

**Analysis of genetic diversity in linseed using molecular markers**

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**Abstract:** Broadening the genetic base of linseed cultivars to maintain, evaluate and utilize germplasm efficiently and effectively, it is essential to investigate the extent of genetic diversity present in the germplasm. The major aim of the study was to analyze the extent and distribution of genetic variation among various linseed genotypes using Random amplified polymorphic DNA (RAPD) markers. Out of 15 RAPD primers used, seven primers had given clear and polymorphic bands. Total number of loci traced by these primers was 68 and all were polymorphic. Polymorphism of these genotypes was 100 %. Amplification product ranged from 250 bp to 3000 bp. Similarity coefficient of selected genotypes ranged from 0.5 (50 %) to 0.93 (93 %). Maximum similarity of 0.93 was shown by LS-11 with LS-17 (93 %). Minimum similarity of 0.50 was shown by LS-19 with LS-32 and LS-04 (50 %). Genetic distance of RAPD for the total pairs of genotypes varied from 0.1 to 0.41. LS-19 is the most diverse genotype showing maximum genetic distance of 0.41 (41 %) from rest of the genotypes. Cluster analysis revealed that genotypes LS-19, LS-32, LS-3 and LS-25 were diverse and promising accessions for use in national variety development program.

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## 1. Introduction

Linseed (*Linum usitatissimum* L.,  $2n = 2x = 30$ ), is an annual self-pollinated crop that is commercially grown as a source of stem fibre and seed oil Cloutier *et al.*, (2012). The fibers are used in textile as well as automobile and construction industries, while flax seeds are used in animal feed and human food, as source of omega-3-fatty acids and biologically active lignans Smy'kal *et al.*, (2012.) Linseed is used in treatment of some provocative human and animal diseases in Pakistan and its oil is mainly utilized in the preparation of paints, printing ink, varnish, resins and several innumerable by-products. The oil cake is a most valuable feeding cake for animals. Linseed imparts numerous health benefits, including anti-hypercholesterolemic and anti-carcinogenic effects and is also beneficial in the development of brain and retinal tissues of infants Payne, (2000). The success of any breeding programme mainly depends upon magnitude of genetic variability in hand and continuous supply of new germplasm for sustainable production Shanmugam and Shreerangaswamy, (1982); Smith *et al.*, (1991). The efficient use of genetic variation can only be optimized, if this diversity is evaluated, quantified and classified in some way Amurrio *et al.*, (1995). Molecular markers act as DNA signposts to locate gene (s) for a trait of interest on a plant chromosome, and are widely used to study the organization of plant genomes and for

the construction of genetic linkage maps. Molecular markers provide a direct measure of genetic diversity and go beyond indirect diversity measures based on agronomic traits or geographic origin Li *et al.*, (2000). Due to advances in the field of molecular genetics a variety of different techniques to analyze genetic variation has emerged during the last few decades Argawal *et al.*, (2008); Kalendar and Schulman, (2006). The use of PCR based markers, such as RAPD is an alternative method in detection of polymorphism in linseed Williams *et al.*, (1990). Being a fast and sensitive method, RAPD can be quickly and efficiently applied to identify useful polymorphisms Ko *et al.*, (1998); Doldi *et al.*, (1997); Ottai *et al.*, (2012); Ali *et al.*, (2014abc); Anwar *et al.*, (2014); Azam *et al.*, (2014); Jehangir *et al.*, (2014); Khan *et al.*, (2014); Shan *et al.*, (2015); Zameer *et al.*, (2015); Zeleňáková *et al.*, (2009). RAPD markers are dominant markers and some loss of information may occur in comparison to the codominant markers. However, RAPD requires no previous knowledge of DNA sequences. In addition, storage of DNA extractions is not as critical with RAPDs, because DNA is relatively more stable than protein that is used for isozyme analysis. RAPD markers are generally randomly distributed throughout the genome and are reported to be efficient and reliable in supporting conventional plant

breeding programs through marker-assisted selection Guthridge *et al.*, (2001).

Keeping in view the importance of genetic diversity in linseed for its yield improvement, the main aim of the study was to analyze the extent and distribution of genetic variation among various linseed genotypes using RAPD.

## 2. Materials and Methods

The study was conducted using 19 linseed genotypes viz. LS-1, LS-2, LS-3, LS-4, LS-8, LS-11, LS-12, LS-13, LS-14, LS-17, LS-19, LS-24, LS-25, LS-26, LS-30, LS-31, LS-32, LS-33 including one check variety (Chandni) in the Biotechnology Laboratory, Department of Plant Breeding and Genetics, PMAS Arid Agriculture University Rawalpindi during 2012-13. Seeds of linseed genotypes were grown in pots in green house of the department of Plant Breeding and Genetics. Fresh leaf samples (8-10) were taken from two weeks old plants and DNA from these samples was extracted following a modified CTAB method for DNA extraction Khan *et al.*, (2004).

