Assessment of genetic diversity in lettuce (*Lactuca sativa* L.) germplasm using RAPD markers

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Abstract

The importance of germplasm characterization is an important link between the conservation and utilization of plant genetic resources in various breeding programmes. In the present study, genetic variability and relationships among 25 *Lactuca sativa* L. genotypes were tested using random amplified polymorphic DNA (RAPD) molecular markers. A total of 45 random decamer oligonucleotide primers were examined to generate RAPD profiles, out of these reproducible patterns were obtained with 22 primers. A total of 87 amplicon were obtained, out of which all were polymorphic and 7 were unique bands. The level of polymorphism across genotypes was 100% as revealed by RAPD. Genetic similarity matrix, based on Jaccard's coefficients ranged from 13.7 to 84.10% indicating a wide genetic base. Dendrogram was constructed by unweighted pair group method with arithmetic averages method. RAPD technology could be useful for identification of different accessions as well as assessing the genetic similarity among different genotypes of lettuce. The study reveals the limited genetic base and the needs to diversify using new sources from the germplasm.

Keywords *Lactuca sativa* L. · RAPD · Genetic diversity · Molecular markers

Introduction

Lettuce (*Lactuca sativa* L.) belongs to the family *Asteraceae* having chromosome number $2n = 18$. The four species of lettuce, i.e., *L. sativa*, *L. serriola*, *L. varisa* and *L. saligna* originated in the mediterranean region. *L. sativa* L. is considered as one of the most important vegetables in the group of leafy vegetables. *L. sativa* is an annual glabrous herb. The inflorescence is composed of 7–15 (35) yellow ligules (florets). The fruit (achene) has 5–7 setose ribs on each side, a beak and a white pappus (Kristkova et al. 2008). Large numbers of leaves are produced of which outer leaves are usually green to dark green and inner leaves are light green to whitish which are tender and crisp. It is especially important as a commercial crop in Asia, North and Central

America, and Europe. China followed by US, Spain, Italy, India and Japan are among the world's largest producers. It is almost exclusively used as a fresh vegetable in salads, but some forms are also cooked. Lettuce is rich in vitamin A and minerals like calcium and iron. It also contains proteins, carbohydrates and vitamin C. Its tender leaves and heads are chopped and used as salad with salt and vinegar. Lettuce is also known to be a sedative, diuretic and expectorant (Srivastava et al. 2016).

Being an economically important cultivated crop, genetic diversity is essential for successful breeding and creation of new cultivars. Genetic diversity can be estimated from pedigree analysis or from multivariate analysis carried out on a large number of plant attributes. Genetic diversity can be estimated using the morphological features, but variation in morphological traits is limited. Moreover, environmental and growing conditions also affect these features. Protein markers such as isozyme have been extensively developed but their use has declined due to lack of polymorphism. DNA based markers are considered the most suitable for estimation of genetic distances and for characterization of cultivars. Developments in DNA based technologies have revolutionized the utilization of molecular markers in genetics and breeding studies (Kumar et al. 2015). RAPD analysis

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is a powerful tool for detection of sequence polymorphism (Welsh and McClelland 1990). Lettuce was investigated for genetic diversity so that it would facilitate different genotypes of lettuce to be identified as genetic donors in different breeding programs and also to avoid confusion regarding the cultivar identity associated with morphological characteristics and multiple cultivar registration. The DNA markers of the RAPD type are a valuable option, because they consist of relatively simple procedures, with the advantage of not being subject to environmental influence or epistatic interactions and offering high sensitivity in detecting variability (Gaur et al. 2016). The present investigation was carried out with 25 genotypes of lettuce to characterize them at molecular level using RAPD technology. The aim of this study was to establish genetic relationship among different genotypes of lettuce.

Materials and methods

Genetic material

Twenty-five lettuce genotypes were obtained from the Department of Vegetable Science, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India (Table 1).

