LL147 and L4147 were found to have maximum similarity (84%) which was closely followed by L4147 and VL103 (82.9%). The lowest similarity was found in between T36 and Precoz (37%) which was followed by IPL525 and T36 (40.9%).

A total of 11 unique bands were also obtained which is present only in a particular genotype. A 900 bp band was obtained in genotype DPL 44 with UBC-808 marker. Similarly the marker UBC-815 amplified two unique bands of 120 bp and 400 bp in case of Sehore 74-3 and IPL525. Table 2 includes the complete list of such genotype specific bands.

1.2 Cluster analysis

The Jaccard’s (1908) similarity coefficients based on ISSR profiles were subjected UPGMA analysis and a dendrogram of 25 lentil genotypes was constructed (Figure 2). It showed two main clusters. Cluster I contains genotypes IPL525, PL234 and PL5 which are tolerant to rust and wilt disease, the similarity between IPL525 and PL 234 is 68.10%. Cluster IIA contains genotypes DPL15, WBL58 and Sehore74-3. The similarity value between WBL 58 and Sehore74-3 is 75.30%. These two genotypes were large seeded and had ash green colour foliage with high pubescence. The cluster II B contains 4 sub-clusters; sub cluster IIB1 contains two small seeded varieties PL406 and L-9-12. The similarity value between these genotypes is 77.7%. In Sub cluster II B2, NDL 1 and LH 84-8 genotypes were grouped together; the reason for this closeness may be due to a common parent L-9-12 in
their pedigree. The similarity value as per similarity matrix table is 76.50%. Four genotypes JL-1, LL-147, L-4147 and VL-103 came together in same cluster (sub cluster II B3) and the similarity value of LL-147 with L-4147, L-4147 with VL-103 and LL-147 with VL-103 are 84%, 82.9% and 77.39% respectively. This cluster showed bootstrap replication of 36 in major cluster and 50 in sub cluster. Sub cluster II B4 comprised of genotypes LH-82-6 & DPL-62 with similarity value 78.90%. The bootstrap value of this cluster is 63.0. Both genotypes are tolerant to rust and wilt diseases also.

Table 2 Pedigree and morphological description of lentil genotypes used in study

| S.No | Varieties | Pedigree | Origin | Special feature |
|------|-----------|----------|--------|----------------|
| 1    | IPL525    | PL639 X Precoz | IIPR, Kanpur | Extra early |
| 2    | Precoz    | Argentina Variety | USA | Extra bold and rust resistance |
| 3    | DPL15     | PL406 X L4076 | IIPR, Kanpur | Tolerant to rust &wilt |
| 4    | WBL58     | JLS2 X T-36 | Berhampur, (WB) | Extra bold, drought tolerant |
| 5    | Sehore74-3 | Local Selection Form,Sehore (MP) | JNKVV | Bold seed |
| 6    | PL234     | Selection from P 230 | GBPUAT, Pantnagar | Medium bold seed, rust and wilt tolerant |
| 7    | PL5       | L4060 X LG171 | GBPUAT, Pantnagar | Rust and wilt resistance |
| 8    | K75       | Local Selection From Bundelkhand | CSAUAT, Kanpur | Wide adaptability |
| 9    | L4076     | PL234 X PL639 | IIPR, Kanpur | Bold seed tolerant to rust |
| 10   | PL406     | Selection from P495 | GBPUAT, Pantnagar | Small seeded, rust resistant & tolerant to wilt |
| 11   | L-9-12    | Selection From Local | PAU, Ludhiana | Small seed, wide adaptability tolerant to rust |
| 12   | IPL81     | K75 X PL639 | IIPR, Kanpur | Tolerant to rust & wilt |
| 13   | NDL1      | Precoz X L-9-12 | NDAUT, Faizabad | Bold seed, resistant to rust |
| 14   | Sapna (LH-84-8) | L9-12 X JLS2 | CCSHAU, Hisar | Resistant to rust |
| 15   | JL-1      | Selection From Local | JNKVV, Jabalpur | Medium bold seed, early maturity |
| 16   | LL147     | PL28467 X NP21 | PAU, Ludhiana | Small seed, resistant to rust |
| 17   | L4147     | (L3875 X P4) PKL1 | IARI, New Delhi | Resistant to rust |
| 18   | VL 103    | Local Selection From Almora Hills | VPKAS, Almora | Tolerant to rust |
| 19   | T-36      | Badaun (UP) | CSAUAT, Kanpur | Wide adaptability |
| 20   | LH-82-6   | USA-2 | CCSHAU, Hisar | Tolerant to rust & wilt |
| 21   | DPL 62    | JLS1 X LG171 | IIPR, Kanpur | Resistant to rust & wilt |
| 22   | ILL6002   | ILL4349 X ILL4605 | ICARDA | Extra bold & drought tolerant |
| 23   | ILL-8114  | Pakistan Variety | ICARDA | - |
| 24   | P-2016    | DPL61 X DPL60 | IIPR, Kanpur | Extra bold, yellow coat |
| 25   | DPL-44    | L9-12 X PRECOZ | IIPR, Kanpur | Extra bold, yellow coat, rust resistant |