### DNA Extraction

Fresh and newly emerged leaves of two weeks old linseed plants were collected and used for DNA extraction. Approximately one to two leaves were taken in an autoclaved mortar and crushed to fine paste using 2-3 ml CTAB buffer (Table 1) and transferred to 1.5 ml eppendorf tubes. The homogenate was incubated at 65 °C for 45 min in water bath (I 800-A, Irmeco GmbH, Germany). An equal volume of chloroform:isoamylalcohol (Table 2) was added and inverted gently. After vortexing, the mixture was centrifuged at 12000 rpm for 20 min at 25 °C. The aqueous phase (supernatant) was transferred to a fresh tube and mixed with an equal volume of ice chilled isopropanol. It was mixed by inverting the tubes very gently for 4-5 times. After mixing, these tubes were placed at 4 °C for 20 min. DNA was recovered as a pellet by centrifugation at 12000 rpm for 10 min at 4 °C and washed with 70 % ethanol. Pellet was dried at room temperature for an hour and resuspended in 100 µl TE buffer (Table 3). To remove RNA, DNA was treated with 1 µl RNase at 37 °C for 1 hour. After RNase treatment, DNA samples were run on 1 % gel to check the quality of DNA and then stored at -20 °C. For Polymerase Chain Reaction (PCR) 1:1 dilution of DNA was made in ddH<sub>2</sub>O.

### Random Amplified Polymorphic DNA Analysis

For polymerase chain reaction GL decamer primers (Gene Link., USA) were used. The PCR reaction mixture (20µl) contained 1 µl of 20ng

genomic DNA, 2µl 10X reaction buffer, 0.4µl dNTPs, 1.6µl MgCl<sub>2</sub> (25mM), 2µl of primer and 0.2µl of Taq Polymerase.

### Primer selection

15 RAPD primers (A series) (Table 4) were used in the study for RAPD analysis and seven polymorphic primers gave scorable bands to amplify the genomic DNA of linseed germplasm (Table 6).

### Optimization of PCR conditions

For RAPD analysis the amplification was performed in programmable thermal cycler (Eppendorf AG 22331, Hamburg, Germany), which was set for one cycle of 5 min at 94 °C, 1 min at 94°C, 1 min at 36 °C and 2 min at 72 °C. Then from step 2 to 4 repeated with a number of cycles forty, then after this 10 min for 72 °C and then held at 4 °C.

### Agarose Gel Electrophoresis

The quality of DNA was checked by running it on 1.5 % agarose gel in TBE buffer (Table 5) and stained with Ethidium bromide solution. The stained gel was photographed by using Gel documentation system (Kodak EDAS 290) The genetic ruler 1kb DNA Ladder (Fermentas) for RAPDs was used for sizing and approximate quantification of wide range double stranded DNA fragments on agarose gel. The DNA was further quantified and dilution was made for further RAPD analysis.

### Statistical analysis

Photographs from ethidium bromide stained agarose gel were used to score RAPD data for analysis. The presence of a particular band was scored as 1 and absence as 0 for each of the 19 varieties with all the random primers. Bands with same mobility were treated as identical fragments. Ambiguous bands that could not be clearly distinguished were not scored. The position of PCR bands were compared with molecular weight standards. All the data analysis was performed by using the software package Multivariate statistical package, version 3.13p (1985-2007). After processing the gel images, all pair wise similarity values were calculated with a similarity coefficient. Then the similarity matrix was converted into Dendrogram using UPGMA (i.e. unweighted pair group method with the arithmetic average) clustering algorithm.

Arithmetic Means (UPGMA) procedure was calculated given by Nei and Li (1979).

$$GD_{xy} = 1 - \frac{d_{xy}}{d_x + d_y - d_{xy}}$$

Where,  $GD_{xy}$  = Genetic distance between two genotypes,  $d_{xy}$  = Total number of common loci (bands) in two genotypes,  $d_x$  = Total number of loci (bands) in genotype 1,  $d_y$  = Total number of loci (bands) in genotype 2

**Table 1: 2X CTAB**

Chemicals	Mol. Wt.	Final Conc.	100ml
Tris Base (pH 8.0)	121.14 g/mol	100mM	10ml
0.5M Na <sub>2</sub> EDTA (pH 8.0)	372.24 g/mol	20mM	4ml
CTAB	364.4 g/mol	2 %	2g
NaCl	58.44 g/mol	1.4M	8.18g
PVP	40,000 g/mol	1 %	1g
ddH <sub>2</sub> O		Final volume 100ml	

Stored at room temperature

**Table 2: Chloroform:Isoamylalcohol**

Components	Ratio	100 ml.
Chloroform	24	96
Isoamylalcohol	1	4

Stored in screw tight bottle at 4 °C

**Table 3: Tris EDTA (TE) Buffer**

Components	Mol. Wt.	2000 ml.
1M Tris HCl (pH 8.0)	157.6 g/mol	20 ml.
0.5 M EDTA (pH 8.0)	372.24 g/mol	04 ml.
ddH <sub>2</sub> O		Final volume 100ml

