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*Full Length Research Paper*

# Estimation of genetic variability in Linseed (*Linum usitatissimum* L.) using molecular marker: Molecular characterization of linseed genotypes

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***Linum usitatissimum,* L. accessions were analyzed using RAPD (Random Amplified Polymorphic DNA) markers to assess the genetic variability among 20 genotypes. For optimization of sampling, development of appropriate breeding strategy and estimation of genetic diversity RAPD is an efficient and cost effective molecular tool. Different primers were used for the analysis yielding a total of 83 loci, out of which 51 were polymorphic, showing 64% of overall polymorphism. A dendrogram was generated based on the similarity matrix by the Un weighted Pair Group Method with Arithmetic Mean (UPGMA), wherein the flax genotypes were grouped in five major clusters. The maximum similarity was observed between genotypes V-38 and V-40 (98.8%). While the most diverse genotypes were V-28 and V-21 (71.08%). Analysis has given no evidential grouping based on straight pedigrees relationships, but wider genetic background of genotype can be seen. The most diverse genotypes were identified and suggested their use in breeding programs and mapping the linseed genetic pool.**

**Key words:** Linseed, RAPD, genetic diversity, molecular markers, cluster analysis, polymorphism.

## INTRODUCTION

Linseed (*Linum usitatissimum* L.) has been cultivated for oil and fiber several years ago (Zohary and Hopf, 2000). It is also used in treatment of some inflammatory human and animal diseases in Pakistan and its oil is mainly utilized in the preparation of paint, printing ink and innumerable by-products (Akbar et al., 2003). Linseed is very rich in oil and after extracting oil the refuse called seed cake is a well known fattening food for cattles. These seeds contain gum, acetic acid, acetate and muriate of potash, and other salts. They were taken as food by the ancient Greeks and Romans. An infusion of the seeds has long been given as linseed tea for soothing a sore chest or throat in severe catarrh and pulmonary complaints. Crushed seed is also used for making poultices.

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The cultivated form of linseed is annual self pollinating diploid plant with a karyotype of 2n=30. Morphological (Diederichsen and Hammer, 1995) genetic (Gill and Yermanos, 1967) and molecular (Fu et al., 2002) evidence suggest that the wild progenitor of cultivated linseed is pale linseed (*Linum angustifolium* L.). It is the sole specie of agricultural importance within the family Linaceae and belongs to the founder crops that initiated agriculture in the old world (Zohary, 1999) grown for its fibers and oil or both (dual purpose).

Assessment of genetic variability is the first step in any crop improvement programme. Diversity analysis is an essential process for clear and sound identification of the genetic relatedness of the available genetic resources. It is also required for effective choice of parents for subsequent crossing and selection of the progenies. Morphological or phenotypic descriptors have traditionally been used to distinguish one accession from the other but they are subjected to environmental influences, time con-

suming and they must be assessed during a fixed vegetative phase of the crop (Swanepoel, 1999). Analysis of plant genomes by using DNA markers allow the breeders to rapidly develop crop varieties with enhanced productivity. Genomic studies enable researchers to organize genome and also providing a number of practical applications, like variety identification through DNA fingerprinting and developing genetic maps that facilitate the indirect selection of economic traits (e.g. disease resistance) without cumbersome screening or cloning of important genes in evolutionary and phylogenetic studies (Guthridge et al., 2001 and Khan et al., 2002).

Molecular markers techniques are the modern tools for precise estimation of genetic variation in crop species. Among them, RAPD markers, generated by the Polymerase Chain Reaction (PCR) have widely been used since 1990s to assess intra specific genetic variation at molecular level (Williams et al., 1990). So collection and molecular characterization of linseed germplasm from various geographic regions is required, not only for the study of linseed domestication but also for the exploration of new sources of genetic diversity. The objective of proposed study was to determine genetic diversity among linseed genotypes to design a concrete breeding programme for future.