Cluster IIC contains two lentil varieties K-75 and L-4076. The similarity value as per similarity matrix table value is 73.50%. Finally the cluster II D contains 2 sub clusters. P-2016, ILL-8114 and ILL-6002 were grouped into same sub cluster II D1. The breeding material P-2016 is derived from DPL44, which is the derivative of Precoz. Both ILL-6002 and P-2016 are extra large seeded and with light green foliage. ILL-6002 had similarity value with P-2016 & ILL-8114 as 74.50% and 78.40% respectively. The variety DPL-44 showing extra bold yellow seed coat character of sub cluster II D2 is highly similar with Cluster II D1 with similarity value of 66.6%.

This exotic cultivar Precoz which is of Argentine origin was sharply isolated from the clusters. This variety is distinct with others morphologically also due to light green foliage, extra large seeded, low pubescence and resistant to rust. The genotype T-36 is isolated one but it is similar in character to the cluster II. IPL-81, which is derived from K75 and PL639, positioned itself between sub-clusters IIC and IID the first parent was grouped in the adjacent cluster IIC.

1.3 Multi scaling analysis with two and three dimensions

The two dimensional plot obtained through multidimensional analysis revealed the spatial separation of genotypes with each other (Figure 3; Figure 4). The Sehore74-3 and JL1, both varieties are very near to each other which
might be due to their origin and adaptation from same place Jabalpur, in India. Morphologically also they are similar to each other in relation to foliage colour, size and hairiness. The maximum distance was found between genotype T-36 and Precoz which is also evident by their genetic similarity (37%), which is lowest among all pair of genotypes. The next distant pair was IPL525 and T36, with genetic similarity of 40.9%. The average similarity was found to be 69.32% among all genotypes based on ISSR marker data.

The genotype LL147 and L4147 were appears closely in three dimensional plot which proves that they are highly similar which is also clear by their high genetic similarity value (84%) followed by L4147 and VL103 (82.9%) (Figure 3; Figure 4). The maximum distance appears between T-36 and Precoz (37.0%) which was followed by IPL525 and T36 (40.9%). The similar kind of result was obtained in two-dimensional analysis also. The average similarity was found to be 69.32% among all the genotypes based on ISSR data, which indicated relatively narrow genetic base of lentil.

2 Discussion
Genetic variations in crop plants has continued to narrow down due to continuous selection pressure for specific traits i.e. yield, thus rendering them more vulnerable to disease and insect epidemics and jeopardizing the potential for long term sustained genetic improvement.

Therefore, it is extremely important to study the genetic relationship of the existing modern-day genotypes in comparison with their ancestors and related species. This will not only provide information on their genetic distance and phylogenetic relationship but will also indicate a chance of finding new and useful genes.