Stored at room temperature

**Table 4: RAPD Primers Used for Assessing Genetic Diversity, Their Oligo Names and Sequences.**

Count	Oligo Names	Sequences
1	G1 Decamer A-01	CAGGCCCTTC
2	G1 Decamer A-02	TGCCGAGCTG
3	G1 Decamer A-03	AGTCAGCCAC
4	G1 Decamer A-04	AATCGGGCTG
5	G1 Decamer A-05	AGGGGTCTTG
6	G1 Decamer A-06	GGTCCCTGAC
7	G1 Decamer A-07	GAAACGGGTG
8	G1 Decamer A-08	GTGACGTAGG
9	G1 Decamer A-09	GGGTAACGCC
10	G1 Decamer A-10	GTGATCGCAG
11	G1 Decamer A-11	CAATCGCCGT
12	G1 Decamer A-12	TCGGCGATAG
13	G1 Decamer A-13	CAGCACCCAC
14	G1 Decamer A-14	TCTGTGCTGG
15	G1 Decamer A-15	TTCCGAACCC

**Table 5: Tris Borate EDTA (TBE)**

Components	Mol. Wt.	10X	1X
Tris Base	121.3 g/mol	108 g	Take 100 ml. 10X TBE + 900 ml. dd. H <sub>2</sub> O
Boric Acid	61.83 g/mol	55 g	
EDTA	372.24 g/mol	9.3 g	
ddH <sub>2</sub> O			Final Volume 1000 ml.

Stored at room temperature

### 3. Results and Discussions

#### Molecular Evaluation

#### Molecular Diagnostic for RAPD Primers

The selected genotypes were subjected to molecular characterization using PCR based Random

Amplified Polymorphic DNA (RAPDs) Decamer primers. Out of 15 RAPD primers used, seven primers had given clear and polymorphic bands. To detect genetic polymorphism at DNA level selected primers were applied on linseed genotypes. RAPD

bands were scored as present (1) or absent (0) for all of the linseed genotypes. Each band was assumed to represent a unique genetic locus; the presence of a RAPD band was interpreted as either a heterozygote or dominant homozygote and the absence of a RAPD band as a recessive homozygote. The pattern and extent of RAPD variation were analyzed with respect to primer, polymorphic locus, and cultivar. RAPD variations within accessions were examined and comparisons of RAPD variation were made among genotypes. The comparisons were based on the proportion of fixed recessive loci over the 68 variable RAPD loci observed for the 19 genotypes (Table 6), rather than heterozygosity (Weir, 1990).

Total number of loci traced by these primers was 68 and all were polymorphic. The percentage of polymorphism among these genotypes was 100 %. The size of amplification product ranged from 250 bp to 3000 bp. The selected Random Amplified Polymorphic DNA (RAPDs) Decamer primers generated 8 to 12 visible RAPD bands. The highest number (12) of scorable bands was obtained with primer OPA-02 while the lowest number (8) was

obtained with primer OPA-08, OPA-11 and OPA-12. Maximum genotypes (17) were amplified by primer OPA-02 with 62 scorable bands while minimum genotypes (3) by OPA-12 with 11 scorable bands. Different primers showed variation to detect polymorphism. Genotype LS-4 and LS-19 was amplified by maximum number of primers (6) whereas genotype LS-11, LS-17 and LS-24 were amplified by two primers. Primer OPA-02 showed highest polymorphism (18 %). Primer OPA-08, OPA-11 and OPA-12 showed lowest polymorphism (12 %).

**Polymorphism % = (polymorphic bands / Total number of loci) × 100**

Gel electrophoresis patterns of OPA-02, OPA-03, OPA-08, OPA-09, OPA-10, OPA-11 and OPA-12 RAPD primers are depicted in Figure 1, 2, 3, 4, 5, 6 and 7 respectively as representative pictures. Efficiency of these primers to amplify the genotypes ranged from 17 genotypes by primer OPA-02, 12 genotypes by OPA-03, 8 genotypes by OPA-08, 9 genotypes by OPA-09, 11 genotypes by OPA-10, 10 genotypes by OPA-11 and 3 genotypes by OPA-12.

**Table 6: Sequence of selected decamer RAPD primers for amplification and their polymorphism revealed**

Oligo Names	Sequences (5'-3')	Loci/ fragments	Polymorphic Bands	No. of genotypes amplified
GI Decamer A-02	TGCCGAGCTG	12	12	17
GI Decamer A-03	AGTCAGCCAC	11	11	12
GI Decamer A-08	GTGACGTAGG	08	08	8
GI Decamer A-09	GGGTAACGCC	11	11	9
GI Decamer A-10	GTGATCGCAG	10	10	11
GI Decamer A-11	CAATCGCCGT	8	8	10
GI Decamer A-12	TCGGCGATAG	8	8	3
Total		68	68	
Average		9.7	9.7	
Range		8 - 12	8 - 12	



**Figure 1:** manifests the amplification profile of 19 genotypes out of which 17 genotypes gave scorable bands (62). The size of amplification product ranged from 250 bp to 1200 bp for OPA-02 (TGCCGAGCTG). It gave 12 polymorphic loci. Scorable bands were detected for all linseed genotypes except LS-2 and LS-30. Maximum Scorable bands (7) were given by genotype LS-32 and minimum numbers of bands (2) was detected in genotype LS-24. Total genomic DNA from remaining 02 genotypes did not amplify.