DNA extraction and RAPD assay

Healthy leaf samples (1 g/plant) were obtained from all the 25 lettuce genotypes which were ground individually in liquid nitrogen and were isolated by CTAB method (Doyle and Doyle 1990) with some modifications. The PCR amplification conditions were as described by Williams et al. (1990) with minor modifications. RAPD analysis was carried out by 45 random decamer oligonucleotide primers (Sigma) in thermocycler (BIORAD MJ Mini DNA Amplification System). Amplifications were carried out in a 20 µl reaction mixture containing PCR Buffer 2 µl; 0.56 µl MgCl₂; 2.0 µl dNTP; 0.3 µl *Taq polymerase* enzyme, 0.2 µl of each primer, 11.94 µl distilled water and 3.0 µl DNA samples of different genotypes of lettuce. Thermal profile consisted of 32 cycles of 94 °C for 30 Seconds, 36 °C for 30 Seconds and 72 °C for 1 min followed by extension for 10 min at 72 °C. The amplified products were electrophoresed on 1.2% agarose gel in 1× TAE buffer and stained using ethidium bromide. The size of the fragment was estimated using Gene ruler 1 kb DNA ladder (Bangalore Genei, Bangalore, India). After electrophoresis, the gel was photographed under ultraviolet light using Gel Documentation System (Alpha Imager, USA).

Table 1 List of lettuce (*Lactuca sativa* L.) genotypes used in present investigation

Code	Genotype name
G1	CGN 10944-2
G2	CGN-14651
G3	CGN-05169
G4	UHF-Sel.-07
G5	CGN 10944-3
G6	CGN-05198
G7	UHF-Sel.-03
G8	CGN-05167
G9	CGN-20721
G10	CGN-04987
G11	CGN 10944-1
G12	CGN-04990
G13	CGN-04933
G14	CGN-04934
G15	CGN-14629
G16	UHF-Sel.-06
G17	UHF-Sel.-04
G18	CGN-19009
G19	CGN-09373
G20	CGN-11358
G21	Great lakes
G22	CGN-19088
G23	CGN-04543
G24	UHF-Sel.-01
G25	UHF-Sel.-02

Data analysis

Each amplified band was coded by the primer used and its size is given in base pair. Data analysis was carried out only for those primers which gives scorable patterns for the genotypes under study. Co-migrating bands were considered to represent the same locus and thus treated as a same band while scoring. The presence of an amplified product was designated as “1” and the absence was marked as “0”. NTSYS-pc, version 2.02 or version 1.8 (Numerical Taxonomy System Exeter Software) was used to perform cluster analysis of the complete RAPD data. Similarity between accessions was estimated using Jaccard's coefficient and calculated as: $J = A/(N - D)$, where A is the number of positive matches (i.e., the presence of bands in both the samples), D is the number of negative matches (i.e. the absence of bands in both the samples), N is the total sample size including the number of matches and mismatches. Similarity estimates were analyzed by unweighted pair group method with arithmetic averages (UPGMA) and the resulting clusters were expressed as dendrogram in the lettuce genotypes.

Results

RAPD studies

RAPD studies of lettuce genotypes (*L. sativa* L.) were carried out by a total of 45 random decamer oligonucleotide primers. Only 22 primers were informative and gave scorable banding pattern and polymorphism. The total number of amplicons was 87, out of which 7 were found to be genotype-specific bands. The percentage of total polymorphism was 100% and no monomorphic band was obtained. The seven genotype-specific amplified bands were present in lettuce genotype G1 (CGN 10944-2) with primers OPA-05, OPA-07₃₀₀₀, OPA-07₂₀₀₀, OPA-07₁₇₅₃, OPA-10, in genotype G24 (UHF-Sel.-01) with primer OPB-04 and in genotype G16 (UHF-Sel.-06) with primer OPB-17. Such informative primers identified either singly or in combination may be of great use to identify unknown genotypes and to monitor the designed crosses involving these genotypes (Tables 2, 3; Figs. 1, 2). UPGMA analysis of RAPD markers and pairwise genetic similarity among 25 genotypes of lettuce was estimated and ranged from 0 to 1, indicating that the genetic distance among the genotypes

tested was high and the genotypes were grouped into two major clusters. The maximum value obtained was 0.841 and minimum value obtained was 0.137.

Similarity index

Similarity index was calculated considering all the bands obtained by 22 random decamer primers. The least similarity coefficient value (13.7%) was observed between genotypes G7 (UHF-Sel.-03) and G17 (UHF-Sel.-04) and the maximum value of similarity (84.1%) was observed between genotypes G14 (CGN-04934) and G15 (CGN-14629).