Figure 2 Dendrogram of 25 lentil cultivars depicting genetic relationship derived from UPGMA cluster analysis using the Jaccard’s similarity coefficients based on 156 amplicons from 24 ISSR markers
In the present study, genomic diversity was studied in twenty-five lentil genotypes (Indian/Exotic genotypes and collection). Out of 100 ISSR markers tested, 24 produced unambiguous amplicons. Amplified markers produced easily scorable bands ranging from 160 to 3000 bp in length. A total of 156 ISSR fragments were amplified with an average of 6.5 amplicons per marker. The most informative markers were selected on the basis of extent band polymorphism, detected by the individual marker. Also, 11 unique bands were found only in particular genotypes. A 900 bp band was obtained in genotype DPL-44 with marker UBC-808. Similarly, the marker UBC-815 amplified two unique bands of 1120 bp and 400 bp in case of Sehore74-3 and IPL525.

Figure 3 Two dimensional scaled plot of Lentil cultivars

Figure 4 Three dimensional scaled plot of Lentil cultivars
These unique bands specific to different genotypes can serve as potential diagnostic markers in identification and differentiation of genotypes and therefore may be of immense importance in Intellectual Property Right (IPR) issues related to Plant Variety Protection and Farmer’s Right (PVP & FR) Act.

Efficiency of markers and their utility in terms of polymorphism and quantitative estimation could be expressed in mean heterozygosity and marker index (Choudhury et al., 2007). The average Hav, (Hav)_p, and MI were found to be 0.579, 0.124 and 0.640, respectively. The minimum (0.16) and maximum (0.88) PIC value were found with markers UBC-861 and UBC-824, while the lowest (0.056) and highest (0.490) heterozygosity were found with markers UBC- 815 and UBC-858 respectively. The average genetic distance coefficient value among the all genotypes was 0.31 based on ISSR markers, which indicated a limited degree of genetic variation in the lentil material. The genotype LL147 and L4147 were found to have maximum similarity (84%) which was closely followed by L4147 and VL103 (82.9%). The lowest similarity was found between T36 and Precoz (37%) which was followed by IPL525 and T36 (40.9%) The average similarity was found to be 69.32 %.

The information on genetic diversity among these lentil cultivars will be helpful to lentil breeders in selection of appropriate hybridizing parents in developing superior cultivars.

3 Materials and Methods

3.1 Plant materials

The experimental material comprised of 25 lentil genotypes from different lentil growing states of India and other countries (Table 2). All seed material for this study was obtained from the germplasm unit of Indian Institute of Pulses Research, Kanpur (India).

3.2 DNA Isolation and PCR amplification

Seeds of 25 lentil genotypes were germinated under etiolated conditions on paper towel soaked in sterilized water. One-week-old seedlings were ground in preheated CTAB buffer and incubated at 60°C for 1 h. The aqueous phase containing DNA was separated using chloroform: isooamyl alcohol (24: 1) (Abdelnoor et al., 1995). The DNA was precipitated with chilled isopropanol and the pellet was dissolved in 100 µl of T10E1 buffer. The RNA was eliminated by adding 0.5 U of RNAse. DNA concentrations were quantified by measuring absorbance using Hoefer® Dyna Quant® 200 DNA fluorometer (Amersham Biosciences, Piscataway, NJ, USA) and stocks were maintained at 25 ng/µl.

One hundred ISSR markers of the UBC series were used to study genetic similarity in 25 lentil genotypes. PCR reaction mixture (20 µl) consisted of 20 ng of template DNA, 1X PCR buffer, dNTPs (Banglore Genei, Bangalore) 2.5 nM each, 10 pM primerand 0.6 U of Taq DNA polymerase (Banglore Genei, Bangalore). The thermal cycling program was carried out in a PTC 200 thermal cycler (MJ Research, Biorad). The PCR program had an initial denaturation step at 94°C for 2 min, followed by 41 cycles of denaturation at 94°C for 1 min, primer annealing(depending upon the Tm of respective marker) for 1 min and DNA extension at 72°C for 3 min. A final extension step was given at 72°C for 4 min. The amplified DNA fragments were resolved on ethidium bromide stained agarose gel (2%) in 1X TAE buffer at 50 V. A 100 bp DNA ladder (MBI Fermentas) was used as a molecular weight marker for determining the molecular weight of the amplified products.