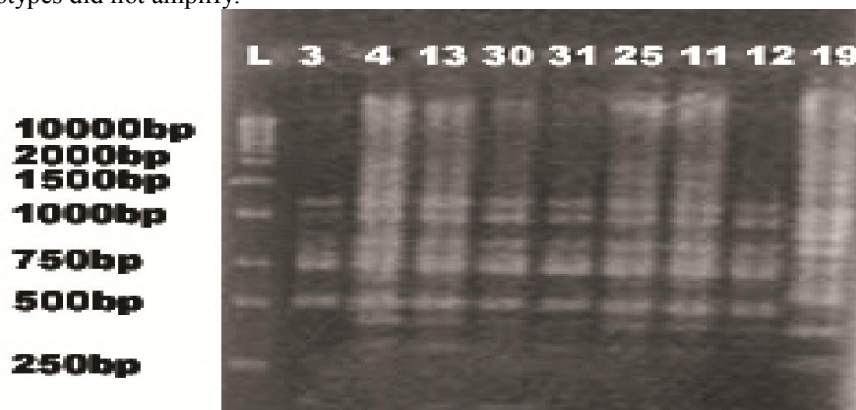




**Figure 2:** shows the amplification profile of 19 genotypes out of which 12 genotypes gave scorable bands (50). The size of amplification product ranged from 250 bp to 1200 bp for OPA-03 (AGTCAGCCAC) . It gave 11 polymorphic loci. Scorable bands were detected for all linseed genotypes except LS-14, LS-31, LS-11, LS-8, LS-12, LS-17 and LS-31. Maximum Scorable bands (5) were given by genotype LS-2 and minimum numbers of bands (2) were detected in LS-30. Total genomic DNA from remaining 07 genotypes did not amplify.



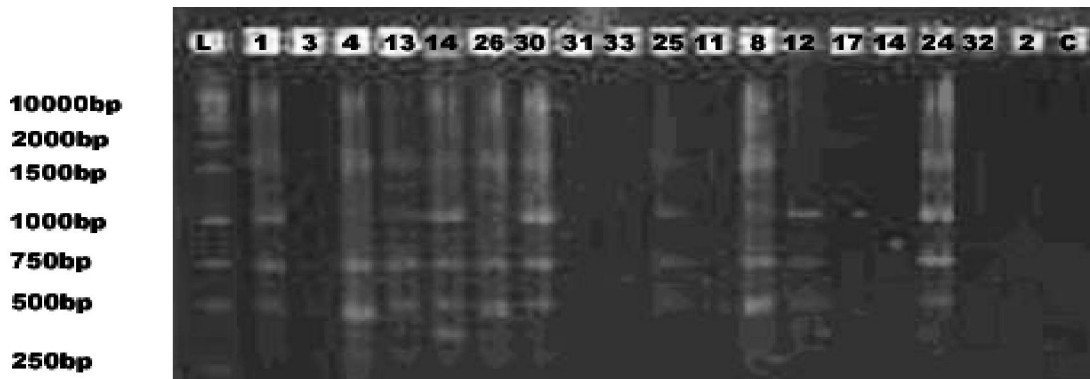
**Figure 3:** shows the amplification profile of 19 genotypes out of which 08 genotypes gave scorable bands (22). The size of amplification product ranged from 250 bp to 1200 bp for OPA-08 (GTGACGTAGG). It gave 8 polymorphic loci. Scorable bands were detected for all linseed genotypes except LS-1, LS-3, LS-13, LS-26, LS-33, LS-25, LS-11, LS-19, LS-32, LS-2 and chandni. Maximum Scorable bands (4) were given by genotype LS-4, LS-14, LS-30 and LS-12 and minimum numbers of bands (1) were detected in LS-8, LS-17 and LS-31. Total genomic DNA from remaining 11 genotypes did not amplify.



**Figure 4:** shows the amplification profile of 19 genotypes out of which 09 genotypes gave scorable bands (51). The size of amplification product ranged from 250 bp to 1500 bp for OPA-09 (GGGTAACGCC). It gave 11 polymorphic loci. LS-1, LS-2, LS-8, LS-14, LS-17, LS-24, LS-26, LS-32, LS-33 and chandni genotypes were not amplified by OPA-09. Maximum Scorable bands (10) were given by genotype LS-19 and minimum numbers of bands (4) were detected in LS-3. Total genomic DNA from remaining 10 genotypes did not amplify.



**Figure 5:** shows the amplification profile of 19 genotypes out of which 11 genotypes gave scorable bands (28). The size of amplification product ranged from 400 bp to 1500 bp for OPA-10 (GTGATCGCAG). It gave 10 polymorphic loci. LS-4, LS-26, LS- 30, LS-25, LS-11, LS-8, LS-32 and chandni genotypes were not amplified by OPA-10. Maximum Scorable bands (7) were given by genotype LS-2 and minimum numbers of bands (1) were detected in LS-12. Total genomic DNA from remaining 08 genotypes did not amplify.



**Figure 6:** shows the amplification profile of 19 genotypes out of which 10 genotypes gave scorable bands (51). The size of amplification product ranged from 400 bp to 2000 bp for OPA-11 (CAATCGCCGT). It gave 8 polymorphic loci. Scorable bands were detected for all linseed genotypes except LS-2, LS-3, LS-11, LS-17, LS-19, LS-31, LS-32, LS-33 and chandni. Maximum Scorable bands (5) were given by genotype LS-14 and minimum numbers of bands (2) were detected in LS-25. Total genomic DNA from remaining 09 genotypes did not amplify.