## MATERIALS AND METHODS

For the present study twenty accessions of linseed were collected from the Ayub Agriculture Research Institute (AARI) Faisalabad (Table 1). Seeds of linseed germplasm were sown in pots under dark conditions and controlled temperature ranging from 26-28$^{o}$C and humidity.

Fresh leaf samples (2g) from etiolated potted linseed plants were collected two weeks after germination and immediately stored at -80˚C until DNA extraction. For molecular studies DNA from these samples was extracted following a modified CTAB method (Khan et al., 2004). The DNA quality was checked electrophoretically and spectrophotometrically. Four series (A, I, J, K) of RAPD primers were used in the study for RAPD analysis and twenty polymorphic primers were selected to amplify the genomic DNA of linseed germplasm (Table 2). Following PCR profile was optimized for proper amplification of RAPD fingerprint. Hot start at 95˚C for 5 min, forty cycles of Denaturation at 95˚C for 1 min, Primer annealing at 34˚C for 1 min and Extension at 72˚C for 2 min. Final extension at 72˚C for 10 min.

The fingerprints were examined under ultra violet transilluminator and photographed using SyneGene Gel Documentation System. The data generated from the detection of polymorphic fragments were analyzed using popgen32 software (Ver. 1.44) (Yeh et al., 2000). All amplification products were scored as present (1) or absent (0) for each of the 20 accessions with all the random primers. Ambiguous bands that could not be clearly distinguished were not scored. The bands were counted by starting from top to bottom of the lanes. Genetic similarity between all the 20 varieties was estimated by simple matching co-efficient.

## RESULTS AND DISCUSSION

### Genetic diversity based on RAPD analysis

Twenty linseed genotypes were analyzed by 20 RAPD decamers. Each primer-template produced different, easily detectable bands of variable intensities. Indistinct bands were considered as non-specific amplification and these were ignored. The bands reproducible over repeated runs with sufficient intensity to detect presence or absence with confidence were used for fingerprinting. RAPD bands were scored as present (1) or absent (0) for all of the flax accessions. Considering all the primers and linseed genotypes 61% of overall polymorphism was observed.

The polymorphism percentage was lower than that obtained by Fu (2002), who found 84% polymorphism in linseed genotypes. This level of polymorphism observed in linseed is comparable to the reports of several RAPD studies by various workers. Reports from Lakhanpaul et al. (2000), Chattopadhyay et al. (2008) and Betal et al. (2004) showed 64%, 72.38%, and 63.17% polymorphism, respectively. Number of bands produced per genotype ranged from 45 to 77 with an average of 60 bands per genotype. Linseed accession V-28 produced the maximum number of bands (77) while V-36 gave the minimum number of bands (45) as shown in figure 1a and 1b.

The selected decamer primers generated 3 to 6 apparent RAPD bands with an average of 5 bands per primer. Among the 20 primers, two primers (J-11 and A-11) generated no polymorphic bands. The low degree of similarity (monomorphic bands) indicated high divergence between the varieties. The maximum number of fragments were produced by the primer GL K-18 (8), while the lower frequency of fragments were produced by the primer GL J-11 (3), GL I-03 (3), GL K-13 (3) and GL A-11 (3) as shown in figure 2.

### Genetic Relationship among Linseed Genotypes

Multivariate analysis was conducted to generate a similarity matrix using Popgen 32 software, version 1.44 (yeh et al., 2000) based on Nei's Unweighted Paired Group of Arithmetic Means Average (UPGMA) to estimate genetic distance and relatedness of linseed germplasm. Dendrogram drawn for the genetic distances is shown in the Figure 3. The maximum similarity was ob-