3.3 Scoring and Data analysis

The amplification profiles of each marker in different genotypes were scored and recorded as presence [1] or absence [0] of bands and binary quantitative data matrix was constructed. Unique alleles were defined as thosedetected in only one genotype. The presence and absence of alleles for each marker was recorded for all genotypes and then converted into genetic similarity matrix using Jaccard (1908) similarity coefficient in NTSYS-PC 2.1 software. (Rohlf, 1998). The similarity coefficients were used to construct a dendrogram depicting genetic relationship using unweighted pair group mean average (UPGMA) method (Sneath and Sokal, 1973). The Polymorphism Information Content (PIC) values were calculated following the formula described by Botstein et al. (1980):
Polymorphism information content

\( (\text{PIC}_i) = 1 - \sum_{j=1}^{n} P_{ij} \)

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

The arithmetic mean heterozygosity for a marker (Hav) was calculated by Hav = Hn / n \((n= \text{number of markers on loci analyzed})\) (Powell et al., 1996). Heterozygosity for a marker (Hn) = 1 -\( p_i^2 \), where \( p_i \) is the allele frequency of \( i^{th} \) allele (Nei et al., 1979). The average heterozygosity for polymorphic markers was derived by (Hav) \( p = \delta H_n / np \) ( \( np = \text{no. of polymorphic markers or loci} \)). Marker Index was calculated as the product of two functions: DI (Diversity index) and EMR (Effective Multiplex Ratio). DI of a primer is defined as 1 -\( p_i^2 \), where \( p_i \) is the frequency of the ith allele (band). EMR of a primer is defined as “the product of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay” (Prevost and Wilkinson, 1999).

References

Abdelnoor R.V., Barros E.G., and Moreira M.A., 1995, Determination of genetic diversity within Brazilian soybean germplasm using random amplified polymorphic DNA techniques and comparative analysis with pedigree data, Braz. J. Genet., 18(2): 265-273

Abe J., Xu D.H., Suzuki Y., Kanazawa A., and Shimamoto Y., 2003, Soybean germplasm pools in Asia revealed by nuclear SSRs, Theor. Appl. Genet., 106(3): 445-453

Abo-Elwafa A., Murai K., and Shimada T., 1995, Intra- and inter-specific variations in Lens revealed by RAPD markers, Theor. Appl. Genet., 90(3-4): 335-340

Ahmad M., and McNeil D.L., 1996, Comparison of crossability, RAPD, SDS-PAGE and morphological markers for revealing genetic relationships within and among Lens species, Theor. Appl. Genet., 93(5-6): 788-793

Basheer-Salimia R., Camilli B., Scacchi S., Noli E. and Awad M., 2015, Assessment of genetic diversity in lentils (Lens culinaris Medik.) based on SNPs, Genet. Mol. Res., 14(2): 5870-5878

Botstein B., White R.L., Skolnick M., Davis R.W., 1980, Molecular markers in plant genome analysis, Am. J. Hum. Genet., 32: 314-331

Choudhury P.R., Singh I.P., Verma A.K., George B., and Datta S., 2007, Identification of genotype specific markers and assessment of genetic relatedness among pigeonpea cultivars using RAPD, J. Food Leg., 20(1): 12-15

Datta S., Kaashyap M., and Kumar S., 2010a, Amplification of chickpea-specific SSR primers in Cajanus species and their validity in diversity analysis, Plant Breed., 129(3): 334-340