Dendrogram

From the dendrogram obtained after RAPD analysis, it is clear that all the cultivars of lettuce could be grouped into two main clusters: A and B. The cluster A contains only one genotype G7 (UHF-Sel.-03) whereas the cluster B is further divided into subclusters B1 and B2. Cluster B1 was further divided into B1a and B1b. Cluster B1a contains genotypes G9 (CGN-20721), G18 (CGN-19009) and G8 (CGN-05167). Cluster B1b contains genotypes G23 (CGN-04543), G19 (CGN-09373), G20 (CGN-11358), G10 (CGN-04987),

Table 2 Total number of amplified fragments and number of polymorphic fragments generated by PCR using 22 informative random decamer oligonucleotide primers

RAPD primers name	Sequence (5'–3')	Total number of amplified bands	Total number of polymorphic bands	Genotype specific bands
OPA-01	CAGGCCCTTC	5	5	0
OPA-03	CAGCCACAGT	7	7	0
OPA-04	AATCGGGCTG	3	3	0
OPA-05	AGGGGTCTTG	4	4	1
OPA-07	GAAACGGGTG	7	7	3
OPA-09	GGGTAACGCC	2	2	0
OPA-10	GTGATCGCGC	4	4	1
OPA-11	CAATCGCCGT	6	6	0
OPA-15	TTCCGAACCC	4	4	0
OPA-16	AGCCAGCGAA	5	5	0
OPA-17	GACCGCTTGT	3	3	0
OPA-18	AGGTGACCGT	3	3	0
OPB-03	CATTGACGCA	3	3	0
OPB-04	GGA CTGGAGT	5	5	1
OPB-06	TGCTCTGCCC	4	4	0
OPB-08	GTCCACACGG	1	1	0
OPB-10	CTGCTGGGAC	4	4	0
OPB-11	G TAGACCCGT	2	2	0
OPB-17	AGGGAACGAG	4	4	1
OPC-09	CTCACCGTCC	3	3	0
OPC-10	TGTCTGGGTC	4	4	0
OPC-19	GTTGCCAGCC	4	4	0
Total		87	87	7

Table 3 Summary of RAPD amplified fragments from 25 genotypes of lettuce (*Lactuca sativa* L.) using 22 random decamer oligonucleotide primers

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25	Total
Number of bands scored	58	27	31	34	39	45	16	39	37	44	47	34	45	34	45	41	42	37	30	42	36	39	32	41	35	950
Number of polymorphic bands	58	27	31	34	39	45	16	39	37	44	47	34	45	34	45	41	42	37	30	42	36	39	32	41	35	950
Average number of fragments per primer	2.6	1.2	1.4	1.5	1.7	2.0	0.7	1.7	1.7	2.0	2.1	1.5	2.0	1.5	2.0	1.9	1.9	1.7	1.4	1.9	1.6	1.8	1.5	1.9	1.6	1.9
Average number of polymorphic bands per primer	2.6	1.2	1.4	1.5	1.7	2.0	0.7	1.7	1.7	2.0	2.1	1.5	2.0	1.5	2.0	1.9	1.9	1.7	1.4	1.9	1.6	1.8	1.5	1.9	1.6	1.9
Average of total polymorphic bands (%)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

G3 (CGN-05169), G2 (CGN-14651). Cluster B2 further bifurcated into two main clusters B2a and B2b. B2a further divided into two subclusters which included genotypes G25 (UHF-Sel.-02) and G16 (UHF-Sel.-06.) Cluster B2b separated out into several other subclusters. Under this cluster genotype G12 (CGN-04990) singled out itself from the rest of the genotypes which were grouped within this cluster at 43% genetic similarity. Rest of the genotypes which were grouped within cluster B2 were G22 (CGN-19088), G21 (Great Lakes), G6 (CGN-05198), G13 (CGN-04933), G5 (CGN 10944-3), G11 (CGN 10944-1), G14 (CGN 04934), G15 (CGN 14629), G4 (UHF sel.07), G17 (UHF sel.04), G24 (UHF sel.01) and G1 (CGN10944-2). It is clear that the cultivars grouped into one cluster are closely related to each other and may have originated from same parental line (Fig. 3).