**Table-1.** Linseed genotypes.

| Sr. No. | Line | Parents |
|---|---|---|
| 1 | V 2I | Chandni × LS-30 |
| 2 | V 22 | Jhang |
| 3 | V 23 | LIRAL Prince |
| 4 | V 24 | NC-30 |
| 5 | V 25 | $P_1$-1-9L-5-93 |
| 6 | V 26 | $P_2$-IFR×T-20 |
| 7 | V 27 | $P_3$-IFR×T-20 |
| 8 | V 28 | P-20-21 |
| 9 | V 29 | $P_{22}$-12-7 |
| 10 | V 30 | P-30-17 |
| 11 | V 31 | P-101 |
| 12 | V 32 | P-104 |
| .13 | V 33 | P-105 |
| 14 | V 34 | P-106 |
| 15 | V 35 | P-109-3-48-51 |
| 16 | V 36 | Plate Bold |
| 17 | V 37 | Plate-14 |
| 18 | V 38 | PB-80 |
| 19 | V 39 | Rhori×P-35-20-10 |
| 20 | V 40 | Royal-4 |

**Table 2.** Detail of RAPD primers along with their sequences used in the study.

| Sr. No | Primer Name | Sequence |
|---|---|---|
| 1 | GL DecamerA-07 | GAAACGGGTG |
| 2 | GL DecamerA-11 | CAATCGCCGT |
| 3 | GL DecamerA-15 | TTCCGAACCC |
| 4 | GL DecamerA-18 | AGGTGACCGT |
| 5 | GL DecamerA-20 | GTTGCGATCC |
| 6 | GL DecamerJ-05 | ACATGCCGTG |
| 7 | GL DecamerJ-11 | ACTCCTGCGA |
| 8 | GL Decamer J-17 | ACGCCAGTTC |
| 9 | GL DecamerJ-18 | TGGTCGCAGA |
| 10 | GL DecamerJ-19 | GGACACCACT |
| 11 | GL DecamerJ-20 | AAGCGGCCTC |
| 12 | GL Decamer I-02 | GGAGGAGAGG |
| 13 | GL Decamer I-03 | CAGAAGCCCA |
| 14 | GLDecamerI-06 | AAGGCGGCAG |
| 15 | GL Decamer I-11 | ACATGCCGTG |
| 16 | GL Decamer K-13 | GGTTGTACCC |
| 17 | GL DecamerK-15 | CTCCTGCCAA |
| 18 | GL Decamer K-18 | CCTAGTCGAG |
| 19 | GL DecamerK-19 | CACAGGCGGA |
| 20 | GL Decamer K-20 | GTGTCGCGAG |