Datta S., Mahfooz S., Singh P., Choudhary A.K., Singh F., and Kumar S., 2010b, Cross-genera amplification of informative microsatellite markers from common bean and lentil for the assessment of genetic diversity in pigeonpea, Physiol. Mol. Biol. Plants., 16(2): 123-134

Datta S., Tiwari S., Kaashyap M., Gupta P.P., Choudhary P.R., Kumari J., and Kumar S., 2011, Genetic similarity analysis in lentil using cross-genera legume sequence tagged microsatellite site markers, Crop Science, 51(6): 2412-2422

Datta S., Kaashyap M., Singh P., Gupta P.P., Anjum K.T., Mahfooz S., and Gupta S., 2012, Conservation of microsatellite regions across legume genera enhances marker repertoire and genetic diversity study in phaseolus genotypes, Plant Breed., 131(2): 307-311

Datta S., Singh P., Mahfooz S., Choudhary A.K., Chaturvedi S.K., and Nadarajan N., 2013a, Conservation of genic and genomic microsatellite regions across legume genera allows marker transferability for polymorphism studies in pigeonpea, Aus. J. Crop Sci., 7(13): 1990-1997

Datta S., Singh P., Mahfooz S., Patil P., Choudhary A.K., Agbagwa I.O., and Nadarajan N., 2013b, Novel genic microsatellite markers from Cajanus scarabaeoides and their comparative efficiency in revealing genetic diversity in pigeonpea, J. Genet. 92(1): 1-7

Datta S., Kaashyap M., and Gupta P.P., 2015, Development of EST derived microsatellite markers in chickpea and their validation in diversity analysis, Ind. J. Biotech., 14(1): 55-58

Dixit G.P., and Katiyar P.K., 2004, Genetic base of lentil (Lens culinaris) varieties and breeding lines developed in India, Indian J. Agric. Sci., 74(11): 625-627

Duran Y., Fratini R., Garcia P., and Vega M.P., 2004, An interspecific genetic map of Lens, Theor. Appl. Genet., 108(7): 1265-1273

http://dx.doi.org/10.1007/s00122-003-1542-3 PMid:14676948
Edossa F., Kassahun T., and Endashaw B., 2007, Genetic diversity and population structure of Ethiopian lentil (Lens culinaris Medikus) landraces as revealed by ISSR marker, Afr. J. Biotechnol., 6(12): 1460-1468

Ejiao Y., Erskine W., Bayaa B., Baum M., and Pehu E., 1998, Fusarium vascular wilt in lentil: inheritance and identification of DNA markers for resistance, Plant Breeding, 117(5): 497-499

http://dx.doi.org/10.1111/j.1439-0523.1998.tb01982.x

FAOSTAT. 2011, Agricultural Data on Primary Crops, FAO

Gilbert J.E., Lewis R.V., Wilkinson M.L., and Kaligari P.D.S., 1999, Developing an appropriate strategy to assess genetic variability in plant germplasm collections, Theor. Appl. Genet., 98(6-7): 1125-1131

http://dx.doi.org/10.1007/s001220051176

Hamweih A., Udupa S.M., Sarkar A., Jung C., and Baum M., 2009, Development of new microsatellite markers and their application in the analysis of genetic diversity in lentils, Breed. Sci., 59(1):77-86

http://dx.doi.org/10.1270/jsbbs.59.77

Hamweih A., Udupa S.M., Choutmane W., Sarker A., Dreyer F., Jung C., and Baum M., 2005, A genetic linkage map of Lens sp. based on microsatellite and AFLP markers and the localization of Fusarium vascular wilt resistance, Theor. Appl. Genet., 110(4): 669-677

http://dx.doi.org/10.1007/s00122-004-1892-5 PMid:15650814

Havey M.H., and Muehlbauer F.J., 1989, Variability for fragment length and phylogenies in lentil, Theor. Appl. Genet., 77(6): 839-843

http://dx.doi.org/10.1007/BF00268336 PMid:24232901

Jaccard P., 1908, Nouvelle recherches sur La distribution florale, Bull. Soc. Vaud. Sci. Nat., 44(163): 223-270