**Figure 7:** shows the amplification profile of 19 genotypes out of which 03 genotypes gave scorable bands (11). The size of amplification product ranged from 650bp to 3000bp for OPA-12 (TCGGCGATAG). It gave 8 polymorphic loci. Only genotypes LS-19, LS-25 and LS-33 gave scorable bands. Maximum Scorable bands (5) were given by genotype LS-19 and minimum numbers of bands (2) were detected in LS-25. Total genomic DNA from remaining 16 genotypes did not amplify.

### Similarity Matrix

A similarity matrix was generated from RAPD amplification data to estimate genetic diversity and relatedness among 19 linseed genotypes (Table 7). The value of similarity co-efficient of selected linseed

genotypes ranged from 0.5 (50 %) to 0.93 (93 %). Maximum similarity of 0.93 was shown by LS-11 with LS-17 (93 %) and minimum similarity of 0.50 was shown by LS-19 with LS-32 and LS-04 (50 %).

**Table 7: Genetic similarity index showing Nei's Similarity Coefficient among linseed genotypes generated by UPGMA analysis**

	LS1	LS2	LS3	LS4	LS8	LS11	LS12	LS13	LS14	LS17	LS19	LS24	LS25	LS26	LS30	LS31	LS32	LS33
LS2	0.74																	
LS3	0.73	0.70																
LS4	0.64	0.53	0.69															
LS8	0.73	0.81	0.60	0.54														
LS11	0.86	0.86	0.73	0.61	0.76													
LS12	0.73	0.81	0.71	0.69	0.83	0.76												
LS13	0.74	0.66	0.73	0.81	0.61	0.69	0.76											
LS14	0.77	0.77	0.59	0.70	0.84	0.80	0.76	0.69										
LS17	0.81	0.90	0.74	0.57	0.83	0.93	0.83	0.67	0.76									
LS19	0.54	0.63	0.59	0.50	0.64	0.66	0.67	0.54	0.57	0.73								
LS24	0.80	0.83	0.59	0.59	0.81	0.80	0.79	0.66	0.83	0.79	0.51							
LS25	0.60	0.71	0.70	0.67	0.67	0.63	0.79	0.71	0.63	0.64	0.51	0.71						
LS26	0.89	0.77	0.67	0.70	0.76	0.89	0.76	0.74	0.89	0.84	0.57	0.83	0.63					
LS30	0.66	0.66	0.61	0.76	0.70	0.71	0.76	0.74	0.86	0.67	0.63	0.69	0.66	0.77				
LS31	0.66	0.83	0.64	0.67	0.73	0.69	0.87	0.77	0.71	0.73	0.60	0.74	0.77	0.71	0.74			
LS32	0.70	0.73	0.80	0.54	0.63	0.76	0.63	0.61	0.61	0.77	0.50	0.61	0.61	0.70	0.53	0.56		
LS33	0.81	0.76	0.69	0.54	0.66	0.90	0.66	0.61	0.70	0.83	0.59	0.70	0.59	0.81	0.64	0.59	0.69	
chandani	0.66	0.77	0.67	0.59	0.70	0.71	0.73	0.66	0.66	0.76	0.60	0.66	0.66	0.66	0.60	0.66	0.61	0.67

### Dendrogram Interpretation

Similarities among 19 genotypes examined were calculated from the 68 RAPD loci using the simple match formula Apostol *et al.*, (1993)). This similarity matrix was analyzed using NTSYS-PC 2.01 Rohlf, (1997) and clustered with UPGMA (unweighted pair-group methods using arithmetic averages) algorithm to determine the genetic relationships among the 19 genotypes. Dendrogram shows the precise analysis of genetic distance by doing the grouping of genotypes on the bases of similarities and differences. The dendrogram of RAPD based genetic diversity evaluation clearly indicates four main clusters A, B, C and D. Genetic distances of genotypes are mentioned in table 4.10. LS-19 is the most diverse genotype showing maximum genetic distance of 0.41 (41 %) from rest of the 18 genotypes.

**CLUSTER A:** This cluster includes total two genotypes LS-32 and LS-3 (sequence is in accordance to dendrogram) showed a genetic distance 0.204 (20.4 %) with each other.

**CLUSTER B:** This cluster includes four genotypes chandni, LS-25, LS-31 and LS-12. Among these genotypes chandni and LS-25 are genetically more diverse genotype showing a genetic distance of 0.345 (34.5 %) and 0.225 (22.5 %) respectively with all remaining genotypes. In this cluster LS-12 and LS-31 showed a genetic distance 0.135 (13.5 %) with each other.

**CLUSTER C:** This cluster includes six genotypes LS-4, LS-8, LS-13, LS-14, LS-24 and LS-30. This cluster was further divided into two sub clusters i.e. C1, C2 and C3.

**Sub Cluster C1:** This sub cluster includes two genotypes LS-4 and LS-13 sharing a genetic distance of 0.18 (18 %) with each other.