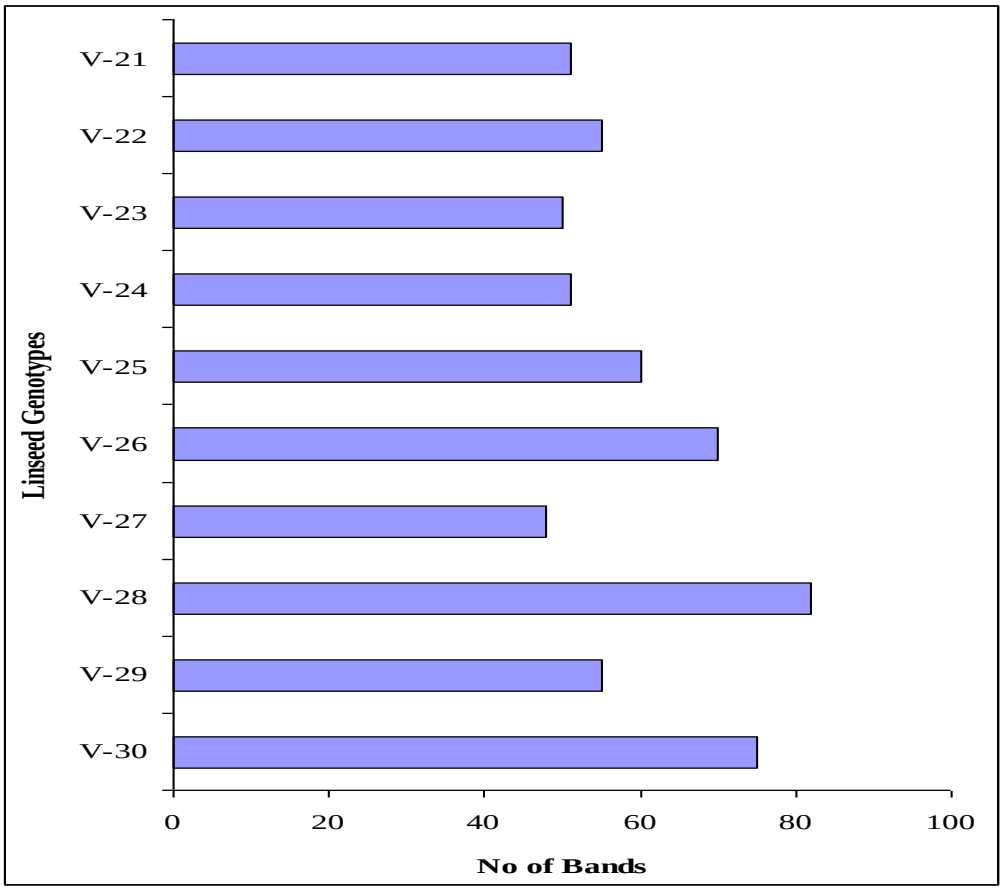


**Figure 1a.** Total number of bands per genotype.

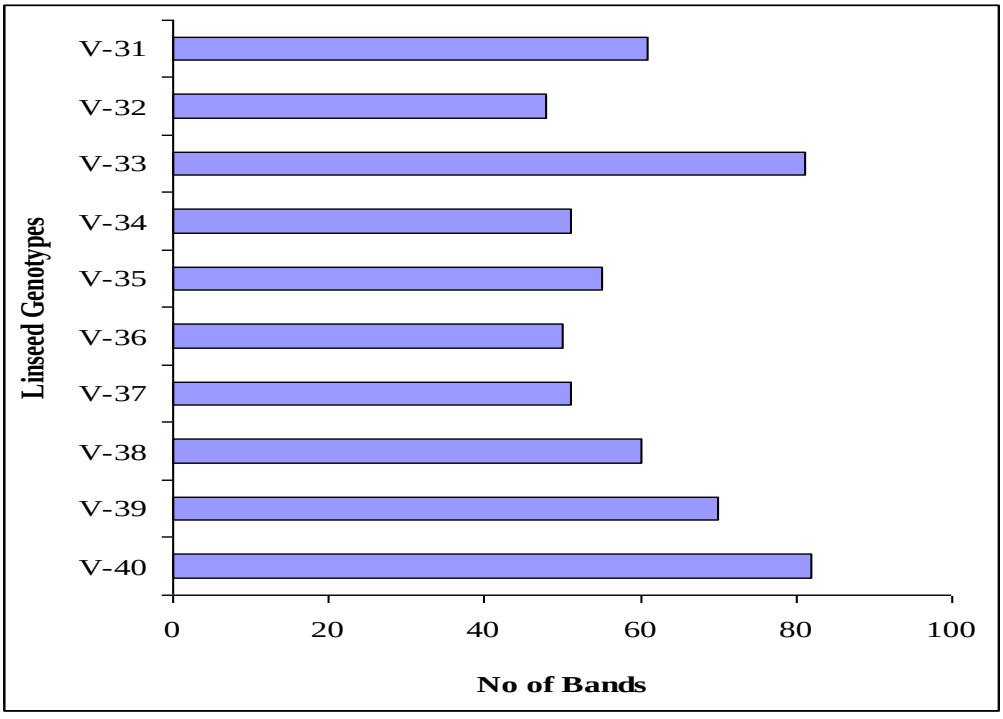


**Figure 1b.** Total number of bands per genotype

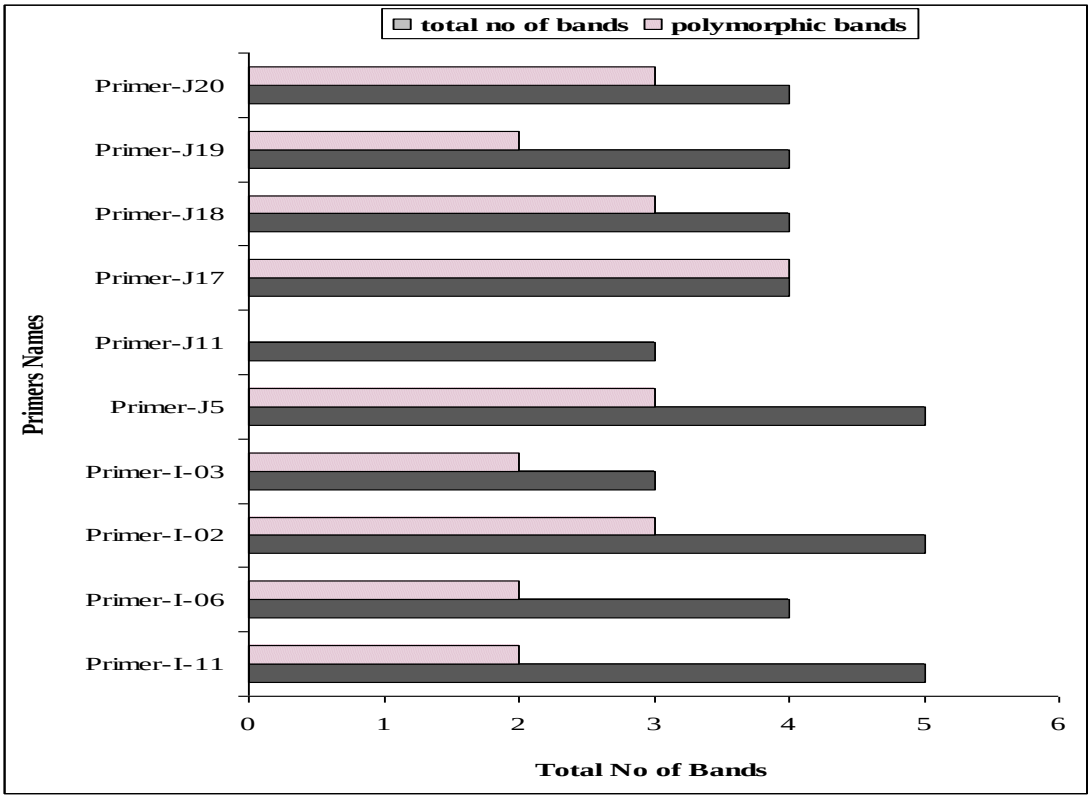


**Figure 2**. Total number of bands per primer.

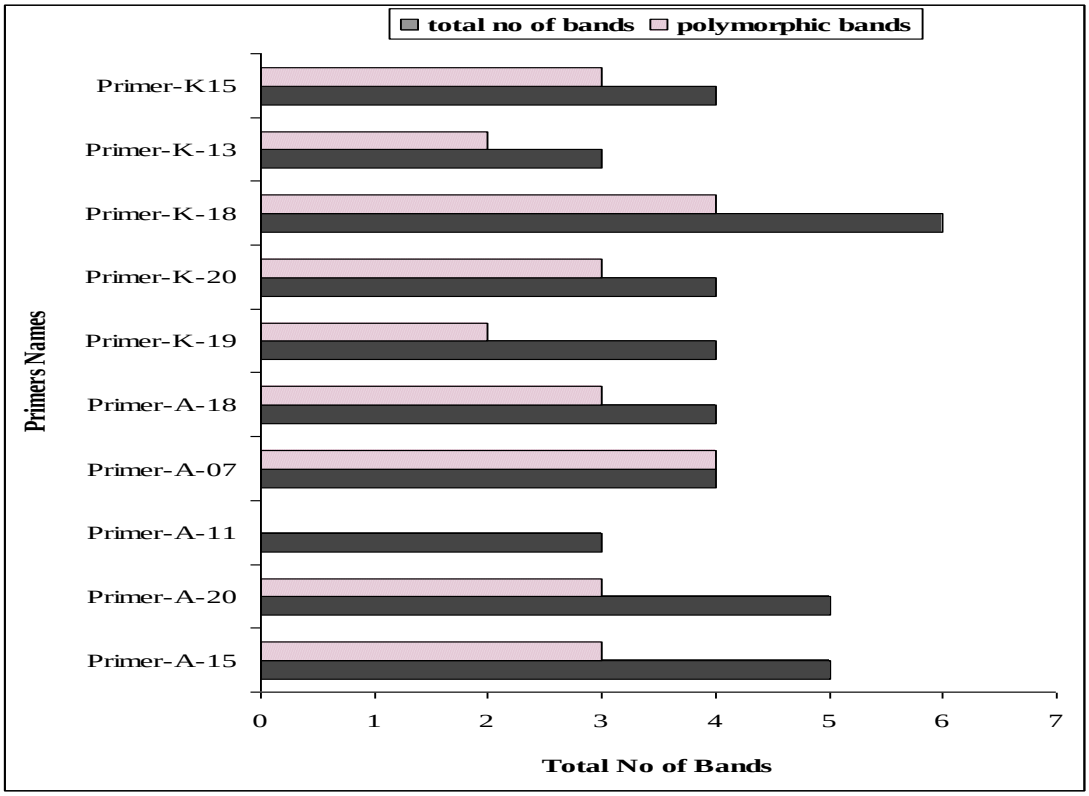


**Figure 3**. Total number of bands per primer.

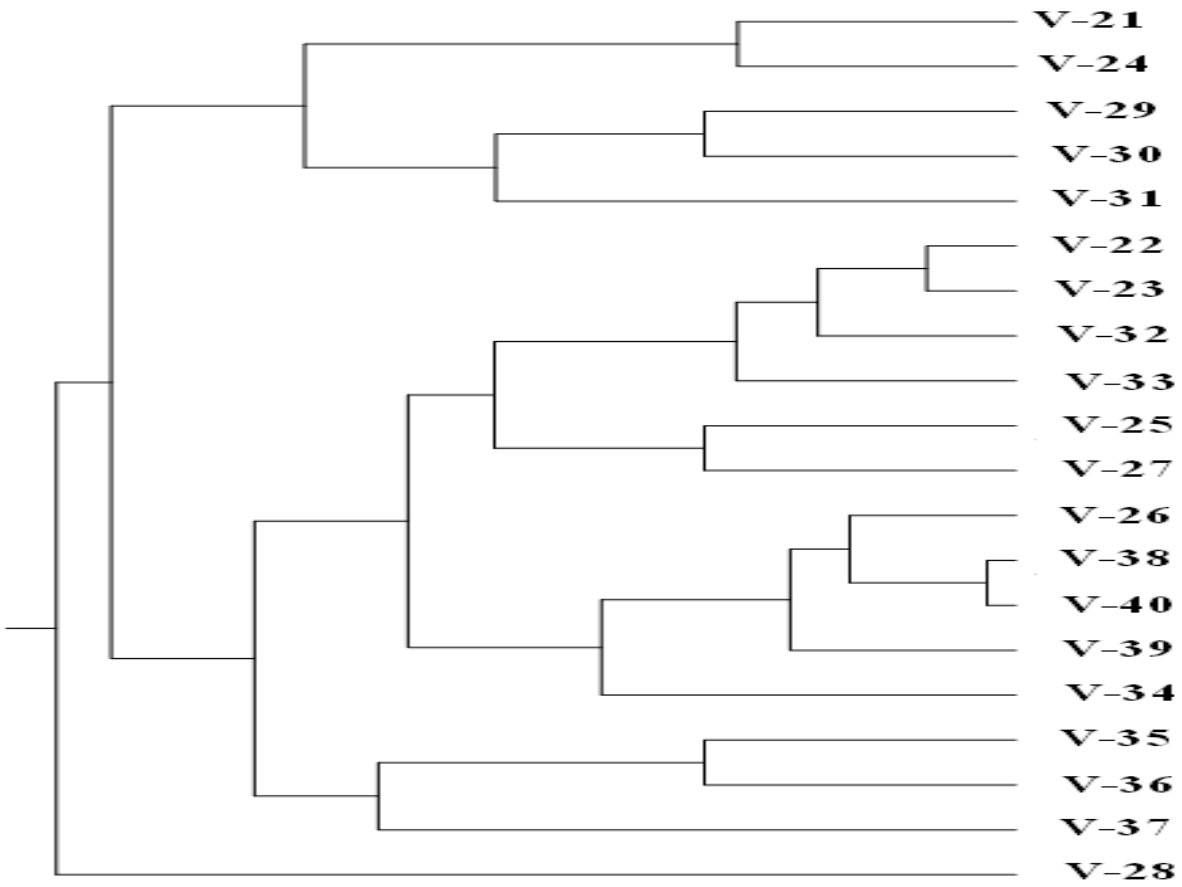


**Figure 4.** Dendrogram of twenty linseed genotypes obtained from similarity matrix.

served between genotypes V-38 and V-40 (98.8%). while the most diverse genotypes were V-28 and V-21 (71.08%).

Cluster analysis classified linseed genotypes into five distinct groups:

Group A consisted of five genotypes i.e., V-21, V-24, V-29, V-30, V-31, in this group V-21 and V-24 showing close similarity among them. In this group V-21, V-24, V-29 and