Kahraman A., Kusmenoglu I., Aydin N., Aydogan A., Erskine W., and Muehlbauer F.J., 2004, QTL mapping of winter hardiness genes in lentil, Crop Sci., 44(1): 13-22

http://dx.doi.org/10.2135/cropscic2004.0013

Kaur S., Cogan N.D., Stephens A., Noy D., Butsch M., and Forster J.W., 1999, Allelic variation at (TAA) and (TAC) repeats in lentil (Lens culinaris Medik.) enable candidate gene selection for boron tolerance, Theor. Appl. Genet. 127(3): 669-677

http://dx.doi.org/10.1007/s00122-002-1283-9 PMid:10628157

Kumar S., Gupta S., Chandra S., and Singh B.B., 2004, How wide is the genetic base of pulse crops, In: Ali M., Singh B.B., Kumar S., and Dhar V., (eds.), Pulses in New Perspective, ISPRD, Kanpur, India, pp.188-210

Nei M., and Li W.H., 1979, Mathematical model for studying genetic variation in terms of restriction endonucleases, Proc. Natl. Acad. Sci., 76(10): 5269-5273

http://dx.doi.org/10.1073/pnas.76.10.5269 PMid:291943 PMCid:PMC413122

Powell W., Mackray G.C., and Provan J., 1996, Polymorphism revealed by simple sequence repeats, Trends in Plant Sci., 1(7): 215-222

http://dx.doi.org/10.1016/S1360-1385(96)86898-0

Prevost A., and Wilkinson M.J., 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars, Theor. Appl. Genet., 98(98): 107-112

http://dx.doi.org/10.1007/s001220051046

Reddy M.R.K., Rathour R., Kumar N., Katoch P., and Sharma T.R., 2010, Cross-genera legume SSR markers for analysis of genetic diversity in Lens species, Plant Breeding, 129(5): 514-518

Rohlff F.J., ed., 1998, NTSYS-pc: Numerical taxonomy and multivariate analysis system, Version 2.1, Exeter Publications, New York, USA

PMcid:PMC24543

Salimath S.S., Oliveira A.C., Godwin I.D., and Bemnetzen J.L., 1995, Assessment of genome origins and genetic diversity in the genus Elesine with DNA markers, Genome, 38(4): 757-763

http://dx.doi.org/10.1139/g95-066 PMid:7672607

Sardana S., Gastam N.K., Kumar D., Sapra R.L., and Mithal S.K., 1998, Genetic divergence in lentil germplasm, Indian J. Plant Genet. Resour., 11(1): 25-30

Sharma S.K., Dawson I.K., and Wauge R., 1995, Relationships among cultivated and wild Lentils revealed by RAPD analysis, Theor. Appl. Genet., 91(4): 647-651

http://dx.doi.org/10.1007/BF00223292 PMid:24169893

Sharma S.K., Knox M.R., and Ellis T.H.N., 1996, AFLP analysis of diversity and phylogeny of Lens and its comparison with RAPD analysis, Theor. Appl. Genet., 93(5-6): 751-758

http://dx.doi.org/10.1007/BF00240722 PMid:24162404

Sneath P.H.A., and Sokal R.R., eds., 1975, Numerical taxonomy: the principles and practice of numerical classification, Freeman and Company, San Francisco, USA

Tullu A., Buchwaldt L., Warkentin T., Taran B., and Van登enberg A., 2003, Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI320937 germplasm of lentil (Lens culinaris Medikus), Theor. Appl. Genet., 106(3): 428-434

PMid:12589542

Udupa S.M., Robertson L.D., Weigand F., Baum M., and Kahl G., 1999, Allelic variation at (TAA), microsatellite loci in a world collection of chickpea (Cicer arietinum L.) germplasm, Mol. Gen. Genet., 261(2): 354-3

http://dx.doi.org/10.1007/s004380050976 PMid:10102371