Discussion

Accurate identification and characterization of different germplasm resources are important for cultivar development, certification and breeder's right protection (Hale et al. 2006). RAPD markers offer a great potential in determining the genetic variation and have been efficiently used to give reliable and reproducible results for estimating the variations. In an attempt to examine the potential of RAPD markers for the ability to assess the variability in lettuce genotypes, a set of 45 random decamer RAPD primer were used for 25 genotypes of lettuce germplasm. In RAPD analysis out of 45 random decamer primers, only 22 showed the amplification. The percentage of polymorphism was obtained 100% with the informative RAPD markers. The result obtained in our study was in accordance with those obtained by Al-Redhaimam et al. (2005) who also achieved considerable degree of polymorphism with all the thirteen primers in 13 lettuce genotypes. Hwang et al. (2002) also recorded 87.9% polymorphism with 35 cultivars of lettuce. However, the results obtained in the present study varied from those of Tardin et al. (2003), who showed that there was 67.03% polymorphism among the amplified fragments. In a total of 82 developed bands 55 were polymorphic. According to Yang et al. (2007), a total of 112 RAPD bands were generated by 11 primers amongst which 69 were polymorphic within *L. sativa*.

During the present studies, seven unique bands were obtained in RAPD. According to Yang et al. (2007) 20 bands were unique in specific cultivars. Among these, three were specific in a plant type: one in Korean leafy type, one in crisp head type, and one in cos type lettuce. From the dendrogram obtained after RAPD analysis, it is clear that all the cultivars of lettuce have been grouped into two main clusters A and B. The cluster A contains only one genotype

Fig. 1 RAPD banding pattern of OPA-10 primer in 25 genotypes of lettuce (*Lactuca sativa* L.)

Fig. 2 RAPD banding pattern of OPB-03 primer in 25 genotypes of lettuce (*Lactuca sativa* L.)

Fig. 3 Dendrogram for 25 genotypes of lettuce (*Lactuca sativa* L.)

G7 (UHF-Sel.-03) which separated out itself from the rest of the genotypes at 23% genetic similarity. The remaining 24 genotypes were grouped in cluster B. Maximum genetic divergence was observed between G7 (UHF-Sel.-03) and G17 (UHF-Sel.-04) and minimum was present in G14 (CGN-04934) and G15 (CGN-14629). The results of Al-Redhaimam et al. (2005) reported UPGMA analysis of 13 lettuce genotypes based on 83 RAPD markers differentiated into two major groups, which were in accordance with those obtained in the present study. According to Yang et al. (2007) in the phylogenetic analysis among *Lactuca* species, *L. saligna*, *L. serriola*, and *L. georgica* clustered in a sister branch of the *L. sativa* complex. Two *L. virosa* accessions show the highest intra-specific relationships. *L. perennis* outlied from all the other *Lactuca* species at a genetic similarity of 0.53. Forty-four lettuce accessions were subdivided into discrete branches according to plant type: crisphead, butterhead, and stem type, with some exceptions. The leafy- and cos-type accessions were intermingled in other groups with no discrete branch indicating that these are more diverse than others. The clustering varied from those obtained by Tardin et al. (2003) and Kesseli et al. (1994) who obtained six and eight genotype groups, respectively. The RAPD technique was found useful in revealing genetic variation among different genotypes of lettuce. All the 22 primers of RAPD have clearly differentiated 25 lettuce genotypes. The informative primers can thus be efficiently used for the identification of genotypes and to study the genetic relationship among them. The primers which gave unique band(s) should be employed for assessment of intraspecific diversity and variety/genotype identification in lettuce.

From the data obtained, it can be concluded that the RAPD technology can be useful in current breeding programmes allowing the identification of different genotypes as well as assessing the genetic similarity among different genotypes of lettuce which would facilitate their use as identified genetic donors in future breeding programme. It can also be concluded that RAPD analysis can be useful in certification scheme for releasing certified plant material. The application of this technique may alleviate some of the confusion of cultivar identity associated with morphological characteristics and multiple cultivar registration.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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