V-30 clustered together while the remaining 1 genotype V-31 remained unclustered showing a distinct behaviour from the rest of the Group-A genotypes.

Group-B consisted of six accessions i.e., V-22, V-23, V-32, V-33, V-25 and V-27. In this group, V-22 & V-23 and V-25 & V-27 clustered together while V-32 and V-33 remained unclustered showing a distinct behaviour from other four genotypes of this group.

Group-C consisted of five linseed accessions i.e., V-26, V-38, V-40, V-39 and V-24. In this group, V-38 and V-40 clustered together showing a close relationship to each other while V-26, V-39 and V-24 remain unclustered having distinct behaviour from other two genotypes of this group.

Group D comprised of four accessions viz; V-35, 36, V-37 and V-28 wherein, V-35, 36 vcxz1 `clustered together, while V-37 and V-28 remained unclustered showing a distinct behaviour from other accessions of Group-D (Figure 4).

The mean number of bands per primer obtained in this study was higher than reported by Karuppanapandian et al. (2006) and Lavanya et al. (2008). The level of polymorphism observed in the study was higher than that reported in previous studies Chattopadhyay et al. (2008) and Betal et al. (2004). The high levels of diversity observed in this study probably were associated with the extensive range of genetic diversity represented in the panel of linseed genotypes.

Genetic diversity reflects a rich history of selection, migration, recombination and mating system. Additionally, the nucleotide diversity across a genome is the source of most of the phenotype variation.

## CONCLUSION

A primary conclusion of the present study is that RAPD showed high polymorphism i.e., 64%. Such a high polymorphism could be attributed to the nature of the genetic material under investigation. The extent of polymorphism differs substantially between accessions.

RAPD markers have found a wide range of applications in

gene mapping, population genetics, molecular evolutionary genetics, plant and animal breeding mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers. Despite the reproducibility problem, the RAPD method would probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labour (Bardakci, 2001).

It was concluded that the RAPD technology is well suited to DNA fingerprinting because it is easy and cheap method, requiring less amount of DNA and less technicalities but it suffers from lack of precision of genetic distance estimates and reproducibility, to a certain degree, due to mismatch annealing.

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