**Sub Cluster C2:** This sub cluster includes two genotypes LS-30 and LS-14 with the genetic distance of 0.14 (14 %) with each other.

**Sub Cluster C3:** This sub cluster includes two genotypes LS-24 and LS-8 with the genetic distance of 0.18 (18 %) with each other.

**CLUSTER D:** This cluster includes six genotypes LS-33, LS-17, LS-11, LS-2, LS-26 and LS-1. This cluster was further divided into two sub clusters i.e. D1 and D2.

**Sub Cluster D1:** This sub cluster includes four genotypes LS-33, LS-17, LS-11 and LS-2. Among these genotypes LS-33 is genetically more diverse genotype showing a genetic distance of 0.17 (17 %) with all remaining genotypes. In this cluster LS-17 and LS-11 showed a genetic distance 0.07 (7 %) with each other. LS-2 showed a genetic distance of 0.11 (11 %) with all remaining genotypes.

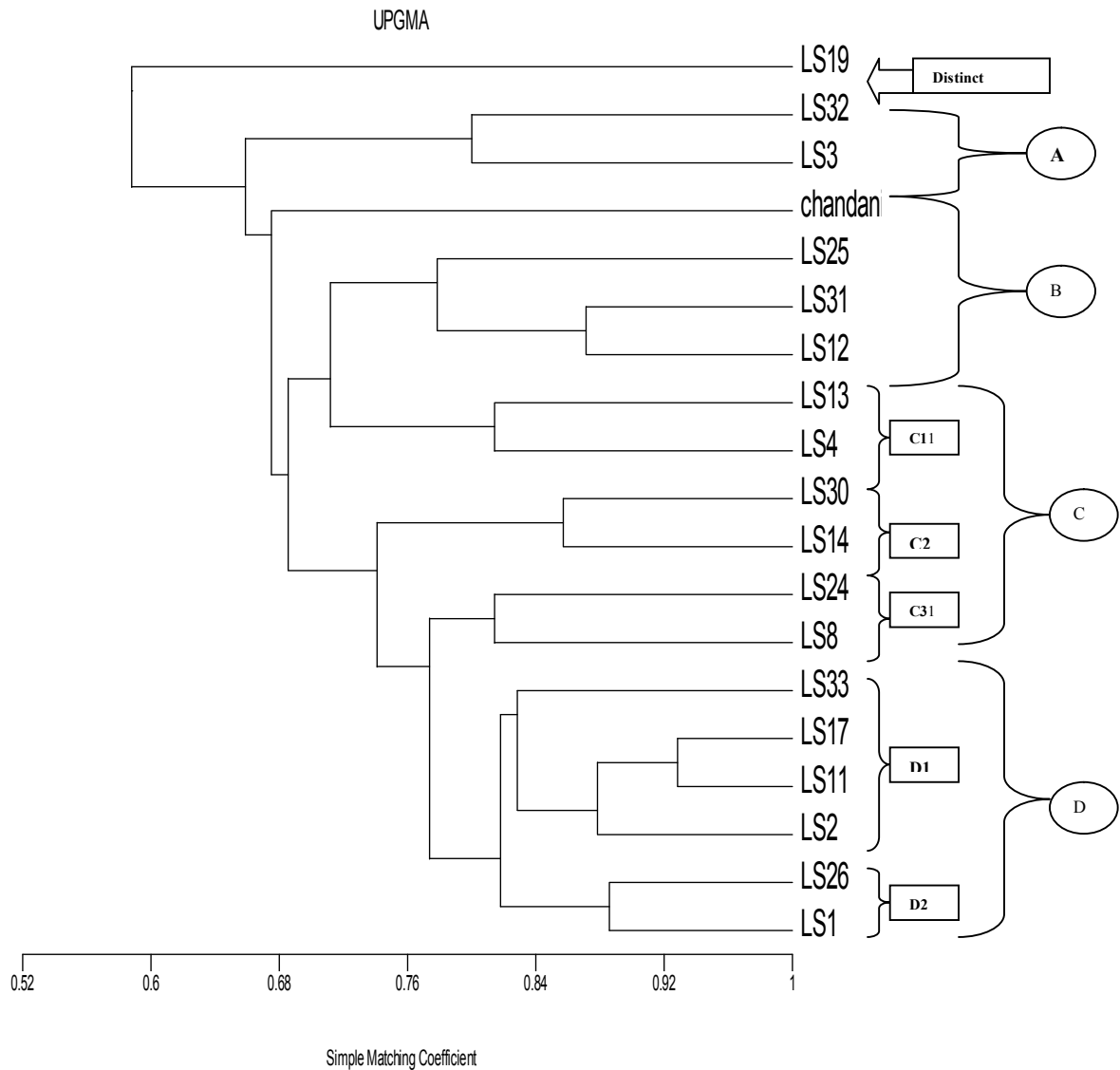
**Sub Cluster D2:** This sub cluster includes two genotypes LS-26 and LS-1 with the genetic distance of 0.1 (10 %) with each other.

The genetic variability of plants results from mutation, selection and random migration. Mutation pressure and selection pressure are major factors changing the level of genetic equilibrium Max *et al.*, (1978). Sources of polymorphism in RAPD assay may be due to deletion, addition or substitution of bases within the priming sites sequences Williams *et al.*, (1990). The objectives of the present study were to describe the levels and patterns of genetic diversity

among linseed genotypes using RAPD markers. This diversity analyses was based on 15 RAPD primers. For analysis of diversity levels, the consideration of estimated genetic distance is very important, as well emphasized by other researchers Roldan-Ruiz *et al.*, (2001). Genetic diversity values of RAPD varied from 0.1 to 0.41. It was evident from the RAPD cluster that genotypes LS-19 had shown maximum diversity with genetic distance of 41 % while diversity of remaining genotypes lies between ranges of 10 – 22.5 %.

In conclusion, our results provide bases for better flax germplasm management, core collection

establishment and exploration of diversity in breeding Smy'kal *et al.*, (2012). Fu *et al.* (2003) reported one or two polymorphic bands per primer indicated that the RAPD variations within linseed accessions were low. In contrast, RAPD showed a high level of polymorphism and a high number of clearly amplified bands among accessions in our study. Our findings are supported by Fu *et al.*, (2005); Žiarovská *et al.*, (2012) where he reported that a large amount of genetic variability exists in the world collection of cultivated linseed.



**Figure 8: Dendrogram constructed through UPGMA of linseed genotypes**



**References**

1. Agarwal M, Shrivastava N and Padh H. (2008). Advances in molecular marker techniques and their application in plant sciences. *Pl. Cell Rep.* 27: 617-631.
2. Ali A, Muzaffar A, Awan MF, Ud Din S, Nasir IA. 2014. Genetically Modified Foods: Engineered tomato with extra advantages. *Adv. Life Sci.*, 1 (3): 139-152.
3. Ali MA, Rehman I, Iqbal A, Din S, Rao AQ, Latif A, Samiullah TR, Azam S, Husnain T. (2014). Nanotechnology, a new frontier in Agriculture. *Adv. life sci.*, 1(3): 129-138.
4. Ali Q, Ahsan M, Ali F, Aslam M, Khan NH, Munzoor M, Mustafa HSB, Muhammad S. 2013. Heritability, heterosis and heterobeltiosis studies for morphological traits of maize (*Zea mays L.*) seedlings. *Adv. life sci.*, 1(1): 52-63.
5. Ali Q, Ahsan M, Tahir, MHN and Basra SMA. Study of gene action for various physiological traits in *Zea mays*. *Rep and Opinion* 2014;6(4):71-83.
6. Ali Q, Ali A, Ahsan M, Ali S, Khan NH, Muhammad S, Abbas HG, Nasir IA, Husnain T. 2014c. Line  $\times$  Tester analysis for morpho-physiological traits of *Zea mays L.* seedlings. *Adv. life sci.*, 1(4): 242-253.
7. Amurrio, J. M., A. A. De Ron and A. C. Zeven. (1995). Numerical taxonomy of Iberian pea landraces based on quantitative and qualitative characters. *Euphytica* 82: 195-205.
8. Anwar M, Hasan E, Bibi T, Mustafa HSB, Mahmood T, Ali M, 2013. TH-6: a high yielding cultivar of sesame released for general cultivation in Punjab *Adv. life sci.*, 1(1): 44-57.
9. Apostol, B. L., W. C. I. V. Black, B. R. Miller, P. Reiter and B. J. Beaty. (1993). Estimation of the number of full sibling families at an oviposition site using RAPD-PCR markers: applications to the mosquito *Aedes aegypti*. *Theor. and Appl. Genet.*, 86: 991-1000.
10. Azam S, Samiullah TR, Yasmeen A, Din S, Iqbal A, Rao AQ, Nasir IA, Rashid B, Shahid AA, Ahmad M, Husnain T. 2013. Dissemination of Bt cotton in cotton growing belt of Pakistan. *Adv. life sci.*, 1(1): 18-26.
11. Butt SJ, Varis S, Nasir IA, Sheraz S, Shahid A, Ali Q. Micro Propagation in Advanced Vegetable Production: A Review. (2015). *Adv. Life Sci.* 2(2). pp: 48-57.
12. Cloutier, S., R. Ragupathy, E. Miranda, N. Radovanovic, E. Reimer, A. Walichnowski, K. Ward, G. Rowland, S. Duguid and M. Banik. (2012). Integrated consensus genetic and physical maps of flax (*Linum usitatissimum L.*) *Theor. Appl. Genet.*, 125: 1783–1795.
13. Dar AI, Saleem F, Ahmad M, Tariq M, Khan A, Ali A, Tabassum B, Ali Q, Khan GA, Rashid B, Nasir IA, Husnain T. Characterization and efficiency assessment of PGPR for enhancement of rice (*Oryza sativa L.*) yield. (2014). *Adv. Life Sci.* 2(1). pp: 38-45.
14. Doldi, M. L., J. Volmann and T. Lelley. (1997). Genetic diversity in soybean as determined by RAPD and microsatellite analysis. *Pl. Br.*, 116: 331-335.
15. Fu, Y. B. (2005). Geographic patterns of RAPD variation in cultivated flax. *Crop Sci.*, 45: 1084-1091.
16. Fu, Y. B., G. G. Rowland, S. D. Duguid and K. W. Richards. (2003). RAPD analysis of 54 North American flax cultivars. *Crop Sci.*, 43: 1510-1515.
17. Guthridge, K. M., M. Dupal, R. Kolliker, E. S. Jones, K. F. Smith and J. W. Forster. (2001). AFLP analysis of genetic diversity within and between populations of perennial ryegrass (*Lolium perenne L.*) *Euphytica*, 122: 191-201.
18. Jahangir GZ, Nasir IA, Iqbal M. Disease free and rapid mass production of sugarcane cultivars. (2014). *Adv. life sci.*, 1(3): 171-180.
19. Kalendar R and A. H. Schulman (2006). IRAP and REMAP for retrotransposon- based genotyping and fingerprinting. *Nat Protoc.* 1: 2478-2484.
20. Khan JA, Afroz S, Arshad HMI, Sarwar N, Anwar HS, Saleem K, Babar MM, Jamil FF (2014). Biochemical basis of resistance in rice against Bacterial leaf blight disease caused by *Xanthomonas oryzae pv. oryzae*. *Adv. life sci.*, 1(3): 181-190.
21. Khan, M. A., M. Munir, S. Ajmal, M. A. Rajput, B. R. Kazi, S. Junejo and B. A. Abro. (2004). Heritability estimates of quantitative characters in barley under rainfed conditions. *Indus J. Pl. Sci.*, 3: 173-175.
22. Ko, M. K., J. Yang, Y. H. Jin, C. H. Lee and B. J. Oh. (1998). Genetic relationships of *Viola* species evaluated by Random Amplified Polymorphic DNA Analysis. *J. Hort. Sci. and Biotech.*, 74: 601-605.
23. Li, Y. C., T. Fahima, J. H. Peng, M. S. Röder, V. M. Kirzhner, A. Beiles, A. B. Korol and E. Nevo. (2000). Edaphitic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. *Theor. Appl. Genet.*, 101: 1029-1038.
24. Nei, N. and W. Li. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.*, 76: 5269-5273.

25. Nevo, E., Golenberg, E., Beilies, A., Brown, A. H. D. and D. Zohary. (1982). Genetic diversity and environmental associations of wild wheat. *Triticum diococcoides* in Israel. *Theor. Appl. Genet.*, 62: 241-254.
26. Ottai, M.E.S., M.A.A. Al-Kordy, R.M. Hussein and M.S. Hassanein. (2012). Genetic Diversity among Romanian Fiber Flax Varieties under Egyptian Conditions. *Aust. J. Basic and Appl. Sci.*, 6: 162-168.
27. Payne, T. J. (2000). Promoting better health with flaxseed in bread. *Cereal Foods World*. 45: 102-104.
28. Rohlf, F. J. (1997). NTSYS-pc 2.1. Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, NY.
29. Roldan-Ruiz, I., F. A van Eeuwijk, T. J. Gilliland, P. Dubreuil, C. Dillmann, J. Lallemand, M. De Lose and C. P. Baril. (2001). A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theor. Appl. Genet.*, 103: 1138-1150.
30. Shan MA, Tahira F, Shafique M, Hussain M, Perveen R, et al (2015). Estimation of Different Biochemical Intensities in Drinking Water from Eastern Region of Lahore City. *Adv. Life Sci.* 2(3). pp: 131-134.
31. Shanmugam, A. S. and S. R. Shreerangswamy. (1982). Multivariate analyses of genetic divergence in blackgram (*vigna mungo* L.). *Indian J. Genet. Pl. Br.*, 54: 6-9.
32. Smith, S. E., A. A. Doss and M. Warburton. (1991). Morphological and agronomic variation in North African Arabian Alfalfas. *Crop Sci.*, 31: 1159-1163.
33. Smykal P., N. Bacova-Kerteszo, R. Kalendar, J. Corander, A. H. Schulman and M. Pavelek. (2012). Genetic diversity of cultivated flax (*Linum usitatissimum* L.) germplasm assessed by retrotransposon-based markers. *Theor. Appl. Genet.* 122:1385-1397.
34. Tariq M, Ali Q, Khan A, Khan GA, Rashid B, Rahi MS, Ali, A, Nasir IA, Husnain T. (2014). Yield potential study of *Capsicum annum* L. under the application of PGPR. *Adv. life sci.*, 1(4): 202-207.
35. Weir, B. S. (1990). Genetic data analysis. Sinauer Associates, Inc., Sunderland.
36. Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S.V. Tingey. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531-6535.
37. Zameer M, Mahmood S, Mushtaq Z, Tabasum B, Ali Q, et al. (2015). Detection of bacterial load in drinking water samples by 16s rRNA ribotyping and RAPD analysis. *Adv. Life Sci.* 2(3). pp: 135-141.
38. Zeleňáková, L., M. Šesták and R. Židek. (2009). Monitoring of sheep milk and milk products adulteration on common European food market. *Potravinárstvo*. 3: 69-74.
39. Žiarovská, J., K. Ražná, S. Senková, V. Štefúnová and M. Bežo. (2012). Variability of *Linum usitatissimum* L. based on molecular markers. *ARPN J. of Agric. and Bio. Sci.*, 7: 50-58.